Bioenerg. Res. (2014) 7:120–131
DOI 10.1007/s12155-013-9355-y

Biochar as a Substitute for Vermiculite in Potting Mix for Hybrid Poplar

William L. Headlee · Catherine E. Brewer · Richard B. Hall

Published online: 16 July 2013

Abstract The purpose of this study was to evaluate biochar as a substitute for vermiculite in potting mixes for unrooted vegetative cuttings of hybrid poplar as represented by the clone 'NM6' (*Populus nigra* L. × *Populus suaveolens* Fischer subsp. *maximowiczii* A. Henry). We compared three treatments (peat moss (control), peat moss mixed with vermiculite, and peat moss mixed with biochar) at three times (pre-experiment, pre-fertilizer, and post-fertilizer). The biochar and vermiculite mixes had significantly higher cation exchange capacity (CEC) and pre-experiment exchangeable K than the control. Trees grown in the biochar and vermiculite mixes had significantly higher shoot K than the control at pre-fertilizer and post-fertilizer and significantly higher shoot and total biomass at post-fertilizer. The biochar mix was also associated with lower root biomass and higher shoot/root biomass ratio than the vermiculite mix at post-fertilizer. Vector analysis indicated that all treatments were deficient in N at pre-fertilizer, and the control was also deficient in K at pre-fertilizer and post-fertilizer. Linear regression confirmed that shoot biomass was strongly correlated (R^2=0.97) with N and K uptake (in addition to initial cutting diameter), also, root biomass was strongly correlated (R^2=0.96) with potting mix CEC (in addition to shoot biomass). Luxury consumption of K at pre-fertilizer indicates that the increases in shoot and total biomass observed with the biochar and vermiculite treatments arise from this nutrient being "pre-loaded" in both mixes. We conclude that biochar provides benefits equivalent to vermiculite in terms of key nutrient availability and total biomass productivity.

Keywords Biochar · Biomass · Cation exchange capacity · Fertilizer · Populus · Pyrolysis

Abbreviations

CEC Cation exchange capacity
ECEC Effective cation exchange capacity

Introduction

Biochar is a high-carbon, porous coproduct of biomass fast pyrolysis for the production of renewable bio-oil [1, 2]. Biochar's porosity results in high surface areas for biochar particles, which can serve a number of functions such as adsorbing nutrients and increasing cation exchange capacity (CEC) in soils [3–5]. Other potential benefits of adding biochar to the soil include increased water holding capacity [6, 7], higher pH [8], increased levels of certain plant nutrients [9, 10], and reduced nitrogen leaching and/or volatilization [11–13].

While much research to date has focused on applying biochar to agricultural soils, biochar's properties also make it useful for greenhouse applications. For example, Dumroese et al. [14] found that peat moss amended with biochar pellets showed improved hydraulic water conductivity and water

W. L. Headlee (✉) · R. B. Hall
Department of Natural Resource Ecology and Management, Iowa State University, 339 Science II, Ames, IA 50011-3221, USA
e-mail: wheadlee@iastate.edu

R. B. Hall
e-mail: rbhall@iastate.edu

C. E. Brewer
Center for Sustainable Environmental Technologies, Iowa State University, Biorenewables Research Laboratory, Ames, IA 50011, USA

Present Address:
C. E. Brewer
Department of Chemical Engineering, New Mexico State University, P.O. Box 30001, MSC 3805, Las Cruces, NM 88033, USA
e-mail: cbrewer@nmsu.edu

availability, and Graber et al. [15] found that a potting mix amended with biochar enhanced tomato and pepper plant growth. Moreover, greenhouse applications present important advantages over field applications; for instance, avoiding the issues of reduced herbicide efficacy that may occur with field applications [16–18]. In addition, daily greenhouse watering may help to further capitalize on biochar's nutrient adsorption properties, as an environment of alternating saturated and unsaturated conditions has been shown to enhance the CEC of wood-derived biochars [19, 20].

The purpose of this study was to evaluate biochar as a substitute for vermiculite, a nonrenewable resource used in greenhouses to improve the CEC of potting mixes, for growing hybrid poplar trees. Hybrid poplars were selected because they have rapid growth under greenhouse conditions, they are readily propagated from vegetative cuttings which reduce the inherent variability of the test plants, and they have demonstrated potential as short rotation woody crops for bioenergy production in the region [21, 22]. The clone 'NM6' is a fast-rooting hybrid which is easy to clonally propagate [23] and is expected to be representative of hybrid poplars utilized in the region. While unrooted cuttings are typically used for field planting, greenhouse production of the cloned plants is often used in the initial phase of scaling up a new selection for commercial use; in addition, poplars serve as model plants for woody species in general [24, 25], many of which are reared in the greenhouse before being planted in the field.

The hypotheses tested were (1) hybrid poplar cuttings grown in a potting mix containing biochar would have similar productivity as those grown in a potting mix containing vermiculite, (2) the cuttings grown in these mixes would have higher productivity than those grown in a mix without biochar or vermiculite, and (3) the differences in productivity among the treatments would be explained by the ability of the potting mixes to adsorb plant nutrients from the soil solution (i.e., CEC). To test these hypotheses, three treatments were evaluated: peat moss (control), peat moss mixed with vermiculite, and peat moss mixed with biochar. Chemical properties (pH, CEC, and ECEC) and nutrient content (total N and exchangeable K, Ca, Mg, and Na) of the potting mixes were measured at three times (pre-experiment, pre-fertilizer, and post-fertilizer) to gauge their inherent nutrient content and their ability to adsorb nutrients. Trees were destructively sampled at pre-fertilizer and post-fertilizer to determine the effects of treatment and time on the amount of shoot (stem+leaves), root, cutting, and total (shoot+root+cutting) biomass produced, as well as shoot/root biomass ratio. In addition, nutrient (N, K, Ca, Mg, and Na) concentrations and contents were analyzed for each plant tissue.

