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Effects of Mixed Hardwood and Sugarcane Biochar as Bark-Based Substrate Substitutes on Container Plants Production and Nutrient Leaching

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Abstract: Biochar (BC) has the potential to replace bark-based commercial substrates in the production of container plants. A greenhouse experiment was conducted to evaluate the potential of mixed hardwood biochar (HB) and sugarcane bagasse biochar (SBB) to replace the bark-based commercial substrate. A bark-based commercial substrate was incorporated with either HB at 50% (vol.) or SBB at 50% and 70% (vol.), with a bark-based commercial substrate being used as the control. The total porosity (TP) and container capacity (CC) of all SBB-incorporated mixes were slightly higher than the recommended value, while, the others were within the recommended range. Both tomato and basil plants grown in the BC-incorporated mixes had a similar or higher growth index (GI), leaf greenness (indicated by soil-plant analyses development), and yield than the control. The leachate of all mixes had the highest NO₃-N concentration in the first week after transplantation (1 WAT). All BC-incorporated mixes grown with both tomato and basil had similar NO₃-N concentration to the control (except 50% SBB at 1 and 5 WAT, and 50% HB at 5 WAT with tomato plants; 50% SBB at 5 WAT with basil plants). In conclusion, HB could replace bark-based substrates at 50% and SBB at 70% for both tomato and basil plant growth, without negative effects.

Keywords: biochar; NO₃-N; plant; substrate; container; production

1. Introduction

Both tomato and basil are important crops and 95% of tomato and basil are produced in soilless cultivation systems using different horticultural growing substrates [1]. Tomato is one of the most important horticulture crops, with a total production estimated to be at 164 MT worldwide [2]. Tomato can be grown in coconut fiber, and perlite alone or in mixture with peat, and produce good yields [3]. Additionally, 50% coco-peat mixed with 50% perlite was recommended for tomato seedling production [4]. Basil is an annual herb that is commercially important for its medical and culinary purposes [5,6]. Basil plants can be grown in 75% sphagnum peat moss mixed with 25% coarse perlite [7]. Additionally, the mix of 60% sphagnum peat and 10% biochar with compost, has proven to be suitable for basil production [8].

Container plant production has become a major source of N leaching and runoff that can be a potential contamination source [9,10]. Container plant production requires a large amount of fertilizer, with nitrogen as the key component, making container plant production a major source of

N leaching or runoff [9]. The leachate of N can be a potential contamination source for surface and underground water, resulting in environmental and health concerns [11]. $\text{NO}_3\text{-N}$, the main form for plants absorption, contributes in large to the N leaching and runoff in soilless production systems.

Bark has become one of the most commonly used container organic components in horticulture [12]. The reason for bark being commonly used in horticulture is because it has suitable properties for container plants to grow well and it is easy to get access to [13,14]. Compared to peat moss, another most commonly used container component, bark, is a byproduct of the forestry industry, is less expensive because it is available locally and does not require extra shipping costs [15,16]. In the USA, Douglas fir bark is mainly used in the pacific northwest, while pine bark is mainly used in the southwest [17,18].

Although bark has been a good container component, besides peat moss, its inconstant and unpredictable supply in recent years has limited its usage in horticulture industry [16,19,20]. Bark supply competes with many other markets, including alternatives of industrial fuel, timber production, housing and paper market, all of which prevent bark from being a constant source for the horticulture industry [20–22]. Since the supply of bark is fluctuating and unpredictable, it would be beneficial for the horticulture industry to explore less expensive and more constant alternatives with similar properties [16,22].

Biochar (BC), a by-product from thermochemical biomass decomposition under an oxygen-depleted or oxygen-limited environment [23–25] with specific time and temperature conditions and from certain carbon-rich raw materials, can be a potential alternative to common substrates for plant growth, as has been documented in many trials [16,26–29]. Research has shown that BC can increase water and nutrient holding capacity, ameliorate substrate acidity, and provide suitable environments for plants [30–32]. It, thus, improves greenhouse crop growth, yield, and quality, under appropriate conditions [32–36].

