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Research Paper

Substitution of peat moss with softwood biochar for soil-free marigold growth

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ABSTRACT

Peat moss has historically been a key component of soil-free substrates in the greenhouse and nursery industries. However, the increasing expense of peat, negative impacts of peat mining on wetland ecosystems, and growing perception of peat as unsustainable have led to investigation for alternatives. Biochar (BC) is a promising substitute for peat, yet the majority of studies examine additions of BC to peat-based substrates rather than replacing the peat component or employ relatively low substitution rates. Furthermore, at high substitution rates the alkalinity common to many BCs may increase substrate pH and adversely impact plant production. We evaluated BC substitution for peat and pH adjustment of resulting substrates on marigold (*Tagetes erecta* L.) performance under standard greenhouse conditions. A high pH (10.9) softwood BC (800 °C) was substituted for peat in a standard 70:30 (v/v) peat:perlite mixture at 10% total volume increments. Substrate pH was either not adjusted or adjusted to pH 5.8 using a BC by-product, pyroligneous acid (PLA). Germination was inhibited in pH adjusted substrates with high BC substitution (50–70% total substrate volume) likely due to higher dosages of PLA needed to neutralize pH. At harvest (flowering stage, 9 weeks) the initial pH gradient (4.4–10.4) in substrates that were not pH adjusted had converged to pH 5.6–7.5, and BC substitution for peat did not negatively impact marigold biomass or flowering. At low substitution rates (10–30% total substrate volume), marigold biomass and leaf SPAD values were greater than the control peat-perlite mixture (0% BC). This study demonstrates that softwood BC can be considered as a full replacement for peat in soil-free substrates, and even at high rates (70% total substrate volume) does not require pH adjustment for marigold production. Crop- and BC-specific considerations and economic potential should be investigated for wider application.

1. Introduction

Soil-free substrates are the basis for greenhouse and nursery industries. Such substrates typically have an inorganic and organic component (Bilderback et al., 2005). The organic component provides high porosity, low bulk density, and nutrient retention (e.g., water, nutrient ions) (Raviv et al., 1986), which makes *Sphagnum* peat moss a strongly suitable option with widespread use (Carlile et al., 2015; Robinson and Lamb, 1975). However, increasing expense and competing uses for peat (Caron et al., 2015), impacts of its harvest on wetland ecosystems (Barkham, 1993; Robertson, 1993), including loss of peat bogs as a key global C sink (Cleary et al., 2005), and its perception as unsustainable (Caron et al., 2015) have spurred recent investigations of substitutes for peat in soil-free substrates, including biomass waste products such as compost and sawdust (e.g., Ceglie et al., 2015; Maas and Adamson, 1972; Wright et al., 2009; Álvarez et al., 2017).

Biochar (BC) has been recently proposed as a strong candidate to substitute for peat because of its high porosity, low density and high cation-exchange capacity (Steiner and Harttung, 2014; Vaughn et al., 2015; Kern et al., 2017). Biochar is a carbon (C)-rich material produced by pyrolysis of biomass and has been a major subject of study as a soil amendment in the last decade (Lehmann and Joseph, 2015). In addition to providing high nutrient and water retention, replacing peat with BC could offset or reverse the C footprint of soil-free substrates into a net C sink (Woolf et al., 2010). Evidence to-date suggests neutral or positive effects of BC use in substrates on nutrient availability and plant growth (as reviewed by Singh et al., 2014), though many studies examine additions of BC to peat-based substrates, rather than replacing a substrate component such as peat (i.e., substitution).

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Evaluating effects of high BC substitution rates on substrate properties and plant growth is necessary to understand the extent to which BC can replace peat. At low amendment (1–5%) or substitution (< 25%) rates, BC has been found to maintain or improve plant growth as a result of increased nutrient availability (Headlee et al., 2014), reduced nutrient and water loss (Altland and Locke, 2013; Beck et al., 2011; Graber et al., 2010), and amelioration of peat acidity (Bedussi et al., 2015), though these effects may be BC-specific due to feedstock and pyrolysis influences on BC properties (McBeath et al., 2015; Zhao et al., 2013).

However, at high substitution rates, substrate properties conducive to plant growth may be compromised. In particular, the high pH of many BCs (Lehmann and Joseph, 2012; Mukome et al., 2013) could result in BC-substituted substrates with pH values unfavorable to plant growth. For example, pelleted wood BC (720–755 °C) substitution for peat (< 15% (v/v) required adjustment of pH due to the liming effect of the BC (Vaughn et al., 2013). The neutral to alkaline pH of BCs and their liming potential (Glaser et al. 2002; Hass et al., 2012; Van Zwieten et al., 2010) means that BC substitution for peat can increase pH beyond optimum for plant growth in potting media (Fryda and Visser, 2015; Steiner and Harttung, 2014; Vaughn et al., 2013). Explicit evaluation of BC effects on substrate pH and plant performance provides a basis to improve design of BC-based substrates and inform trade-offs in this application of BC (Jeffery et al., 2015).

The objective of this study was to determine the effects of BC substitution for peat and substrate pH on greenhouse production, using marigold (*Tagetes erecta* L.) as a model crop. In the United States, the wholesale value of marigolds plants was 30.3 million USD in 2015 (NASS, 2015). Softwood BC was substituted for peat in a typical 70:30 (v/v) peat:perlite mixture at 10%v increments. Since many BCs are alkaline and will increase pH of substrates in proportion to the degree of substitution, the effect of adjusting pH of substrates to typical soil-free substrate values (pH 5.8) was also evaluated. Marigold germination and growth were measured over 9 weeks. We hypothesized that under greenhouse conditions (i.e., fertigation), marigold germination and growth (height, biomass, N uptake) would be more sensitive to BC substitution at higher rates and that this would be due to elevated substrate pH. Additionally, we hypothesized that pH adjustment of BC substrates would increase the extent to which this softwood BC could be substituted for peat without compromising plant growth.

2. Materials and methods

2.1. Biochar characterization

The softwood BC used in this study is manufactured by Pacific Biochar (Blacklite Class I, Pahoa, HI) by gasification (800 °C) of conifer timber species from the northern California Sierra Nevada: Douglas fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), ponderosa pine (*Pinus ponderosa subsp. Ponderosa*), western red cedar (*Thuja plicata*), red fir (*Abies magnifica*), white fir (*Abies concolor*), and grand fir (*Abies amabilis*). The softwood BC was then treated by a greenhouse producer (Greener Latitudes, Petaluma, CA) by washing the BC with a proprietary mixture of seaweed extract, magnesium sulfate, and chitin.