The potting mix properties and plant nutrients responsible for treatment effects on biomass parameters were identified and evaluated using a three-step process. In the first step, analysis of variance (ANOVA) was used to identify significant differences in potting mix properties and nutrients, the concentrations and contents of nutrients in the plants, and biomass production. For the second step, vector analysis was used to identify the nutrients responsible for producing the observed biomass responses. In vector analysis, foliar parameters (i.e., least squares means of shoot nutrient concentrations, shoot nutrient contents, and shoot biomass derived from ANOVA) are simultaneously graphed for each treatment relative to a reference condition (i.e., control), whereby the direction and magnitude of the differences from the reference condition indicate the nature and strength of nutrient responses. This method allows for the diagnosis of plant nutrient status and has been applied to hybrid poplars in previous studies [26, 27]. Vector analysis is also capable of detecting the transfer of nutrients from the shoots to other tissues; in such cases, possible nutrient transfer was further investigated using ANOVA for root and cutting nutrients. In the third and final step, linear regression was used to evaluate the predictive power of the variables identified by ANOVA and/or vector analysis as having significant effects on biomass production.

Materials and Methods

Biochar Material

The biochar was produced at the Iowa State University BioCentury Research Farm (Boone, IA) on a pilot scale (8 kg hr^{-1}) bubbling fluidized bed fast pyrolysis. The red oak feedstock was ground to a particle size of <600 μm prior to fast pyrolysis at 500 °C. The sand bed was fluidized with N_2. Biochar was collected by cyclone from the product stream with an approximate yield of 12–15 %.

Greenhouse Experiment

The experiment was conducted at the Iowa State University Forestry Greenhouse (Ames, IA) during the spring of 2010. Three potting media treatments were evaluated as follows: peat (100 % peat moss; control), vermiculite mix (75 % peat moss and 25 % vermiculite by volume), and biochar mix (75 % peat moss and 25 % biochar by volume). A randomized complete block design was used. Treatments were randomly assigned to 236 cm^3 Accelerator® containers (Nursery Supplies Inc., Chambersburg, PA) in each of three trays (blocks). Each tray held 32 containers: 12 peat, 10 vermiculite mix, and 10 biochar mix, for a total of 96 containers (each of which was filled with 225 cm^3 of the assigned mix). Unrooted vegetative cuttings (10 cm long) of the hybrid poplar 'NM6' (*Populus nigra* L. × *Populus suaveolens*

Fischer subsp. *maximowiczii* A. Henry) were soaked in water for 24 h, planted in the containers (one tree per container), and initial cutting diameters were recorded. The trays were placed in a bench-scale humidity tent (consisting of opaque plastic sheeting supported by PVC pipe) for the first 3 weeks and then on open benches for the remainder of the experiment. They were subirrigated continuously over the first 4 weeks and twice daily for 30 min at a time over the remainder of the experiment.

Half of the trees per treatment were destructively sampled after 6 weeks of growth (prior to fertilization; mean height=27.0 cm), and the other half were destructively sampled after 8 weeks (following fertilization; mean height=38.1 cm). Fertilizer solution was prepared by dissolving dry 15-30-15 fertilizer in water (3.6 g L^{-1}); the solution was applied at a rate of 35 mL per tree at the start of the seventh week and 70 mL per tree at the start of the eighth week. Destructive sampling consisted of separating the tree tissues (shoots (stems+leaves), roots, and cuttings) and oven-drying the tissues at 50 °C to obtain the dry weights prior to tissue nutrient analysis. Due to the small amount of root material available for most trees in the pre-fertilizer harvest, root samples from up to five trees were bulked by treatment, resulting in a total of 18 root samples (rather than 48) for the pre-fertilizer harvest.

For the potting media, samples of the unused mixes were collected to determine their pre-experiment chemical properties and nutrient contents. To determine the pre-fertilizer and post-fertilizer effects, the medium from each container was collected during destructive sampling of the trees and bulked by tray for each treatment. The media was then oven-dried at 50 °C prior to analysis of chemical properties and nutrient contents. Because the potting mixes were bulked by tray, the data were evaluated as a completely randomized design, with the three trays serving as replicates (3 treatments×3 sample times×3 replicates=27 total samples).

Laboratory Analyses

Plant tissues and potting mixes were sent to the US Forest Service Institute for Applied Ecosystem Studies in Rhinelander, WI, where they were ground through a 0.5 mm screen prior to analysis. For both the plant tissues and the potting mixes, total N content was determined with a Flash EA1112 N-C analyzer with a model MAS 200 autosampler (Thermo Electron, via CE Elantech, Inc., Lakewood, NJ). For the remaining plant tissue nutrients, atomic emission spectroscopy (AES) was conducted using a Varian Agilent model 240FS atomic absorption spectrophotometer (Agilent Technologies, Englewood, CO) following nitric acid digestion. For the potting mixes, exchangeable base cations (K, Ca, Mg, and Na) were extracted with hexammine cobalt (Co) chloride and analyzed via AES. The base cations were summed to determine CEC (adsorption of base cations), and ECEC (adsorption of all cations) was determined from the difference of the Co level measured compared to the initial Co level, as described by Ciesielski and Sterckeman [28]. Potting mix pH was measured by adding potting mix (1 g) to 5 mL of dilute CaCl solution (0.01 mol L^{-1}), shaking for 1 h, then measuring with an AccuCap combination pH electrode and Accumet model no. XL50 pH meter (Fisher Scientific, Waltham, MA, USA).