Biochar has been considered to be a sustainable component of a growing substrate because it can be derived from various agriculture by-products, such as green waste [33], wood, straw [31,37–40], bark [41], rice hull [42], and wheat straw [31,43]. Additionally, due to the significant variation in pyrolysis conditions, the BC properties could vary significantly, and there is no universal standard for BC addition to plant production and BC's effects on container substrates vary, as a result [28]. Research on BC as a substrate amendment is still in its infant stage [29]. In this present study, a trial was conducted to determine whether two types of BCs had the potential to be a replacement of bark-based substrate amendments for container plant production.

2. Materials and Methods

2.1. Plant Material

Plant seeds (tomato, *Solanum lycopersicum* 'Red RobinTM', Fred C. Gloeckner and Company Inc., Harrison, NY, USA; basil, *Ocimum basilicum*, Johnny's Selected Seeds, Winslow, ME, USA) were sown in 72-cell plug trays (one seed per cell, cell dimension: 5 cm*4 cm*4 cm, depth/length/width; volume: 55 mL) with a commercial germination substrate (BM2 Berger, Saint-Modeste, Quebec, Canada), on 26 February 2019. After the first pair of true leaves expanded, uniform seedlings were transplanted into 6-inch azalea pots (dimension: 10.8 cm* 15.5 cm*11.3 cm, depth/top/bottom diameter; volume: 1330 mL) with a commercial growing substrate (Jolly Gardener, Oldcastle Lawn & Garden Inc., Atlanta, GA, USA) that was incorporated with either sugarcane bagasse biochar (SBB, American Biocarbon LLC White Castle, LA, USA) at two different rates (50% and 70%; by vol.), or with mixed hardwood biochar (HB, Proton Power Inc. Lenoir City, TN, USA) at 50% (by vol.), on 27 March 2019.

The composition used in this study was chosen because a previous study had showed that 70% of HB can be successfully incorporated with peat moss based commercial substrates and with composts for tomato and basil production [29], and 50% of SBB can be used for petunia growth (not published). We wanted to do further tests of HB with different compositions, on tomato and basil, using tests of SBB with different plant species. The main components for the commercial growing substrate was aged pine bark (55%; by vol.), the other ingredients in the substrate were Canadian

sphagnum peat moss, perlite, and vermiculite. The commercial substrate was used as the control. The pH of SBB and of HB were 5.9 and 10.1, respectively (Table 1). The SBB and HB had electrical conductivity (EC) of 753 $\mu\text{S}/\text{cm}$ and 1,058 $\mu\text{S}/\text{cm}$, respectively [44]. During transplanting, slow-release fertilizer Osmocote Plus (15N-4P-10K, Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) was surface-dressed at the rate of 4.8 g/pot for basil and 7.7 g/pot for tomato. All mixes were placed in a greenhouse at Texas A&M University, College Station, TX, USA. The average greenhouse temperature, relative humidity, and dew point were 23.7 °C, 82%, and 19.6 °C, respectively.

Table 1. The pH, electrical conductivity (EC), total porosity (TP), container capacity (CC), air space (AS), and bulk density (BD) of biochars and the substrate mixes used in this study.

| Composition | pH | EC $\mu\text{S}/\text{cm}$ | TP% | CC % | AS % | BD g/cm^3 |
|-----------------------------|------|----------------------------|-------|-------|-------|---------------------------|
| SBB | 5.9 | 753 | 74 | 71 | 3 | 0.11 |
| HB | 10.1 | 1058 | 87 | 66 | 20 | 0.13 |
| 50%SBB + 50%CS | 6.3 | 2073 | 81 | 75 | 7 | 0.13 |
| 50%HB + 50%CS | 7.5 | 1370 | 78 | 62 | 17 | 0.13 |
| 70%SBB + 30%CS | 6.4 | 1830 | 89 | 76 | 13 | 0.14 |
| CS | 6.5 | 1819 | 97 | 85 | 12 | 0.15 |
| Suitable range ^z | - | - | 50–80 | 45–65 | 10–30 | 0.19–0.7 |

Note: SBB = Sugarcane Bagasse Biochar; HB = Mixed hardwood Biochar; and CS = Commercial bark-based growing mix; ^z Recommended physical properties of container substrate by Yeager et al. [45].