The final BC used in this study is characterized by a pH of 10.85, EC of 515.6 ± 0.1 μS cm^{-1} (1:10 m/v water), total organic C of 653 ± 8 mg g^{-1}, C:N of 120.9 ± 2.9, CEC of 19.0 $cmol_c$ kg^{-1}, 166 mg g^{-1} ash content, water holding capacity (WHC) of 2.38 ± 5 g g^{-1}, 0.5 mol L^{-1} $NaHCO_3$-extractable P of 179.0 ± 2.9 μg g^{-1}, and 2 mol L^{-1} KCl-extractable ammonium-N of 0.04 ± 0.01 μg g^{-1} and nitrate-N of 1.67 ± 0.07 μg g^{-1}.

2.2. Substitution treatments and properties

Eight substrates were formulated to encompass a range of BC substitution for peat at 10% increments in a 70:30 peat:perlite mixture (Table 1). A 70:30 peat:perlite mixture is typical of soil-free substrates for greenhouse production (e.g., Arenas et al., 2002; De Boodt and Verdonck, 1971; Huang et al., 2010; Iannotti et al., 1994; Tsakaldimi, 2006), and similar proportions of organic and inorganic components have been used in investigations on substitution of the peat component by organic matter alternatives (Hidalgo et al., 2006; Peet et al., 2008; Sasse and Sands, 1997; Zhang et al., 2004). Additionally, in a preliminary trial, total above-ground biomass of marigold was greatest in 70:30 organic matter:perlite mixtures for a variety of organic components (e.g., softwood BC, torrefied wood, redwood bark). *Sphagnum* peat moss (Black Gold®) and perlite were sourced from Sun Gro (Agawam, MA) and Supreme Perlite Co. (Portland, OR), respectively.

Table 1
Soil-free substrates representing a range of biochar (BC) substitution for *Sphagnum* peat moss. BC was produced by 800 °C gasification of softwood (8 species). Substrate pH was measured 1:10 (m/v) in water.

BC (% vol).	Substrate composition (%BC-Peat-Perlite)	Substrate pH	
		no pH adj	pH adj
0	0-70-30	4.4	5.8
10	10-60-30	5.6	5.8
20	20-50-30	6.6	5.8
30	30-40-30	7.7	5.8
40	40-30-30	8.2	5.8
50	50-20-30	9.3	5.8
60	60-10-30	9.7	5.8
70	70-0-30	10.4	5.8

To examine effects of pH on substrate suitability for plant growth, pH was adjusted for one set of replicated experimental substrates (0–70% BC) to a target pH of 5.8, as this pH is considered optimum and is standard in soil-free substrates (Vaughn et al., 2013; Verdonck et al., 1982). Calcium hydroxide [$Ca(OH)_2$] was used to increase substrate pH (0 and 10% BC) to pH 5.8, and pyroligneous acid (PLA) produced by pyrolysis of almond shell (pH 2.30, 0.59 mol L^{-1}) (Corigin, LLC, Livermore, CA) was used to decrease substrate pH (20–70% BC) to pH 5.8. Titration of substrates in water (1:2 m/v) was performed using 24 h equilibration to estimate PLA or $Ca(OH)_2$ requirement of substrates to reach the target pH. Substrate pH was then adjusted by mixing substrate with $Ca(OH)_2$ or PLA in 18.9 L polypropylene containers with deionized water (11% v/v). Substrate pH was determined as described above at 1, 2, and 6 days following PLA addition and adjusted as needed with $Ca(OH)_2$ or PLA to ensure that substrates were pH 5.8 ± 0.2 prior to sowing. Water-holding capacity (WHC) of substrates was estimated gravimetrically by difference between oven-dried (105 °C) substrates and substrates 2 h after draining from a state of saturation (Flannery and Busscher, 1982; Priha and Smolander, 1999).

2.3. Plant growth experiment

Marigold (*Tagetes erecta* L.) var. ‘Crackerjack’ seeds (Botanical Interests, Inc., Broomfield, CO) were sown (n = 10) directly in 0.7 L of substrate pre-fertigated to 100% WHC using 0.5% Hoagland solution (pH 6.4) in 1.2 L polypropylene pots in a greenhouse at the UC Davis Plant Growth Facility. Pots were arranged 18 cm apart in a completely randomized block design with four replicates per substrate-pH treatment (n = 16 treatment combinations total) (Table 1). Pots were drip fertigated with 0.5% Hoagland solution at 66 mL d^{-1} for weeks 1–6 and 99 mL d^{-1} for weeks 7–9.

Multiple measurements of plant growth were evaluated in order to comprehensively assess the potential of BC as an alternative to peat in soil-free substrates (Barrett et al., 2016). Germination rates were determined by daily counts for the first 10 days following sowing, after which seedlings were thinned to 1 per pot. Seedlings were transplanted into pots that had zero germination. Replacement seedlings were used from substrates with equivalent %BC but no pH adjustment and were

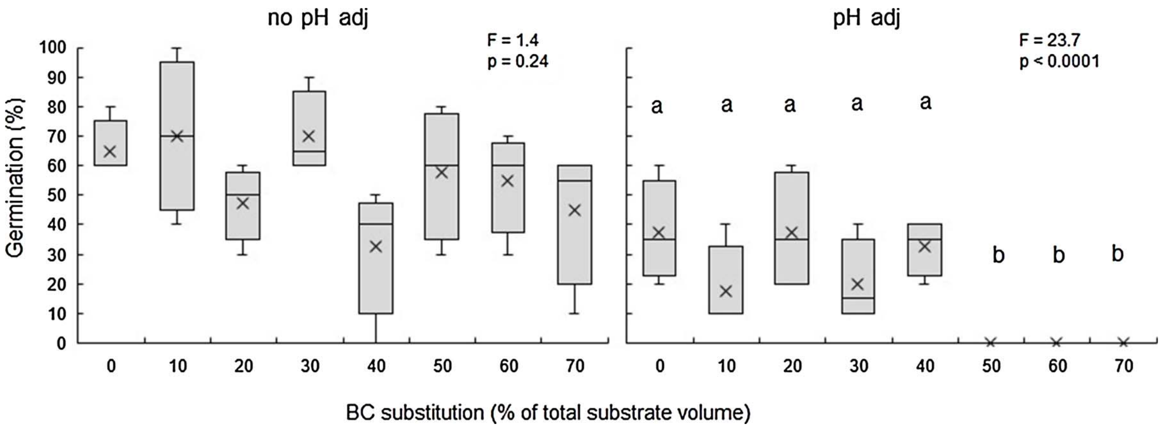

Fig. 1. Germination of marigold across a gradient of softwood biochar substitution for peat moss in soil-free substrates, (a) not adjusted for pH and (b) adjusted to pH 5.8 ± 0.2 at the initiation of the greenhouse growth experiment. Post-hoc analysis of differences among treatments were performed by Tukey's test ($p < 0.05$).

the same age (sowing date) as seedlings in the experimental trial.