Analysis of Variance

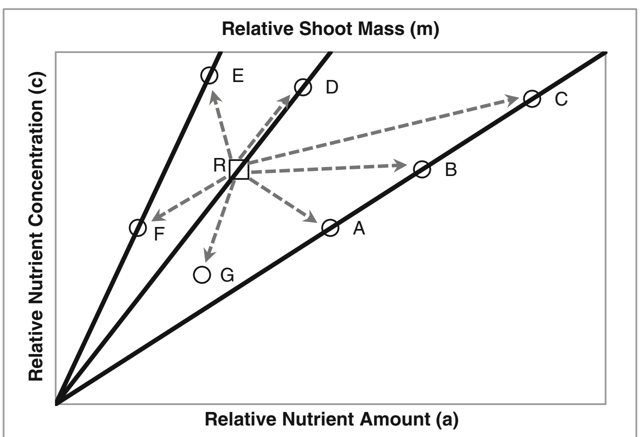
All data were evaluated as a two-way factorial (treatment× time) with ANOVA using PROC GLM in SAS (SAS Institute Inc., Cary, NC). Whenever treatment, time, or treatment×time interactions were found to be significant ($P<0.05$), multiple comparisons analyses (with Tukey's adjustment) were conducted to identify statistically significant differences between the adjusted least squares means. Each tree tissue was evaluated for differences in biomass (i.e., dry weight) and nutrient concentrations, with initial cutting diameter as a covariate based on previous research showing the influence of cutting size on early growth and survival of poplar clones [29]. In addition, the nutrient content of each tissue (determined by multiplying the measured nutrient concentration by the dry weight of the tissue) and total biomass (determined by summing the dry weights of the tissues) were similarly evaluated, again with initial cutting diameter as a covariate. For shoot/root biomass ratio, shoot biomass was used as the covariate based on previous research showing the influence of tree development on biomass allocation [30]. The potting mixes were tested for differences in pH, CEC, ECEC, and nutrient concentrations and contents. Nutrient contents (in milligrams per container) were calculated by multiplying the measured nutrient concentrations (in milligram per kilogram) by the bulk density (in kilograms per container) of the mix; CEC and ECEC (in milliequivalent per container) were similarly calculated by multiplying the measured value (in milliequivalent per kilograms) by the potting media bulk density (in kilograms per container).

Vector Analysis

Vector analysis was conducted using the adjusted least squares means of the shoot parameters (specifically shoot biomass, nutrient concentrations, and nutrient contents). The pre-fertilizer peat treatment was used as the reference condition (relative value=100 for all shoot parameters), and the relative values for all other treatment×time combinations were calculated by dividing the measured value by that of the reference condition and multiplying by 100. These relative values were graphed for each nutrient to compare treatment effects on nutrient status based on the typical interpretations of vector analysis diagrams (Fig. 1, adapted from Lteif et al. [27]). The interpretations reflect the nutrient status of the treatment plants and can be summarized as follows: (A) increased biomass with increased nutrient content and decreased nutrient concentration (growth dilution), (B) increased biomass with increased nutrient content and no change in nutrient concentration (sufficiency), (C) increased biomass with increased nutrient content and concentration (deficiency), (D) no change in biomass with increased nutrient content and concentration (luxury consumption), (E) decreased biomass with increased nutrient concentration and increased or decreased nutrient content (toxicity), (F) decreased biomass with decreased nutrient concentration and content (antagonism), and (G) little or no increase in biomass with decreased nutrient concentration and content (retranslocation). For a more thorough description of vector analysis and its potential applications, see Haase and Rose [31] and Imo and Timmer [32].

Linear Regression

To evaluate the predictive power of variables identified by ANOVA and/or vector analysis as having significant effects on biomass parameters, linear regression was conducted using PROC GLM in SAS. Specifically, coefficients of determination (R^2) values were determined for biomass parameter



Vector	Relative Change			Nutritional Effect	Possible Diagnosis
	m	c	a		
A	+	-	+	Dilution	Growth dilution
B	+	o	+	Sufficiency	Steady-state
C	+	+	+	Deficiency	Limiting
D	o	+	+	Luxury consumption	Accumulation
E	-	+	+/-	Excess	Toxic accumulation
F	-	-	-	Excess	Antagonistic
G	o/+	-	-	Depletion	Retranslocation

Fig. 1 Example of vector analysis diagram and the interpretations associated with shifts in shoot biomass (*m*), shoot nutrient concentration (*c*), and shoot nutrient amount (*a*), for each vector (*A*–*G*) relative to the reference condition (*R*); adapted from Lteif et al. [27]

models which used the identified variables, and the statistical significance of each variable within the model was also determined using Type III sums of squares. As previously noted, potting mixes were bulked by tray; thus, when potting mix variables were used in linear regression, the biomass parameters were averaged by tray to maintain consistency with the potting mix data.

Results

Potting Mix Properties and Nutrients

Analyses of the potting mixes' chemical properties and nutrient contents indicated significant treatment and time effects for several of the parameters, with significant interactions for pH as well as exchangeable K and Na (Table 1). Regarding treatment effects, the biochar and vermiculite mixes had significantly higher CEC and exchangeable Mg than the peat (Table 2). Also, the biochar mix had significantly higher ECEC and exchangeable Ca than both the peat and the vermiculite. The vermiculite mix had significantly lower total N than both the biochar mix and the peat. For the time effects, CEC increased significantly from pre-experiment to pre-fertilizer, along with exchangeable Ca (which was likely introduced via the tap water used for irrigation). ECEC showed a similar trend as CEC, but the differences were not statistically significant; similarly, time effects were not significant for total N or exchangeable Mg.

As noted above, significant treatment × time interactions were found for pH and exchangeable K and Na. The peat showed no significant change in pH over time, whereas the other two treatments both showed significant increases from pre-experiment to pre-fertilizer and significant decreases from pre-fertilizer to post-fertilizer (Fig. 2a). The peat was significantly lower in K than the other two treatments at pre-experiment and did not change significantly at pre-fertilizer, but increased significantly at post-fertilizer; conversely, K decreased significantly for the other two treatments from pre-experiment to pre-fertilizer and did not change significantly from pre-fertilizer to post-fertilizer (Fig. 2b). All treatments increased significantly in Na from pre-experiment to pre-fertilizer, with the peat being significantly lower than the other two treatments at pre-fertilizer; in addition, the biochar mix increased significantly from pre-fertilizer to post-fertilizer, whereas the other two treatments did not change significantly (Fig. 2c).

Shoot Nutrient Concentrations and Contents

Shoot nutrient concentrations showed mainly treatment and/or time effects, with only shoot K concentration showing a significant treatment × time interaction (Table 3). Conversely,

Table 1 *P* values from ANOVA of potting mix chemical properties (pH, CEC, ECEC) and nutrient content (total N and exchangeable K, Ca, Mg, Na). Statistically significant effects ($P<0.05$) are italicized

Effect	pH	CEC	ECEC	N	K	Ca	Mg	Na
Treatment	*<0.0001*	*<0.0001*	*<0.0001*	*0.0008*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Time	*<0.0001*	*<0.0001*	0.0762	0.1206	*0.0003*	*0.0014*	0.4074	*<0.0001*
Trt×Time	*0.0003*	0.4177	0.4138	0.6093	*0.0005*	0.0970	0.1054	*<0.0001*

shoot nutrient contents showed significant treatment×time interactions for all nutrients evaluated.