2.2. Measurements

2.2.1. Potting Mix Physical and Chemical Properties

Mix physical properties—total porosity (TP), container capacity (CC), air space (AS), and bulk density (BD)—were measured according to North Carolina State University Horticultural Substrates Laboratory Porometer [46]. The leachate EC and pH were measured every other week, starting at one week after transplantation (1 WAT), with a portable EC/pH meter (Hanna Instrument, Woonsocket, RI, USA), according to the pour-through method [47].

Nutrient leachate was collected whenever EC and pH were measured and was stored in the refrigerator (4 °C) until analysis. A HQ440d Benchtop Meter and ISENO3181 nitrate electrode (Hach Company, Loveland, CO, USA) were used for leachate $\text{NO}_3\text{-N}$ measurements.

2.2.2. Plant Growth

Plant height and two widest canopy widths (width 1: horizontal, width 2: perpendicular) were measured at 1, 3, 5, and 7 WAT. The plant growth index (GI) was calculated according to the formula: $\text{GI} = \text{plant height}/2 + (\text{width 1} + \text{width 2})/4$ [26]. Plants' leaf greenness was measured at 1 WAT with a portable soil-plant analyses development (SPAD) meter, (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Inc., Plainfield, IL, USA). Each plant's leaf greenness was determined by taking averages of readings from three random mature leaves. Plant stem, leaf, and fruit were harvested separately. After being dried at 80 °C in an oven until a consistent weight was reached, their dry weights (shoot dry weight (SDW), leaf dry weight (LDW), fruit dry weight (FDW)) were measured. Plant roots were washed under running water, after harvest. Root length, root surface area, average root diameter, and the number of root tips were measured by using a root scanner (WinRHIZO, Regent Instruments Canada Inc., Quebec, Canada). Root dry weights (RDW) were determined after being dried at 80 °C in an oven, until a constant weight was reached. Total dry weights (TDW) were calculated by adding up the SDW, LDW, FDW, and RDW.

2.3. Statistical Analysis

This experiment was designed as a completely randomized block design with six replications for each mix. A one-way analysis of variance using JMP Statistical Software (version Pro 14.2.0; SAS Institute, Cary, NC, USA) was used for data analysis. All the means were separated by using

Dunnett's test when treatments were significantly different from control at $p < 0.05$. A principle component analysis (PCA) was conducted to evaluate the relationship between the selected variables and were treated using R programming software (version 3.5.1).

3. Results

3.1. Potting Mix Physical and Chemical Properties

Most of the mixes' physical properties were within the recommended range [45], except for the SBB-incorporated mixes, which had a slightly higher TP and CC than the recommended value (Table 1). The 50% SBB mix had a slightly lower AS, as compared to the recommended value. All the mixes had slightly lower BD in comparison to the recommended value and the commercial mix had the lowest BD among all the mixes.

Tomato and basil plants grown in all BC-incorporated pots had similar EC as compared to the control, throughout the experiment, except for the tomato plants in 50% HB at 1 WAT (Figure 1). The 50% HB mixes with tomato plants had a significantly higher pH than the control at 1, 3, and 7 WAT (Figure 2A). The SBB-incorporated mix with tomato plants (50% at 1 WAT, 70% SBB at 7 WAT) had a significantly lower pH, compared to the control. Plants in all the other BC-incorporated mixes had a similar pH, throughout the experiment. Basil plants grown in 50% HB mixes had a significantly higher pH compared to the control, throughout the experiment (Figure 2B). However, basil plants grown in SBB-incorporated mixes (50% and 70%, at 5 and 7 WAT) had a significantly lower pH, compared to the control.