Weekly measurements over 9 weeks were taken for plant height and for relative chlorophyll content as leaf greenness using a SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Inc., Aurora, IL). SPAD meters measure the difference between red light (540 nm) and infrared light (940 nm) absorbance, and for a given species and cultivar under the same growing conditions (e.g., light, water availability) SPAD values can be used as an indicator of relative chlorophyll content (Monostori et al., 2016; Xiong et al., 2015). To ensure accurate measurement of new leaf tissue, four separate points were consistently measured on the second fully extended leaf from the top of the plant (apical meristem) (Bi et al., 2010; Wang et al., 2014; Yuan et al., 2016). SPAD measurements were taken between the tip and apex of the leaf to better reflect chlorophyll content and reduce measurement variability (Yuan et al., 2016). At early stage flowering in week 9 (day 67 after sowing), above-ground biomass was harvested. Fresh and dry (60 °C for 72 h) biomass was measured individually for shoots, flowers, and buds. Total N was determined separately for non-flowering (shoots) and flowering (flowers + buds) biomass by dry combustion using an elemental analyzer (Costech Analytical Technologies, Inc., Valenica, CA). Total above-ground biomass N was calculated from non-flowering and flowering shoot biomass and N measurements.

2.4. Post-harvest analysis of substrates

To examine fertigation effects on substrate properties over the 9-week growing period, root-free substrates were analyzed for pH, electrical conductivity (EC) (1:2 m/v water), and plant-available nitrogen (N) and phosphorus (P). Available N was determined by extraction (1:5 m/v) with 2 mol L^{-1} KCl with shaking (120 rpm) for 60 min. Ammonium (NH_4^+) and nitrate (NO_3^-) N in the centrifuged extract were measured colorimetrically using the salicylate-hypochlorite method (Verdouw et al., 1978) and vanadium (III) chloride reduction method (Doane and Horwath, 2003), respectively. Available P was determined by extraction (1:20 m/v) with 0.5 mol L^{-1} $NaHCO_3$ at pH 8.5 with shaking (120 rpm) for 30 min, and orthophosphate (PO_4^{3-}) P in the filtered extract was estimated as molybdate-reactive P (Murphy and Riley, 1962). Available N and P in post-harvest substrates were corrected for substrate moisture content, which was determined gravimetrically by drying at 105 °C.

2.5. Statistical analyses

Analyses of variance (ANOVA) was used to analyze differences among the treatments for plant growth and substrate properties. Assumptions of normality and homoscedasticity of residuals were tested with the Shapiro-Wilk and Levene tests, respectively, using SAS Version 9.4 (SAS Institute, Inc., Cary, NC, USA). Data were transformed when possible to meet these assumptions, including log transformation (shoot biomass, harvest index, total above-ground total N, and post-harvest substrate EC), square root transformation (post-harvest substrate ammonium and nitrate) and Poisson transformation for variables with zero values (germination, number of flowers, flower biomass). ANOVA was first performed using an exploratory model to test for potential interactions of substrate and pH adjustment ($p < 0.05$) for each response variable. If there was no interaction, simple mean differences of response variables were evaluated. If there was a significant interaction of BC substitution and pH adjustment, effects were analyzed separately for each factor. Post-hoc analysis of mean differences were performed using Tukey's HSD test ($p < 0.05$). If transformations were not successful, non-parametric analysis was performed (height, SPAD, post-harvest substrate orthophosphate) with JMP Version 11 (SAS Institute, Cary, NC) using a Welch ANOVA, and significant differences in means for BC substitution treatments relative to the non-substituted control (0% BC) were evaluated using the Steel test. Relationships among post-harvest substrate properties were explored using linear correlation analysis (Pearson's R) with PROC CORR in SAS v9.4.

3. Results

3.1. Germination

Germination of marigold seeds was influenced by the degree of BC substitution and pH adjustment (BC × pH $p = 0.0027$) (Fig. 1). Without pH adjustment, BC substrates had no impact on germination ($p = 0.24$). In contrast, germination appeared to differ significantly by BC substitution in pH adjusted substrates ($p < 0.0001$) due to zero germination in the three highest BC substitutions (50–70%), which received greatest amounts of PLA to reduce substrate pH to the target of 5.8.

3.2. Plant growth

Marigold growth was influenced by BC substitution depending on pH adjustment, and this response changed over the 9-week experimental period (Fig. 2, Supplementary Fig. 1 and 2). Initially (week 1), marigold plant heights were negatively influenced by pH adjustment ($p = 0.001$) independently of BC substitution (BC × pH $p = 0.26$). Increasing BC substitution appeared to decrease plant heights at week 1, with lower mean heights (40–49 mm) in 50–70% BC substrates compared to 0% BC (55 mm), though these differences were not statistically significant (Supplementary Fig. 1). By week 9, with over 10-fold increases in plant height, effects of BC substitution depended on initial pH adjustment (BC × pH $p = 0.055$), with significant BC

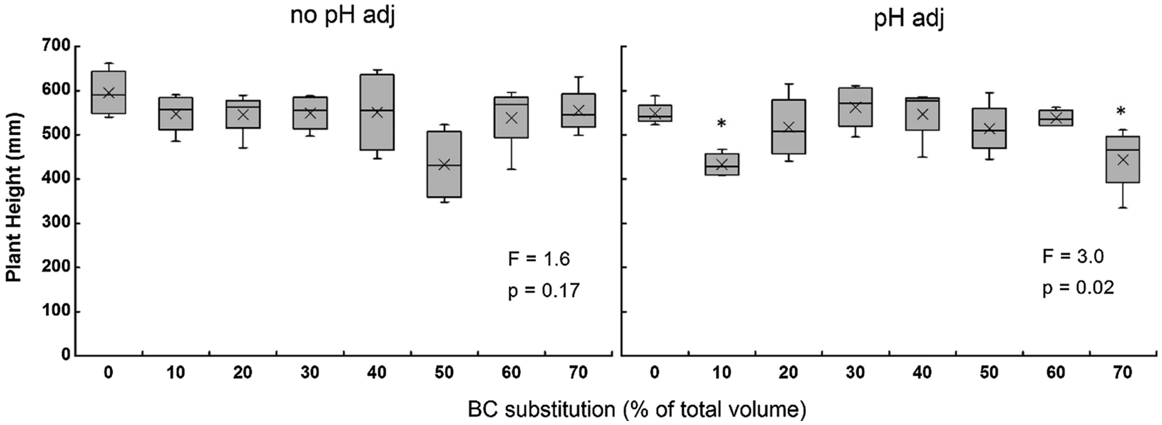

Fig. 2. Height of marigold plants at the conclusion of a 9-week growth trial across a gradient of softwood biochar substitution for peat moss in soil-free substrates (a) not adjusted for pH and (b) adjusted to pH 5.8 ± 0.2 prior to sowing. Post-hoc analysis of differences among BC-containing substrates relative to the 0% BC control were evaluated by Steel's test ($p < 0.05$).

impacts on height only in pH adjusted substrates (Fig. 2). Marigold heights were significantly lower in 10% (434 mm) and 70% (444 mm) BC, compared to 0% BC (549 mm), in substrates that were initially adjusted to pH 5.8.