Shoot N concentration was significantly higher with the peat than with the other two treatments (Table 4). Shoot Ca concentration was significantly lower with the vermiculite treatment than with the others. Shoot Mg concentration for the peat was significantly lower than the vermiculite but significantly higher than the biochar. No significant treatment differences were observed for shoot Na concentration. The treatment×time interaction for shoot K concentration (Fig. 3) shows that the biochar and vermiculite treatments were significantly higher than the peat both pre-fertilizer and post-fertilizer; also, the peat treatment showed a significant increase from pre-fertilizer to post-fertilizer, whereas the other two treatments did not change significantly.

Treatment×time interactions for shoot nutrient contents are illustrated in Fig. 4. For shoot N, Mg, and Na content, no significant differences between treatments were observed at pre-fertilizer, and all treatments increased from pre-fertilizer to post-fertilizer, but at post-fertilizer, the biochar and vermiculite were significantly higher than peat. For shoot Ca content, no significant differences between treatments were observed at pre-fertilizer, and all treatments increased from pre-fertilizer to post-fertilizer, but at post-fertilizer, the biochar was significantly higher than the other two treatments. For shoot K content, the biochar and vermiculite treatments were significantly higher than peat both pre-fertilizer and post-fertilizer, with the absolute difference being larger at post-fertilizer.

Table 2 Adjusted least squares means for potting mix chemical properties and nutrients by treatment and time. Significant differences between means ($P<0.05$) are indicated with different letters within the column. Units of measure for the parameters are: CEC and ECEC (meq container^{-1}); total N and exchangeable Ca and Mg (mg container^{-1}). Results for pH and exchangeable K and Na are not shown here due to significant treatment×time interactions

Effect	CEC	ECEC	N	Ca	Mg
Treatment					
Peat	16.4 b	25.3 b	366 a	742 b	144 b
Vermiculite	20.4 a	24.0 b	289 b	835 b	279 a
Biochar	20.6 a	33.1 a	355 a	1421a	253 a
Time					
Pre-experiment	16.2 b	25.6	318	769 b	236
Pre-fertilizer	20.8 a	28.4	336	1032 a	230
Post-fertilizer	20.5 a	28.3	356	1197 a	211

Tree Biomass Productivity

The biomass productivity data showed significant treatment effects for shoot/root biomass ratio and significant time effects for cutting biomass and shoot/root biomass ratio (Table 5). Significant treatment×time interactions were observed for shoot, root, and total biomass. Regarding treatment effects, the biochar treatment produced a significantly higher shoot/root biomass ratio than the vermiculite and peat treatments, with vermiculite also being significantly higher than peat (Table 6). For time effects, the shoot/root biomass ratio decreased from pre-fertilizer to post-fertilizer, whereas cutting biomass increased from pre-fertilizer to post-fertilizer.

Treatment×time interactions for shoot, root, and total biomass are illustrated in Fig. 5. While the treatments did not differ significantly in shoot or total biomass pre-fertilizer, and all treatments increased significantly from pre-fertilizer to post-fertilizer, the biochar and vermiculite treatments had significantly higher shoot and total biomass post-fertilizer than the peat (Fig. 5a, d). Root biomass also did not differ significantly between treatments pre-fertilizer, and increased for all treatments from pre-fertilizer to post-fertilizer, but at post-fertilizer, the vermiculite treatment had significantly higher root biomass than biochar, while peat was intermediate (Fig. 5b). No significant interactions were detected for cutting biomass (Fig. 5c); the data are included here to illustrate the contribution of cuttings to total biomass. Also, the covariate of initial cutting diameter had a significant influence ($P<0.0001$) on the biomass of all tissues, and the covariate of shoot biomass had a significant influence ($P=0.0044$) on shoot/root biomass ratio (not shown).

Vector Analysis

The vector analysis diagrams (Fig. 6) illustrate the relative shoot nutrient concentrations, shoot nutrient contents, and shoot biomass for each treatment pre-fertilizer (small symbols) and post-fertilizer (large symbols), relative to the pre-fertilizer control (peat). Vectors show the differences between

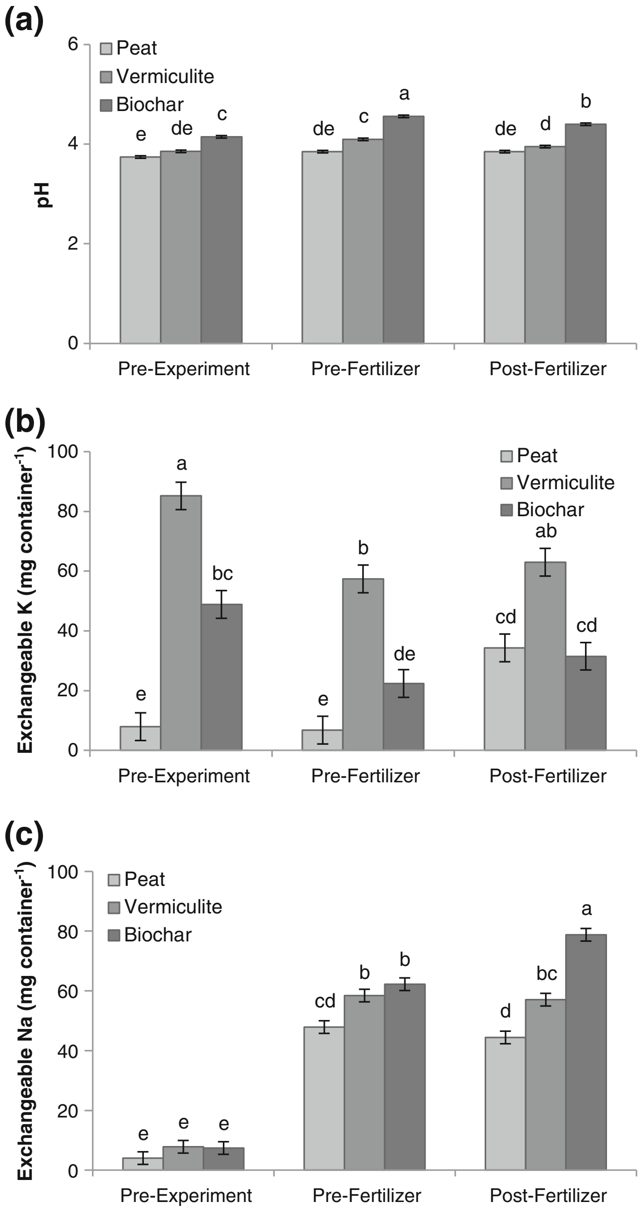


Fig. 2 Adjusted least squares means (±1 standard error) from treatment×time interactions for potting mix **a** pH, **b** exchangeable K, and **c** exchangeable Na. Statistically significant differences ($P<0.05$) are indicated with different letters above the bars