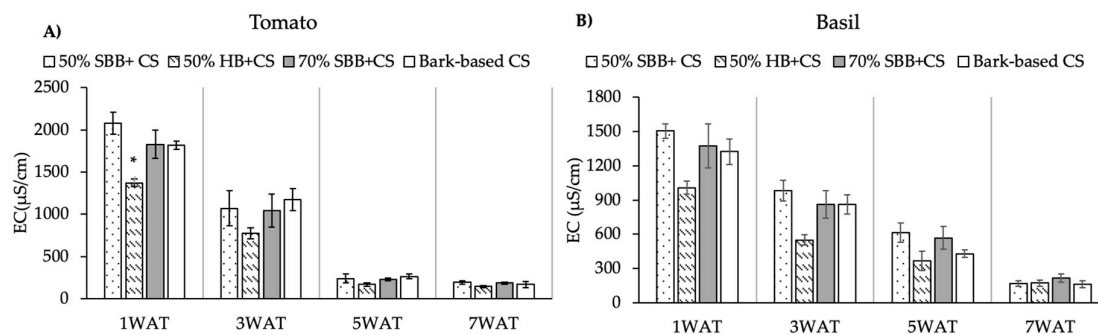


Figure 1. The EC (mean \pm standard error) of potting mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS) with tomato (A) and basil (B) plants at 1, 3, 5, and 7 week(s) after transplanting (WAT). *indicated significant differences from CS using Dunnett's test at $p \leq 0.05$.

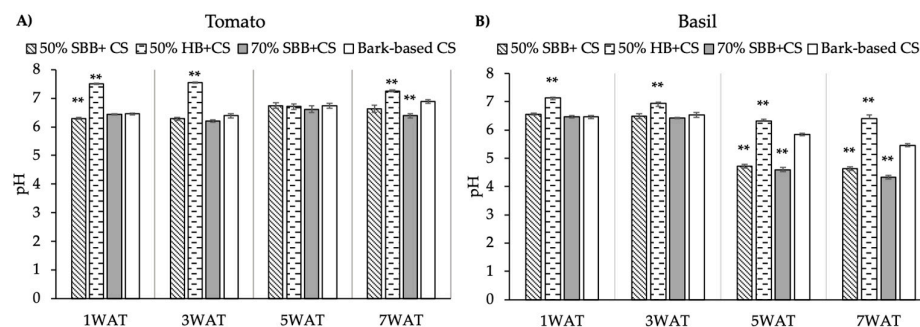


Figure 2. The pH (mean \pm standard error) of container mixes, with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood (HB), and 70% SBB (by vol.) mixed with bark-based commercial

substrate (CS) grown with tomato (A) and basil (B) plants at 1, 3, 5, and 7 week(s) after transplantation (WAT). **indicated significant differences from CS using Dunnett's test at $p \leq 0.01$.

3.2. Leachate $\text{NO}_3\text{-N}$

The leachate of all BC-incorporated mixes (both with tomato and basil plants) had a similar or higher $\text{NO}_3\text{-N}$ concentration compared to the control. The leachate $\text{NO}_3\text{-N}$ concentration generally decreased from 1 WAT to 7 WAT, for each mix (Figure 3).

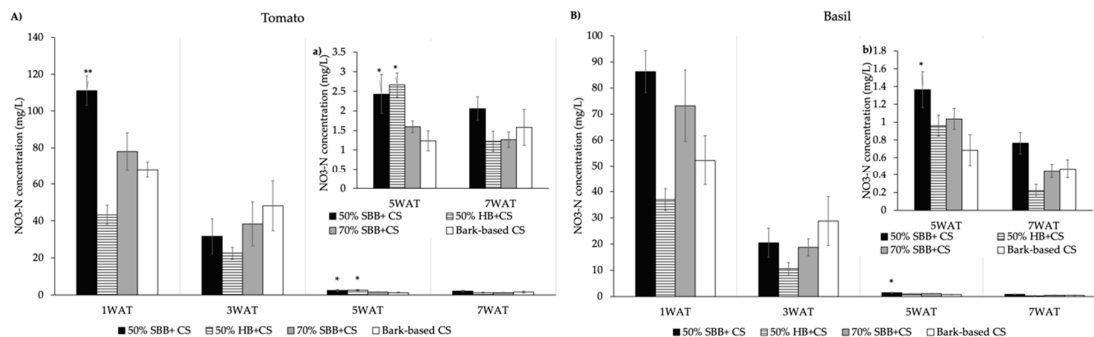


Figure 3. Leachate $\text{NO}_3\text{-N}$ (mean \pm standard error) of tomato (A) and basil (B) plants grown in container mixes with 50% (by vol.) sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB mixed with bark-based commercial substrate (CS). (A,B) Amplified figure for tomato (a) and basil (b) from 5 WAT to 7 WAT. *, **indicated significant differences from CS using Dunnett's test at $p \leq 0.05$ and $p \leq 0.01$, respectively.