An indicator of chlorophyll content, SPAD values indicated sensitivity to higher rates of BC substitution that changed over the 9 weeks of growth, depending on pH adjustment (BC × pH $p < 0.0001$ for weeks 1 and 9) (Fig. 3, Supplementary Fig. 3). For pH unadjusted substrates, SPAD values in high BC treatments (40–70%) were lower in the first week of growth compared to the 0% BC control, whereas plants in pH adjusted substrates with 20% and 60% BC exhibited higher SPAD values (Fig. 3). In week 9, plants exhibited significantly higher SPAD values for all but the lowest rate of BC substitution (20–70%) compared to 0% BC in substrates that did not receive pH adjustment. For substrates with initial pH adjustment, SPAD values were significantly elevated only in 20% BC.

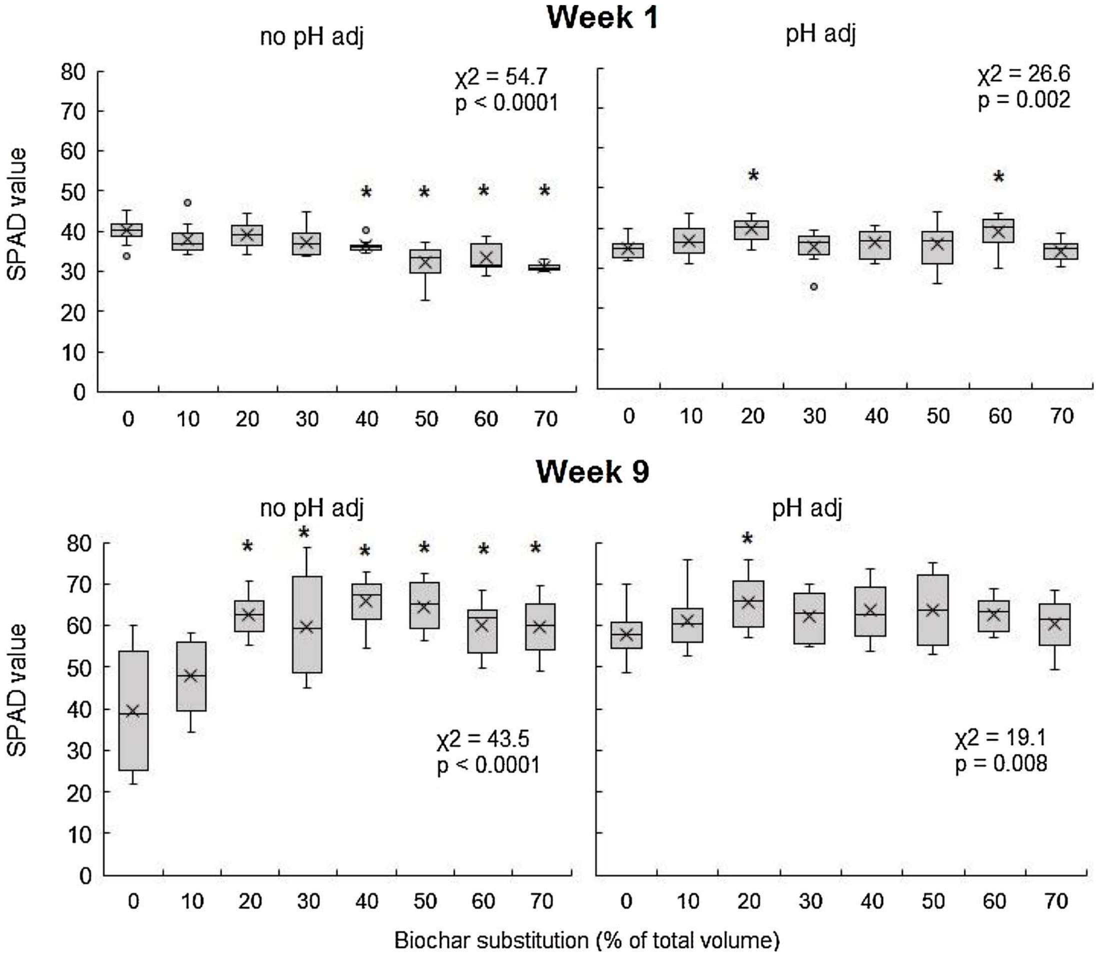

Fig. 3. SPAD values of new leaves (second leaf from the apical meristem) of marigolds at the initiation and conclusion of a 9-week growth trial across a gradient of softwood biochar substitution for peat moss in soil-free substrates (a) not adjusted for pH and (b) adjusted to pH 5.8 ± 0.2 at the initiation of the trial. Post-hoc analysis of differences among BC-containing substrates relative to the 0% BC control were determined by Steel's test ($p < 0.05$).

Table 2
Biomass and flowering of marigolds after 9 weeks of growth across a gradient of softwood biochar substitution for peat moss in soil-free substrates, (a) not adjusted for pH and (b) adjusted to pH 5.8 ± 0.2 at the initiation of the greenhouse growth experiment. F-statistic and significant (*p*) values are shown for biochar substitution and pH adjustment interactions, and as separate factors. Different letters indicate significant differences ($p < 0.05$).

	BC (% vol)	Total biomass (dry, g)	Shoot biomass (dry, g)	Flowering biomass (dry, g)	Number of Flowers	Harvest index (g g^{-1})
no pH adj.	0	24.8 ± 5.3	16.7 ± 5.5 ab	5.1 ± 0.5	6.8 ± 2.1	0.34 ± 0.07
	10	33.2 ± 6.3	23.8 ± 4.8 a	6.3 ± 1.6	8.0 ± 2.3	0.28 ± 0.03
	20	31.9 ± 2.1	22.3 ± 2.7 ab	6.6 ± 2.7	6.8 ± 3.4	0.30 ± 0.08
	30	32.3 ± 1.1	21.8 ± 2.4 ab	7.5 ± 2.4	7.8 ± 3.9	0.33 ± 0.07
	40	31.4 ± 1.3	20.7 ± 1.7 ab	7.6 ± 2.4	6.8 ± 4.6	0.34 ± 0.07
	50	23.8 ± 9.3	14.4 ± 5.2 b	6.3 ± 4.9	6.3 ± 4.6	0.38 ± 0.12
	60	29.1 ± 1.9	20.0 ± 1.9 ab	6.6 ± 1.4	6.0 ± 2.2	0.31 ± 0.04
	70	31.0 ± 4.1	20.3 ± 2.3 ab	7.0 ± 2.1	10.3 ± 3.8	0.34 ± 0.04
	F-stat	2.17	2.9	ns	ns	ns
	p	0.080	0.024			
pH adj	0	30.7 ± 6.7	21.0 ± 5.4 ab	6.4 ± 1.4	10.8 ± 6.2	0.32 ± 0.03
	10	29.3 ± 0.7	19.4 ± 1.1 ab	6.5 ± 1.4	8.0 ± 3.6	0.34 ± 0.04
	20	32.2 ± 4.8	19.7 ± 2.6 ab	9.2 ± 2.4	7.8 ± 5.0	0.39 ± 0.03
	30	32.2 ± 2.4	19.2 ± 1.6 ab	9.8 ± 1.4	8.5 ± 3.3	0.40 ± 0.03
	40	33.7 ± 3.9	22.3 ± 2.0 a	7.9 ± 4.3	8.8 ± 6.6	0.33 ± 0.10
	50	32.9 ± 6.6	21.3 ± 2.7 ab	8.0 ± 5.9	9.5 ± 8.9	0.34 ± 0.13
	60	28.5 ± 3.7	18.7 ± 2.3 ab	6.3 ± 1.9	7.3 ± 4.7	0.34 ± 0.03
	70	23.7 ± 5.2	15.5 ± 1.8 b	4.8 ± 3.6	5.3 ± 4.0	0.33 ± 0.09
	F-stat	2.3	2.3	ns	ns	ns
	p	0.069	0.062			
	BC x pH					
	F-stat	2.7	3.6	0.6	0.9	0.9
	p	0.021	0.012	0.76	0.49	0.55
						0.9
	BC					
	F-stat	ns	ns	1.8	0.4	0.3
	p			0.12	0.88	0.96
						0.9
	pH					
	F-stat	ns	ns	2.3	0.1	1.3
	p			0.13	0.76	0.27