treatments pre-fertilizer (dotted lines) and the changes for each treatment from pre-fertilizer to post-fertilizer (dashed lines).

For N (Fig. 6a), the pre-fertilizer biochar and vermiculite vectors showed a shift towards lower N concentration and slightly lower N content along with slightly higher mass, which is indicative of possible transfer (e.g., retranslocation) from the shoots to other tissues. However, statistical analyses of cuttings and roots did not reflect nutrient transfer within the plant, in that N levels were also lower in the cuttings and roots for these treatments, relative to peat (not shown). At post-fertilizer, all treatments shifted towards higher N concentration, N content, and shoot mass; this indicates that all treatments were deficient in N prior to fertilization. Because the biochar and vermiculite were associated with higher mass but similar N concentrations as peat at post-fertilizer, it can also be concluded that peat was sufficient in N relative to the other two treatments. Thus, the higher shoot productivity for biochar and vermiculite likely is not attributable to a difference in N availability.

The pre-fertilizer biochar and vermiculite vectors showed a shift towards higher K concentration and higher K content along with only slightly higher shoot mass, which is indicative of luxury consumption (Fig. 6b). At post-fertilizer, the biochar and vermiculite treatments shifted towards higher total K content and shoot mass with little change in K concentration, while with peat, all three of these increased; this indicates that the biochar and vermiculite were sufficient in K prior to fertilization, whereas the peat was deficient. In addition, the lower K concentration, K content, and shoot mass for peat at post-fertilizer (relative to the other two treatments) suggests that the trees growing in peat were also K-limited at post-fertilizer. Thus, the higher shoot productivity for biochar and vermiculite is likely related to superior availability of K.

With Ca (Fig. 6c), the pre-fertilizer vermiculite vector showed a shift towards lower Ca concentration and slightly lower Ca content along with slightly higher mass; this indicates possible transfer (e.g., retranslocation) of Ca from the shoots to other tissues. However, statistical analyses of cuttings and roots did not reflect nutrient transfer within the plant, in that Ca levels were also lower in the cuttings and

Table 3 *P* values from ANOVA of shoot nutrient (N, K, Ca, Mg, and Na) concentrations and content. Statistically significant effects ($P<0.05$) are italicized

	Shoot concentration					Shoot content				
Effect	N	K	Ca	Mg	Na	N	K	Ca	Mg	Na
Treatment	*0.0011*	*<0.0001*	*<0.0001*	*<0.0001*	0.1998	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Time	*<0.0001*	0.2190	*<0.0001*	0.7090	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Trt×Time	0.0551	*<0.0001*	0.4567	0.1912	0.4196	*<0.0001*	*<0.0001*	*0.0004*	*<0.0001*	*0.0021*

Table 4 Adjusted least squares means for shoot nutrient concentrations (N, Ca, Mg, and Na; %), by treatment and time. Significant differences between means ($P<0.05$) are indicated with different letters within the column for a given effect. Results for shoot K concentration and shoot nutrient contents (N, K, Ca, Mg, and Na) are not shown here due to significant treatment×time interactions

Effect	N	Ca	Mg	Na
Treatment				
Peat	2.19 a	0.77 a	0.23 b	0.028
Vermiculite	2.03 b	0.64 b	0.26 a	0.026
Biochar	1.97 b	0.77 a	0.21 c	0.028
Time				
Pre-fertilizer	1.60 b	0.68 b	0.23	0.025 b
Post-fertilizer	2.52 a	0.78 a	0.23	0.030 a

roots for the vermiculite treatment, relative to biochar and peat (not shown). At post-fertilizer, all three treatments increased slightly in Ca concentration, with relatively larger increases in Ca content and shoot mass; this is indicative of a slight deficiency for all treatments.

As shown in Fig. 6d, the pre-fertilizer biochar vector showed a shift towards lower Mg concentration but slightly higher Mg content and shoot mass, whereas the vermiculite vector showed a shift towards higher Mg concentration along with slightly higher Mg content and shoot mass; this is indicative of growth dilution for biochar and luxury consumption for vermiculite. At post-fertilizer, all three treatments shifted towards higher Mg content and shoot mass with little change in Mg concentration; this indicates the treatments were sufficient in Mg.

The pre-fertilizer biochar and vermiculite vectors showed a shift toward slightly lower Na concentration along with slightly higher Na content and shoot mass, which indicates slight growth dilution for biochar and vermiculite (Fig. 6e). At post-fertilizer, all three treatments increased slightly in Na concentration, with relatively larger increases in total Na and shoot mass; this is indicative of a slight deficiency for all treatments.