3.3. Plant Growth

In the BC-incorporated mixes, both tomato and basil plants had a similar or higher GI, in comparison to the control, throughout the experiment (Figure 4). Tomato plants in all BC-incorporated mixes had similar SDW and FDW (yield), compared to the control, however, tomato plants in SBB-incorporated mixes had significantly lower TDW, RDW, and LDW compared to the control (Figure 5A). Basil plants grown in all BC-incorporated mixes had similar RDW, SDW (except 50% HB), LDW, FDW, and TDW to the control (Figure 5B). The SPAD of tomato and basil plants grown in all BC-incorporated mixes was no different from the control (Figure 6).

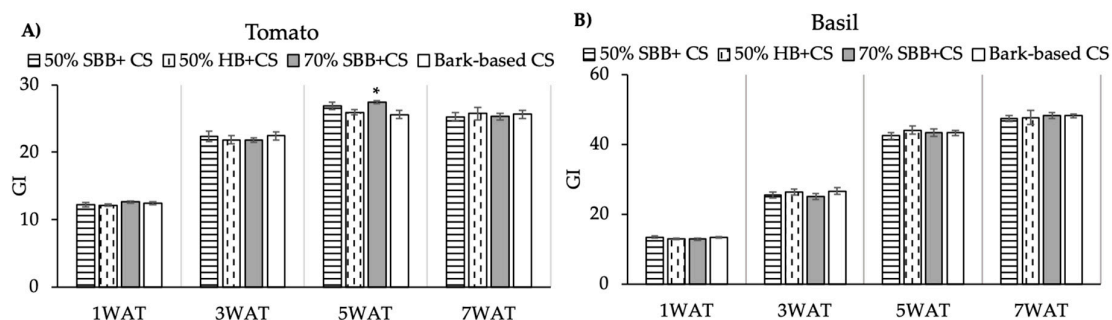


Figure 4. Growth index (mean \pm standard error) of plants tomato (A) and basil (B) grown in container mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS) at 1, 3, 5, and 7 week(s) after transplantation (WAT). *indicated significant differences from CS, using Dunnett's test at $p \leq 0.05$.

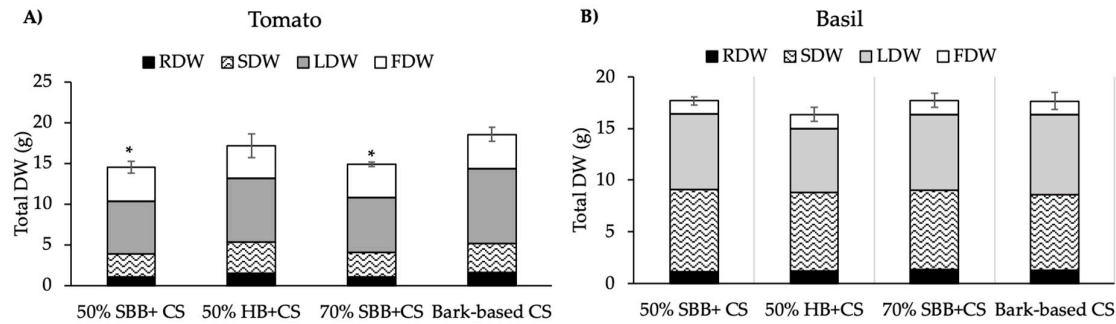


Figure 5. Total dry weight (Total DW = root dry weight (RDW) + shoot dry weight (SDW) + leave dry weight (LDW) + fruit dry weight (FDW); mean \pm standard error) of tomato (A) and basil (B) grown in container mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS). *indicated significant differences on the total DW from CS using Dunnett's test at $p \leq 0.05$.