3.3. Plant biomass and flowering

BC substitution for peat maintained or increased non-flowering shoot biomass (Table 2). Substrate pH adjustment influenced total above-ground biomass response to BC substitution (BC × pH $p = 0.021$), with lower shoot biomass in substrates without pH adjustment at 0 and 70% BC substitution compared to pH adjusted substrates. Total biomass, number of flowers, and the harvest index (i.e., flower mass/total above-ground biomass) were similar across all treatments.

Total N uptake (g N $plant^{-1}$) by marigold plants at week 9 was influenced by BC substitution ($p = 0.0047$) regardless of pH adjustment (BC × pH $p = 0.12$), and pH adjustment did not influence above-ground biomass N ($p = 0.33$). Total above-ground biomass N was significantly higher in 50% BC relative to 70% BC (Fig. 4). A similar pattern occurred for non-flowering shoot N, which accounted for 49–85% of total above-ground biomass N. Flower N and above-ground %N was not influenced by BC substitution nor pH.

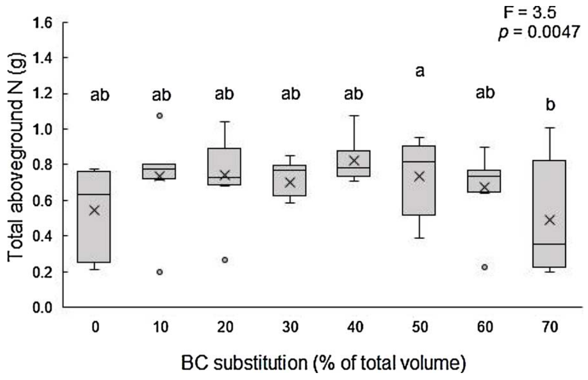

Fig. 4. Above-ground nitrogen content of 9-week old marigold plants grown in soil-free substrates representing a gradient of softwood biochar substitution for peat moss. Values are grouped for BC substrates without and with pH adjustment to pH 5.8 ± 0.2 at the initiation of the trial, because there was no effect of pH adjustment ($p = 0.33$) on above-ground nitrogen content at week 9.

3.4. Post-harvest substrate properties

After 9 weeks of marigold growth under fertigation, substrate pH changed relative to initial pH depending on initial pH adjustment (BC × pH $p < 0.001$) (Table 1, Fig. 5). Post-harvest substrate pH varied significantly for substrates that were not adjusted for pH prior to sowing (pH 4.4–7.4). Substrates initially adjusted to pH 5.8 showed elevated but similar pH across the gradient of BC substitution (pH 6.2–7.0). Post-harvest EC was weakly influenced by the combination of BC substitution and pH adjustment (BC × pH $p = 0.06$), which reflected lower EC in 0% BC relative to BC substrates without pH adjustment (Supplementary Fig. 4). Post-harvest pH and EC were positively correlated ($r = 0.43$, $p = 0.0004$), though EC was less strongly correlated with BC substitution rate ($r = 0.26$, $p = 0.038$).

Extractable N and P of substrates following use for greenhouse production varied by the degree of BC substitution and pH adjustment depending on the nutrient ion (Fig. 6). Extractable NO_3^--N, and to a greater degree extractable NH_4^+-N, tended to decrease with increasing BC substitution in substrates without initial pH adjustment, but did not differ in pH adjusted substrates (BC × pH $p_{NH4\text{-}N} < 0.001$, $p_{NO3\text{-}N} = 0.037$) (Fig. 6a). Though higher BC substrates had lower extractable NO_3^--N ($p = 0.001$), and even more so NH_4^+-N

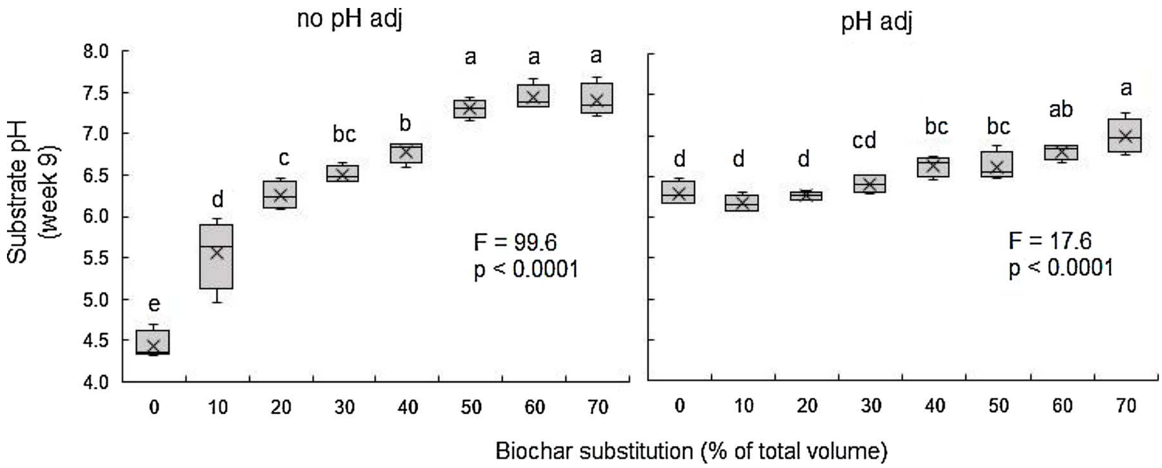

Fig. 5. pH of soil-free substrates in which softwood BC was substituted for peat moss at 0–70% of total substrate volume following 9 weeks of greenhouse growth (including fertigation with 0.5% Hoagland solution). Substrates were used directly (no pH adjustment) or adjusted to pH 5.8 ± 0.2 at the initiation of the trial. Different letters indicate significant differences ($p < 0.05$) among means.