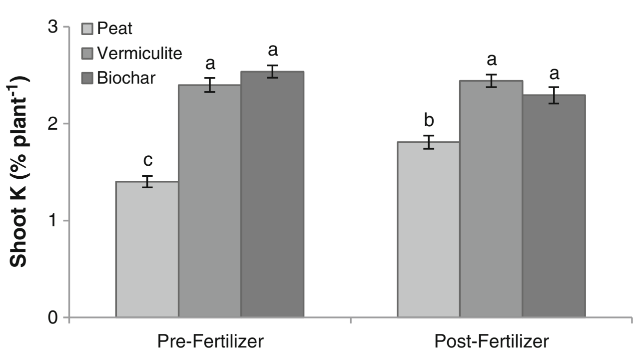


Fig. 3 Adjusted least squares means (±1 standard error) from treatment×time interaction for shoot K concentration. Statistically significant differences ($P<0.05$) are indicated with different letters above the bars

Linear Regression

The ANOVA and vector analysis results suggest that shoot biomass productivity was influenced by N and K uptake, and that initial cutting diameter also had a significant effect. Thus, shoot biomass was modeled with the variables of shoot N content, shoot K content, and initial cutting diameter. The overall model fit was strong ($R^2=0.97$; Fig. 7a), with each of the three variables being statistically significant ($P<0.0001$) predictors of shoot biomass. The resulting equation is:

$$B_S = 0.0863D_i + 0.0229N_S + 0.0114K_S - 0.3015 \quad (1)$$

Where B_S is the shoot biomass (in grams per tree), D_i is the initial diameter of the cutting (in millimeters), N_S is the shoot nitrogen content (in milligrams per tree), and K_S is the shoot potassium content (in milligrams per tree).

The significance of shoot biomass as a covariate in the ANOVA for shoot/root biomass ratio indicates that biomass allocation to the roots was influenced by shoot size. In addition, previous research suggests that increased nutrient availability decreases root biomass allocation (thereby increasing shoot/root ratio) in poplars [33], which in this study is supported by the observed increases in shoot/root ratio for the treatments having higher CEC (i.e., biochar and vermiculite) relative to the control. Based on these observations, root biomass was modeled with the variables of shoot biomass and potting mix CEC. The overall model fit was strong ($R^2=0.96$; Fig. 7b), with both variables being statistically significant ($P\leq0.0002$) predictors of root biomass. The resulting equation is:

$$B_R = 0.0665B_S - 0.00845M_{CEC} + 0.1561 \quad (2)$$

Where B_R is the root biomass (in grams per tree), B_S is the shoot biomass (in grams per tree), and M_{CEC} is the CEC (in milliequivalents per container) of the media.

Predictably, the ANOVA results indicate that initial cutting diameter had a significant effect on cutting biomass. Also, the significant increase in cutting biomass over time demonstrates that a portion of the photosynthate produced by the shoot was allocated to cutting growth. Thus, cutting biomass was modeled with the variables of initial cutting diameter and shoot biomass. The overall model fit was strong ($R^2=0.90$; Fig. 7c), with both variables being statistically significant ($P<0.0001$) predictors of cutting biomass. The resulting equation is:

$$B_C = 0.5825D_i + 0.1397B_S - 3.007 \quad (3)$$

Where B_C is cutting biomass, D_i is the initial diameter of the cutting (in millimeters), and B_S is shoot biomass (in grams per tree). Linear regression was not conducted for the remaining biomass parameters, as they can be determined

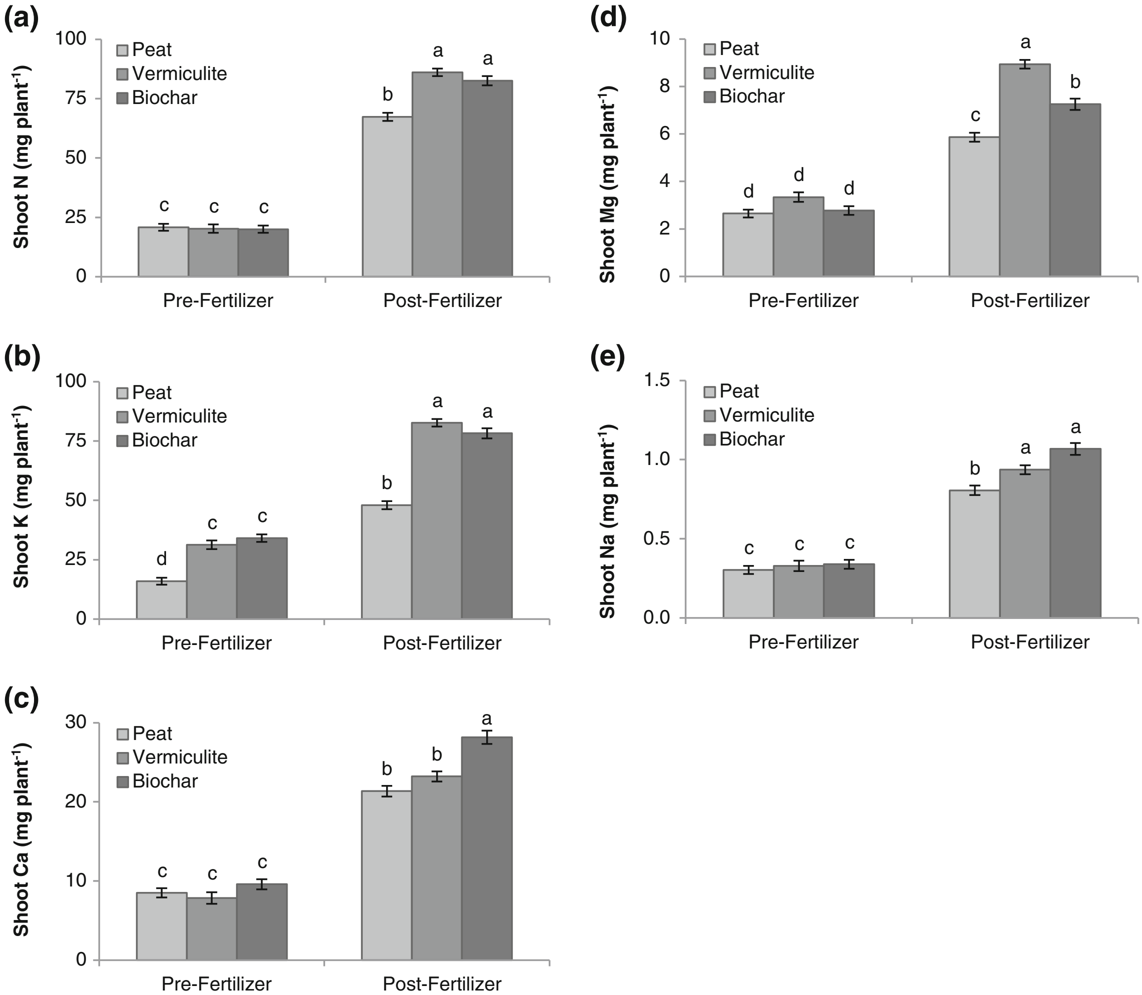


Fig. 4 Adjusted least squares means (±1 standard error) from treatment×time interactions for total shoot content of **a** N, **b** K, **c** Ca, **d** Mg, and **e** Na. Statistically significant differences ($P<0.05$) are indicated with different letters above the bars

by summing equations 1–3 (total biomass) or dividing equation 1 by equation 2 (shoot/root biomass ratio).