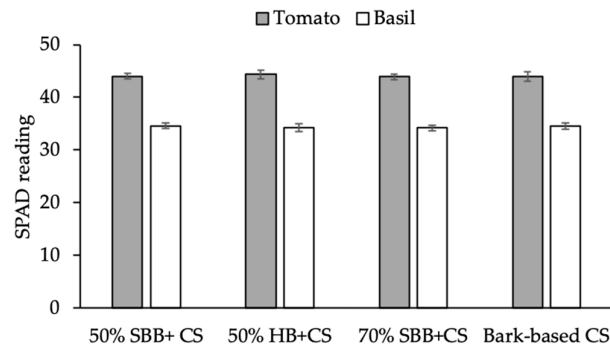


Figure 6. The soil-plant analyses development (SPAD) (mean \pm standard error) of tomato and basil grown in container mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.), mixed with bark-based commercial substrate (CS).

Similar root length, average root diameter, and number of root tips were observed between tomato plants grown in all BC-incorporated mixes and the control (except 50% SBB), however, significantly smaller root surface area of tomato plants grown in all SBB-incorporated mixes were noticed (Table 2). Basil plants grown in all BC-incorporated mixes had significantly shorter root length but bigger diameter than the control. Basil plants in all BC-incorporated mixes had similar root surface area to the control, yet those in 50% BC-incorporated mixes had significantly fewer root tips than the control (Table 2).

Table 2. The root development (mean \pm standard error) of plants grown in potting mixes with 50% sugarcane bagasse biochar (SBB), 50% mixed hardwood biochar (HB), and 70% SBB (by vol.) mixed with bark-based commercial substrate (CS). *, **, and ***indicated significant differences from CS using Dunnett's test at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

| Mixes | Root Length (cm) | Root Surface Area (cm ²) | Average Root Diameter (mm) | Number of Root Tips |
|----------------|------------------|--------------------------------------|----------------------------|---------------------|
| Tomato | | | | |
| 50%SBB + 50%CS | 1214 \pm 60 | 442 \pm 37 * | 1.2 \pm 0.1 | 2650 \pm 94 * |
| 50%HB + 50%CS | 1454 \pm 67 | 557 \pm 24 | 1.2 \pm 0.1 | 3349 \pm 171 |
| 70%SBB + 30%CS | 1234 \pm 74 | 421 \pm 25 * | 1.1 \pm 0.1 | 2970 \pm 196 |
| CS | 1324 \pm 40 | 543 \pm 19 | 1.3 \pm 0.1 | 3227 \pm 157 |
| Basil | | | | |

| | | | | |
|----------------|---------------|----------|---------------|---------------|
| 50%SBB + 50%CS | 1415 ± 48 *** | 819 ± 18 | 1.9 ± 0.1 *** | 3092 ± 166 ** |
| 50%HB + 50%CS | 1887 ± 117 * | 866 ± 23 | 1.5 ± 0.1 * | 3006 ± 149 ** |
| 70%SBB + 30%CS | 1850 ± 115 * | 870 ± 19 | 1.5 ± 0.1 * | 3528 ± 222 |
| CS | 2240 ± 74 | 832 ± 26 | 1.2 ± 0.0 | 4003 ± 80 |

4. Discussion

4.1. Potting Mix Physical and Chemical Properties

Despite the fact that BC can have various effects on substrate properties contingent on the types of feedstocks and the pyrolysis conditions of BC [28,48], many types of BC have been proven to be suitable replacements for commercial growing substrates, without negatively affecting the plant [28,35]. Biochar from fast pyrolysis (pinewood, 450 °C), for instance, could replace commercial substrate at up to 80%, providing suitable properties for the poinsettia and Easter lily growth [26,27]. Biochar from fast pyrolysis (mixed hardwood) could be suitable for tomato and basil plant growth, due to the proper properties it created [29]. Sugarcane bagasse BC and pinewood BC mixes had similar physical properties to commercial growing mix, allowing them to be acceptable for bean and cucurbit seedlings production, even though some of the TP and CC in the SBB-incorporated mixes were slightly higher than the recommended values [44]. Adding pruning residue BC (fast pyrolysis, 500 °C) to soilless mixes can render appropriate physical properties for vegetable production [35,49]. In this study, even though 50% SBB and 70% SBB mixes had slightly higher TP (81%, 89%, respectively) and CC (75%, 76%, respectively) than the recommended value (TP 50%–80% and CC 45%–65%) [45], the growth of tomato and basil plants was not affected, as observed in Webber's study [44].