($p < 0.0001$), 60% BC substrates exhibited the lowest concentrations of both inorganic N forms, with means of 41.6 μg g^{-1} NH_4^+-N and 721.5 μg g^{-1} NO_3^--N (Fig. 6a,b). Regardless of initial pH adjustment, post-harvest substrate pH was negatively correlated with extractable NH_4^+-N ($r = -0.60$, $p < 0.0001$) and NO_3^--N ($r = -0.31$, $p = 0.012$). Post-harvest NH_4^+-N and NO_3^--N were strongly co-correlated ($r = 0.72$, $p < 0.0001$) across all combinations of BC substitution and pH (non)adjustment. Total above-ground biomass and N content were not significantly correlated with extractable inorganic N across all BC substrates ($p < 0.05$), nor for BC substrates analyzed separately by pH adjustment.

Extractable PO_4^{3-}-P concentrations were similarly influenced by initial pH adjustment of substrates (BC × pH $p = 0.023$) (Fig. 6c). In contrast to inorganic N, inorganic P extractable from post-harvest substrates was impacted by BC substitution only in pH adjusted substrates ($p = 0.001$) and was positively correlated with total above-ground biomass ($r = 0.54$, $p < 0.0001$). Extractable inorganic P was not influenced by BC substitution in substrates without initial pH adjustment ($p = 0.21$), and in these substrates, was not associated with total above-ground biomass ($r = 0.12$ $p = 0.52$). Similar to inorganic N, in pH adjusted substrates extractable P decreased markedly (-58%) with increasing BC substitution from 4.5 ± 0.5 mg g^{-1} PO_4^{3-}-P in 0% BC to 2.5 ± 0.7 mg g^{-1} PO_4^{3-}-P in 70% BC.

4. Discussion

4.1. pH adjustment of soil-free substrates with BC

By evaluating an alkaline BC at high volumetric rates in soil-free substrates, this study addresses a potential obstacle to the feasibility of BC-based substrates for plant production (Fryda and Visser, 2015; Vaughn et al., 2013). The present data demonstrate that substituting a softwood BC with strongly alkaline pH (10.9) for peat at high rates in soil-free substrates (up to 70% of total volume) does not require pH adjustment under common greenhouse conditions (e.g., fertigation) because germination, shoot biomass and N content, and flowering of marigold did not significantly differ between substrates with and without initial adjustment to pH 5.8. BC substitution may even improve plant growth, as marigold plants with intermediate BC substitution (50%) exhibited higher relative chlorophyll content (SPAD value) relative to 0% BC (i.e., standard peat-perlite mixture).

These results are in mixed support of the stated hypothesis because BC substitution and pH adjustment effects on marigold depended on the stage of growth. As hypothesized, increasing BC substitution decreased plant height and chlorophyll content in the early stages of marigold growth. Though pH adjustment of BC substrates negatively affected germination and height, this may have been due to phytotoxicity of PLA used to decrease pH of high %BC substrates. By week 9, plant growth (height, biomass, N content) was similar regardless of BC substitution and initial pH adjustment, failing to support the hypotheses that high BC substitution rates would impair plant growth and that this would be alleviated by pH adjustment. However, since fertigation provided excess nutrients, pH was likely less important for nutrient availability.

Equivalent and slightly positive effects of BC substitution at high rates and without pH adjustment can be partially attributed to the convergence of pH over 9 weeks of fertigation and plant growth to pH 4.4–7.4. As this high-temperature softwood BC has a higher pH (10.9) than most BCs (Lehmann and Joseph, 2012; Mukome et al., 2013) and was used at high substitution rates (70%), it represents a ‘worst-case scenario’ liming effect. BCs produced from other feedstocks and/or at lower temperatures may not have as pronounced liming effects. Decreases in substrate pH over time could reflect a number of processes: (1) a residual liming effect of BC, which could also account for the slight upward pH drift of substrates initially corrected to pH 5.8; (2) nitrification; (3) rhizosphere acidification due to cation uptake. Though downward pH drift in peat-based substrates initially limed to a circumneutral pH has been found to be inverse to the base saturation of peat (Rippey, 2005), the 0% BC (70% peat) substrates initially limed to pH 5.8 in this study did not exhibit significant pH changes.

4.2. Substrate and plant N

The availability and plant uptake of N may be impacted by substrate pH, as indicated by extractable inorganic N, relative differences in chlorophyll content, and above-ground plant N. This may explain initial (weeks 1–3) decreased plant height and relative chlorophyll content in high BC substrates with high initial pH (no pH adjustment). Foliar chlorosis in ornamental plants, including marigold, grown in high pH substrates has been induced by liming in peat substrates and could reflect non-N deficiencies such as iron and manganese (Smith et al., 2004; Šrámek and Dubský, 2011). Similar above-ground biomass and total N despite greater relative chlorophyll content in high BC substrates (20–70%, no pH adjustment) by week 9 indicates that initial differences in chlorophyll content by BC substitution did not persist and that initial greater chlorophyll content for marigold in high BC substrates did not necessarily translate to greater biomass and N uptake. A lack of N deficiency under conditions of fertigation is further evidenced by overall high concentrations of available N in substrates at week 9 and by the absence of correlation between available N with marigold above-ground biomass and N content. Elevated chlorophyll content