Discussion

The results of this study demonstrate that biochar is a suitable replacement for vermiculite in potting mix in terms of key nutrient availability and total biomass production. The biochar and vermiculite mixes had significantly higher cation exchange capacity (CEC) and pre-experiment exchangeable K than the peat control (see Table 2 and Fig. 2) and were similar to one another in shoot N and K concentration and content (see Figs. 3 and 4, and Table 4), which resulted in significantly higher shoot and total biomass productivity than the control (see Fig. 5). Vector analysis suggests that the trees growing in the biochar and vermiculite mixes were limited primarily by N, whereas the trees growing in peat were limited by both N and K (see Fig. 6a, b); thus, the improved biomass productivity of the trees grown with biochar and vermiculite appears to be attributable to higher K availability.

The elevated levels of exchangeable K for the biochar and vermiculite treatments relative to peat at pre-experiment and pre-fertilizer (see Fig. 2b), along with the luxury consumption of K associated with these treatments at pre-fertilizer (see Fig. 6b), indicate that the increased availability of K was due

Table 5 *P* values from ANOVA of tree biomass (shoot, B_S; root, B_R; cutting, B_C; and total, B_T) and shoot/root ratio (B_S/B_R). Statistically significant effects ($P<0.05$) are italicized

Effect	B_S	B_R	B_C	B_T	B_S: B_R
Treatment	*<0.0001*	*0.0005*	0.4976	*<0.0001*	*<0.0001*
Time	*<0.0001*	*<0.0001*	*0.0005*	*<0.0001*	*<0.0001*
Trt × time	*<0.0001*	*0.0271*	0.2483	*0.0057*	0.3467

Table 6 Adjusted least squares means for cutting biomass (B_C) and shoot/root biomass ratio (B_S/B_R) by treatment and time. Significant differences between means ($P<0.05$) are indicated with different letters within the column for a given effect. Results for shoot, root, and total biomass are not shown here due to significant treatment×time interactions

Effect	B_C	B_S: B_R
Treatment		
Peat	2.78	15.6 c
Vermiculite	2.83	18.3 b
Biochar	2.87	22.9 a
Time		
Pre-fertilizer	2.71 a	21.4 a
Post-fertilizer	2.94 b	16.5 b

at least in part to the nutrient being "preloaded" in the mixes rather than simply their ability to adsorb K from the soil solution. However, the higher CEC values for both treatments relative to peat (as well as higher ECEC for biochar) suggest that superior availability of K and/or other cations may be sustained over longer periods. Additional research to test this hypothesis is therefore recommended.

Differences between the biochar and vermiculite mixes were also observed, but did not appear to be significant factors in shoot or total biomass productivity. For example, total N was higher in the biochar mix than the vermiculite mix (see Table 2), and exchangeable K was higher in the vermiculite mix than the biochar mix (see Fig. 2); however, these differences did not translate to differences in shoot or total productivity or even to differences in concentrations or contents of the nutrients within the plants. In the case of N, the higher levels for biochar may represent a difference in fixed N rather than available N. Regarding K, it appears that the higher exchangeable K for vermiculite represents a surplus supply.

In addition, the biochar mix had significantly higher exchangeable Ca and post-fertilizer Na than the vermiculite mix (see Table 2 and Fig. 2). This corresponded with higher shoot nutrient concentration and content of Ca for the biochar treatment compared to vermiculite, but no significant differences between the two with regard to Na (see Table 4 and Fig. 4). Although these differences did not appear to significantly impact hybrid poplar growth in this study (see Fig. 5), they may be important for other species which are prone to specific nutrient deficiencies (e.g., Ca in tomatoes) or sensitivities (e.g., Na in beans), and therefore additional research with a wider variety of poplar clones and other crops is recommended. Such studies should account for the initial cutting diameter and changes in biomass allocation associated with plant development, which were found to have significant influences on tree growth in this study and are consistent with previous research [29, 30].