Different initial BC pH (HB: 10.05, SBB: 5.94) resulted in differences in pH levels in the different BC mixes. Mixes with HB (50%, by vol.) and commercial bark-based substrates (initial pH: 6.81) had a pH lower than the initial HB but higher than the initial commercial bark-based substrate. The same was true for all SBB mixes. Since SBB had an acidic initial pH, adding 30% to 50% of the commercial substrate (pH: 6.81) resulted in mixes with a pH that was lower than the commercial substrate but was higher than the SBB.

4.2. Biochar Effects on Leachate $\text{NO}_3\text{-N}$

Plant species, plant stage, and substrate properties can influence $\text{NO}_3\text{-N}$ leaching [9,50,51]. Tomato, as a heavy feeder fertilizer crop, require more nutrients throughout the growing season than other lighter feeder fertilizer crops, such as snapdragon and bedding plants [52,53]. As a result of administering the same amount of fertilizer to different plant species due to their divergent nutrient requirements, the final $\text{NO}_3\text{-N}$ leaching varies. Additionally, the nutrients demand for plant at different stages also vary. During the growing period, plants' requirement for nutrients presents a skewed "s" curve—vegetative periods need less nutrient yet when entering the flowering/fruit-set period, the demand for nutrients increases dramatically [54]. Nitrate leaching can be also affected by soil or substrate texture and normally, coarse textured mixtures lead to more nitrate leaching [55]. Substrate properties affecting nitrate leaching can explain why leachate from 50% HB (in both case of tomato and basil) had the lowest $\text{NO}_3\text{-N}$ concentration (except tomato at 5 WAT), among all mixes.

4.3. Biochar Effects on Plants Growth

Biochar can have positive, null, and negative effects on plant growth [26,56,57], contingent on plant species, BC types, incorporation rates, and the interactions of both. For instance, pinewood BC had positive effects on bell pepper growth [58], similar results were reported on Easter lily, poinsettia, and "Firework" *Gomphrena*. Mixed hardwood BC can positively affect tomato and basil plants growth [16,26,27,29]. The null and negative effects of BC (from tomato crop waste or wood pellet) on tomato plant growth have also been reported [56,57]. This study obtained similar results to some previous studies that found that BC does not negatively affect plant growth at high incorporation rates [16,26,27,29].

There are few studies with detailed information on BC–root systems [59]. Since roots are essential parts for water and nutrients uptake, plants with better roots were desired [59,60], and the effects of BC on root development is an eventuality. In this study, tomato plants grown in all the BC-incorporated mixes had similar root length, root surface area (except 50% and 70% SBB), average root diameter, and number of tips, in comparison to the control. Basil plants had similar root surface area to the control, which can explain why plants grown in BC-incorporated mixes performed as well as those in the control.

4.4. Treatment Factors Determined Plants and Mix Properties

As the effect of biochar on plants and mix properties can be complex and difficult to explain, given the fact that two types of biochars and multiple variables were included in this study, a principal component analysis (PCA) was used to depict variables shaped by different biochars with tomato (Figure 7A) and basil (Figure 7B) plants. For tomato plants, 88.9% of the variability was explained by the first two components (Figure 7A). PC1 accounted for 65.8% variance, with SBB differing from HB and CS. Sugarcane bagasse biochar was associated more with yield (FDW) and $\text{NO}_3\text{-N}$ leaching, while CS and HB was related more to plant growth (RDW, LDW, and GI). PC2 accounted for 23.1% variance, distinguishing the CS and BC mixes. Commercial substrate tended to be affiliated with plant biomass, however, BC mixes appeared to be related to nutrient leaching. For basil plants, the first two components explained 77.1% of the variability (Figure 7B). PC1 accounted for 42.9% variance, SBB 50% differing from HB and CS mixes. A 50% sugarcane bagasse biochar mix showed a greater association with $\text{NO}_3\text{-N}$ leaching and SDW, while CS, 70% SBB, and HB showed a greater relation to plant growth, including root parameters (RDW, root length (RL), root tip (RT), and root surface area (RSA)) and chemical properties of the mixes (EC, pH). PC2 accounted for 34.2% variance, distinguishing between the CS and BC mixes. Commercial substrates tended to be affiliated with plant biomass, however, BC mixes appeared to be related to the chemical properties of the mixes (EC, pH, $\text{NO}_3\text{-N}$).