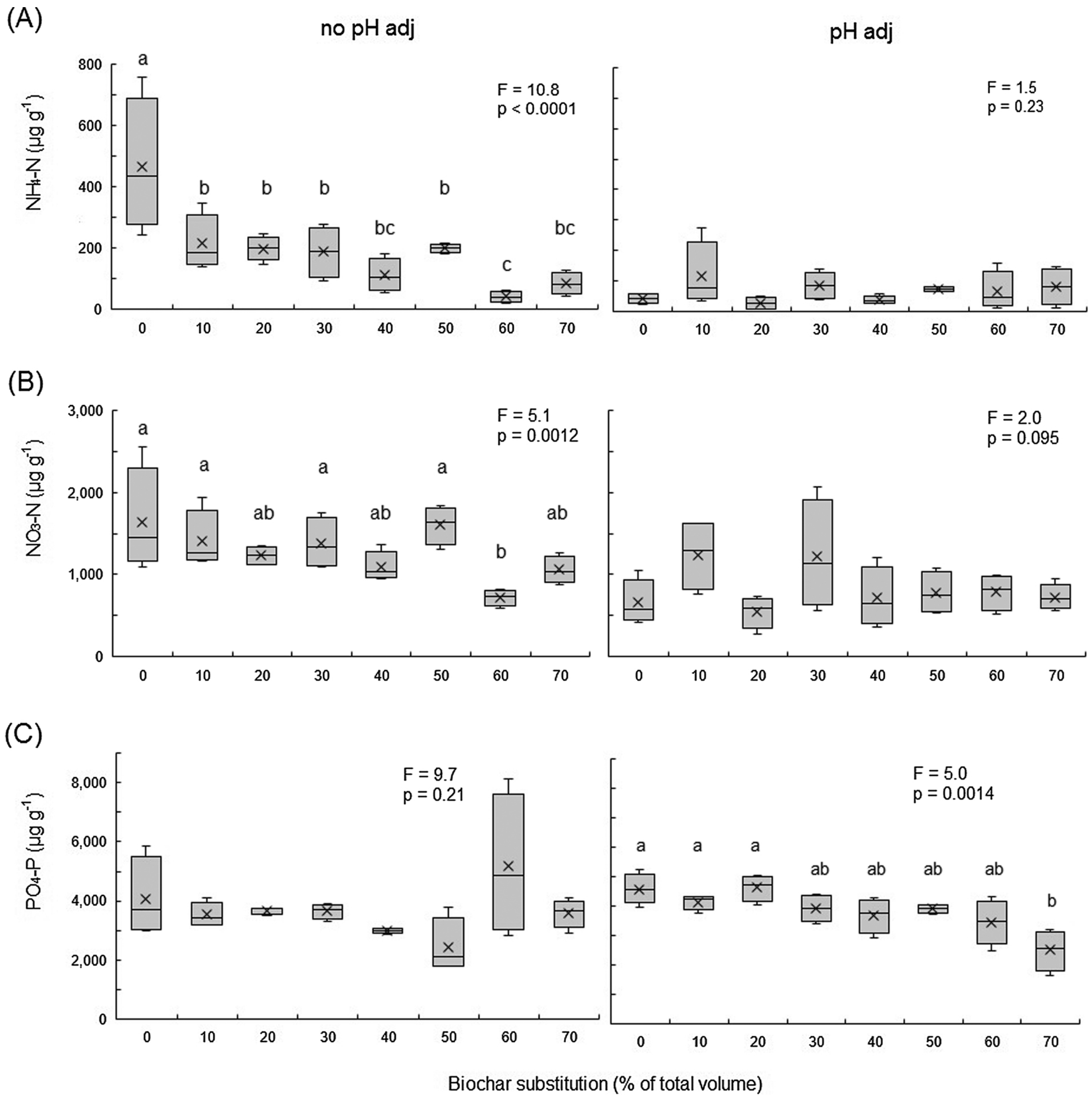

Fig. 6. Available nitrogen (N) and phosphorus (P) in soil-free substrates in which softwood BC was substituted for peat moss at 0–70% of total substrate volume following 9 weeks of greenhouse growth (including fertigation with 0.5% Hoagland solution). Substrates were used directly (no pH adjustment), or adjusted to pH 5.8 ± 0.2 at the initiation of the trial. Properties include (a) 2 mol L^{-1} KCl-extractable NH_3^{+}-N, (b) 2 mol L^{-1} KCl-extractable NO_3^{-}-N, and (c) 0.5 mol L^{-1} $NaHCO_3$-extractable PO_4^{3-}-P. Different letters indicate significant differences ($p < 0.05$) among means.

with BC substitution may therefore reflect enhanced plant access to non-N nutrients.

Available N was inverse to SPAD values in week 9 and did not reflect similar above-ground plant N concentrations. The disparity between marked differences in substrate N availability under conditions of fertigation yet similar above-ground biomass N content could be explained by pH-dependent gaseous losses of N in pH unadjusted substrates (i.e., denitrification) and/or differences in extractability influenced by pH-dependent binding. That extractable inorganic P did not differ as much as inorganic N across pH gradient of pH unadjusted substrates could indicate similar anion exchange capacity of substrates.

High available N and P in substrates challenges the hypothesis that BC substitution can influence marigold growth by affecting availability of nutrients added by fertigation. For example, post-harvest available P was positively correlated with marigold biomass but was two orders of magnitude higher than thresholds of deficiency (Havlin et al., 2013). Though high C:N substrates such as peat can entail sufficient N immobilization so as to compromise plant growth (Belda et al., 2016), N fertilization as in this study would be expected to rapidly alleviate N deficiency. This time-dependent effect may have manifested as lower chlorophyll content (SPAD values) in high BC substrates in week 1 but not week 9. Similarly, N fertilization alleviated slightly lower biomass accumulation of marigolds grown in pine wood-based substrates compared to peat (Wright et al., 2009).

Though the experimental design of this study removed water and nutrient limitations by daily fertigation, the present findings indicate a potential benefit of BC for water availability in soil-free substrates. The increase in WHC with BC substitution that peaked at 30% BC (Supplementary Fig. 5) supports this hypothesized benefit of BC at high rates for soil-free substrates (Steiner and Harttung, 2014), as well as in inorganic matrices like soils (Atkinson et al., 2010).

4.3. Germination and PLA toxicity

Marigold germination and growth response to BC substitution in pH adjusted substrates was likely due to the use of pyroligneous acid (PLA) to decrease pH. An increasing amount of PLA was applied to reduce increasingly elevated pH at high rates of the alkaline BC (pH 10.9) used. Since pH adjusted substrates had the same target pH (5.8), the difference can be attributed to a non-pH effect of the almond shell PLA used in this study. PLAs are a complex mixture of organic compounds of varying biological and phytological activity, including toxicity. These include organic acids (e.g., acetic acid), phenols, ketones phenyl ethers, and furan and pyran derivatives (Mathew and Zakaria, 2015; Wei et al., 2010). The survival and equivalent growth of marigold seedlings transplanted into pH adjusted substrates with no seed germination (50–70% BC) suggests greater sensitivity of seeds than seedlings to PLA effects and is consistent with previous findings of PLA inhibition of germination (e.g., Buss and Mašek, 2014; Rombolà et al., 2015). Parallel *in vitro* experiments (data not shown), revealed full inhibition of marigold and lettuce germination at PLA $\geq$ 2.50% and $\geq$ 1.25% (v/v), respectively, though a similiar response occurred for acetic acid, a major PLA component (Wei et al., 2010) at the same concentration.

Studies indicate mixed effects of PLA on biological activity, with both plant-growth promoting and toxic effects, and antimicrobial effects. For example, PLA improved *in vitro* rooting of pear (*Pyrus pyrifolia*) (Kadota et al., 2002), and at rates of up to 6% increased fruiting of edible mushrooms (*Pleurotus ostreatus*) in sawdust-based substrates (Yoshimura et al., 1995). On the other hand, germination of cress (*Lepidium sativum* L.) was inhibited by exposure to volatiles from pyrolysis, which are captured via condensation in the production of PLA (Buss and Mašek, 2014). Similarly, cress germination was inhibited by BCs with high volatile contents (Rombolà et al., 2015). Like BC, feedstock and production conditions can significantly impact PLA composition and anti-biological activity (Wei et al., 2010; Yatagai et al., 2002), and thus the negative impacts of PLA observed in this study may be specific to the almond shell PLA used here.