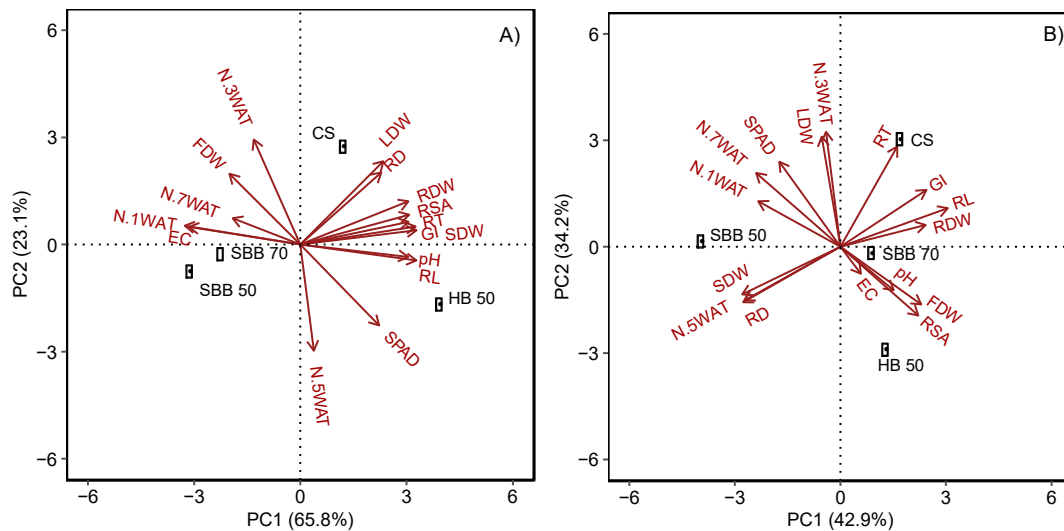


Figure 7. Principal component analysis (PCA) depicting the relationships between selected variables and treatment factors with tomato (A) and basil (B). Selected variables are displayed by arrows and include plant growth parameters—SPAD, growth index (GI), fruit dry weight (FDW), leave dry weight (LDW), shoot dry weight (SDW), root length (RL), root dry weight (RDW), root diameter (RD), root surface area (RSA), and number of root tips (RT); substrate chemical parameters were pH, EC, and $\text{NO}_3\text{-N}$ leachate at different weeks. Treatment factors are displayed by filled grey circles: 50% sugarcane bagasse biochar (SBB 50), 50% mixed hardwood biochar (HB 50), 70% SBB (SBB 70) mixed with bark-based commercial substrate, and bark-based commercial substrate (CS).

5. Conclusions

The mixed hardwood biochar and sugarcane bagasse biochar used in this experiment could be used as bark-based substrate amendments for container plant production. The mixed hardwood biochar could replace the bark-based substrate at 50% and the sugarcane bagasse biochar at 70%, as growing mixes for tomato and basil production. More than 5.4 M ft³ container substrates were used in horticulture industry in 2017 and the current container substrate major components—peat moss and bark are causing serious environmental concerns [61]. As can be seen from the results of this study, if mixed hardwood biochar or sugarcane bagasse biochar was chosen for greenhouse production, around 1.35 M ft³ fewer peat moss or 1.94 M ft³ fewer bark could be used annually (assuming container substrate contains 50% peat moss or bark).

Author Contributions: P.Y. conducted the experiments, collected and analyzed the data, and wrote the manuscript with assistance from all other authors, mainly M.G. L.H. and Q.L. provided technical advice and assistance when the study was conducted. I.M.L. and P.M.W. revised and improved the manuscript. All authors have read and agreed to the published version of the manuscript.

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