4.4. Additional advantages and possibilities of BC substitution for peat

The potential of pyrolyzed biomass in soil-free substrates has been investigated since the mid-20th century. For example, Kono (1956) investigated the utility of charcoal to improve substrate physical properties such as water holding capacity and bulk density for orchid production (Self et al., 1967). However, the rapidly expanding body of knowledge on BC, including the ability to design BCs based on feedstock and pyrolysis conditions, means that BCs can be engineered to target additional benefits for to soil-free substrates.

Significant enrichment in available N and P over the course of 9 weeks of fertigation reflects high input conditions in greenhouse production systems. Compared to peat, the longer decomposition half-life of high-temperature BCs such as the one in this study, and the potential of nutrient ions to bind to BC (Gai et al., 2014; Lehmann and Joseph, 2015; Yao et al., 2012) and re-solubilize when applied to soils (Joseph et al., 2013; Yao et al., 2013) raises the possibility of re-using BC-based substrates as fertilizers.

BC substitution may increase the longevity of peat-based substrates under conditions of high nutrient availability common in their use (Bilderback et al., 2005). Decomposition of peat during long grow periods, in particular under high N additions, can compromise physical and chemical properties (Bilderback et al., 2005; Gómez and Robbins, 2011; Jackson et al., 2009). Partially replacing peat with less decomposable materials (e.g., bark, sawdust with high C:N) can decrease the overall decomposition rate of the remaining peat component of substrates even under N fertilization (Maas and Adamson, 1972), raising the possibility of extending the lifetime of peat-based substrates with partial BC substitution. The availability of BC as a secondary product of bioenergy production (Barrett et al., 2016) and/or waste stream management (Kaudal et al., 2015), as well as lower transportation costs made possible by regional or on-site BC production, could further leverage economic advantages over peat and peat alternatives (e.g., compost).

Recent studies support the unique ability of BC to mediate biological interactions with benefits for greenhouse production such as enhanced pathogen and pest suppression. For example, 1–5% additions of citrus wood BC (450 °C) to peat-based substrates increased expression of pathogen defense genes in strawberry (*Fragaria ananassa* cv Yael) and as a result suppressed fungal disease (Meller Harel et al., 2012); for tomato (*Solanum lycopersicum)* and pepper (*Capsicum annuum*), such additions delayed and reduced disease from fungal pathogens and mites (Elad et al., 2010). However, lower susceptibility of plants to pathogens in soil-free substrates with a BC component may be muted by fertilization (De Tender et al., 2016), and therefore may not be possible under intensive greenhouse production. On the other hand, substrates with a high proportion of BC such as in this study could have detrimental effects on biological processes that support plant productivity (Lehmann et al., 2011), largely due to interference (e.g., sorption) of chemical signals between beneficial microorganisms and host plants (Masiello et al., 2013). As a result, BC could lessen establishment of rhizobial and mychorrizal associations (Warnock et al., 2007) and reduce nodulation in leguminous species (Quilliam et al., 2013). Strong sorption by BC could afford horticultural advantages, however. For example, bulbet organogenesis of grape hyacinth (*Muscari armeniacum*) was enhanced with the use of BC-like material (charcoal) in substrates due to its sorption of inhibitory compounds (Peck and Cumming, 1986). Potential plant health benefits of BC-based substrates are relatively under-investigated in evaluations of peat alternatives, despite one of the main uses of soil-free substrates being the avoidance of plant exposure to pathogens (Barrett et al., 2016).

Finally, the ability to replace peat with BC offers potential economic and environmental benefits. The expense of peat is expected to increase in the coming decades due to production costs, competing uses for peat, and its perception as being unsustainable (Barrett et al., 2016; Carson et al., 2009). Such a perception in part stems from the negative impacts on wetland ecosystems of some peat mining operations (Barkham, 1993; Robertson, 1993), though the sustainability of peat harvesting is a subject of debate (Chapman et al., 2003; Hood, 1999). Peat mining operations and the eventual decomposition of peat after its use in substrates represents a transformation of a terrestrial C sink of global importance into a net C source, with climate change forcing effects (Cleary et al., 2005; Gorham, 1991). Assuming a conservative aerobic decomposition rate for peat in substrates of 5% per annum (Cleary et al., 2005), within one century of mining and use in soil-free substrates 95% of mined peat would be expected to revert from a C sink to source (CO_2). In contrast, high-temperature BCs are thought to generally exhibit lower decomposition rates than undecomposed or humified biomass such as compost and peat (Woolf et al., 2010) and exhibit centennial to millennial residence times (Gurwick et al., 2013). The molar O:C = 0.36 for the BC in this study corresponds to a half-life of 100–1000 years (Spokas, 2010), suggesting that one century after production and use in soil-free substrates, at least 50% of C in the softwood BC in this study would be converted to CO_2. The (re)use of non-peat biomass or even waste in the form of BC in soil-free substrates is an additional strategy for ‘sustainable biochar to mitigate global climate change’ (Woolf et al., 2010) due to its greater stability and ability to preserve a key global C sink.

5. Conclusion

By evaluating an alkaline BC at high substitution rates in soil-free substrates with a common ornamental plant, this study addresses and confirms the feasibility of replacing *Sphagnum* peat moss with BC for greenhouse and nursey plant production. Full substitution of BC (softwood, pH 10.9) for peat in soil-free substrate did not have negative impacts on marigold growth and flowering. Replacement of peat with softwood BC at high rates (up to 70% total substrate volume) yielded an initial pH gradient of up to pH 10.4. However, marigold germination as well as shoot biomass and flowering at harvest (9 weeks) was not negatively impacted. This likely reflects the convergence in substrate pH across the BC substitution gradient (0–70% total volume, at 10% increments) from 4.4–10.4 to 5.6–7.5 by week 9. Similar above-ground biomass and total N at harvest despite greater relative chlorophyll content in 20–70% BC substrates suggested that initial differences in chlorophyll content (SPAD value) due to BC substitution did not persist, though greater chlorophyll content of marigold leaves in high BC substrates did not necessarily translate to greater biomass and N uptake. Despite the high pH of the softwood BC, adjustment of BC-substituted substrates to pH 5.8 prior to sowing did not improve marigold performance. The use of PLA, a common by-product of BC production, to adjust substrate pH may have reduced germination in high BC substrates because these received high amounts of PLA. Analysis of substrate nutrient availability at harvest indicated interactive effects of BC substitution and initial pH adjustment on available N and P, suggesting that under nutrient-constrained conditions (e.g., no or low fertigation) the degree of BC substitution and initial pH adjustment could impact N and P availability. As BCs can differ greatly in properties such as pH, additional BCs should be investigated for their potential to fully replace peat moss in soil-free substrates.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.10.053.

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