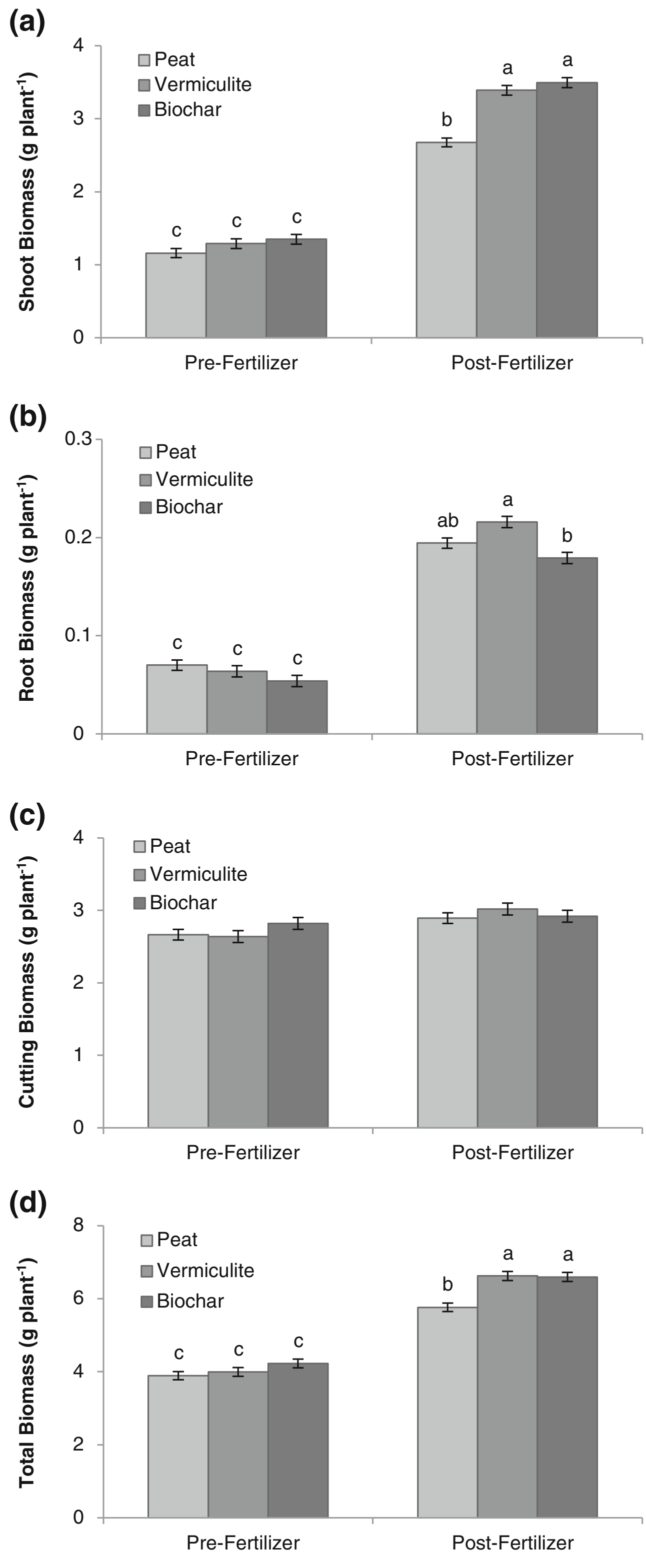


Fig. 5 Adjusted least squares means (±1 standard error) from treatment×time interactions for **a** shoot, **b** root, **c** cutting, and **d** total (shoot+root+cutting) biomass. Statistically significant differences ($P<0.05$) are indicated with different letters above the bars. Though nonsignificant, the interaction for cuttings is shown here to demonstrate the contribution to total biomass

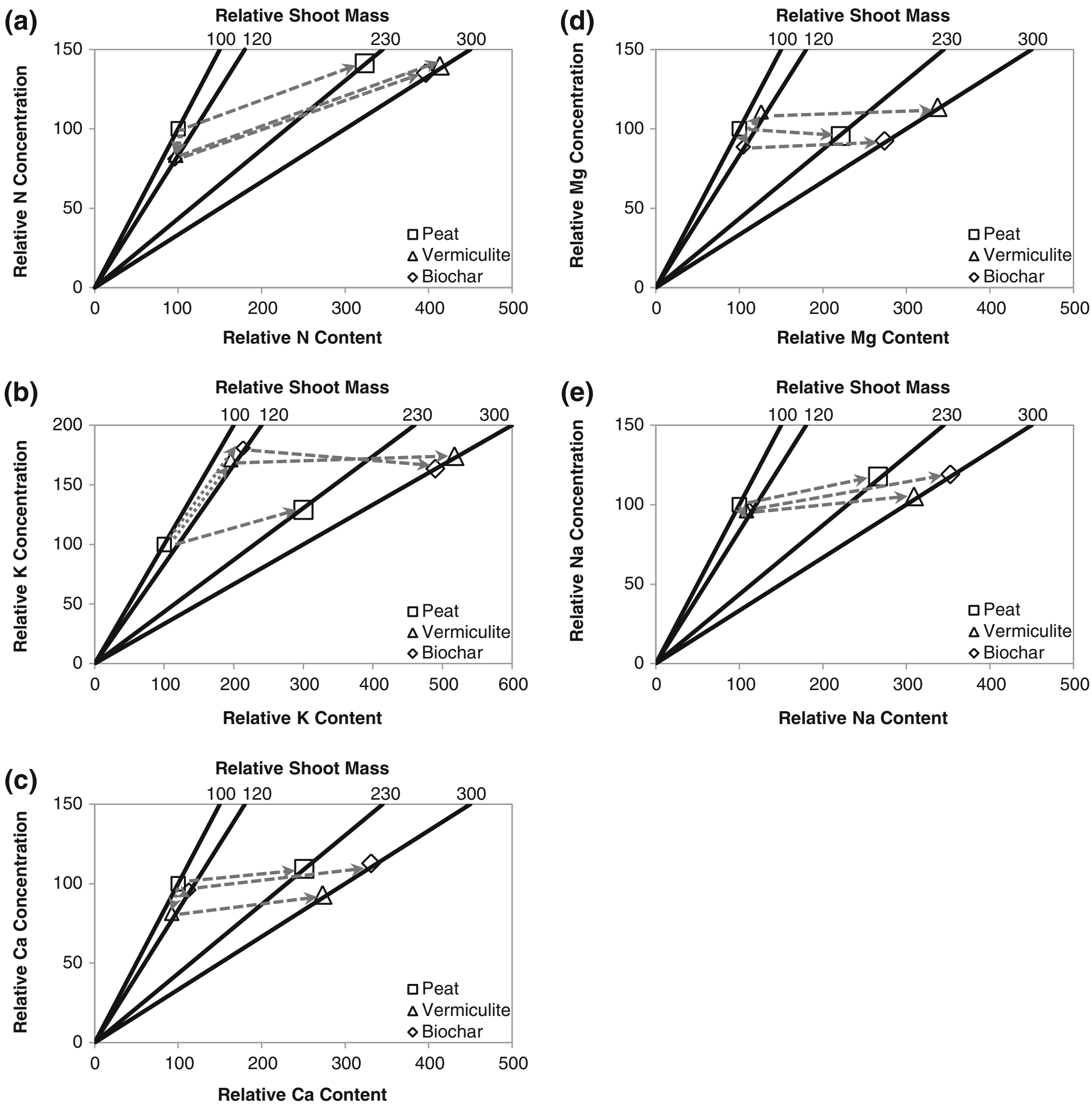


Fig. 6 Vector diagrams showing relative shifts associated with biochar (*diamond*), vermiculite (*triangle*), and peat (*white square*) treatments pre-fertilizer (*small symbols*) and post-fertilizer (*large symbols*) for **a** N, **b** K, **c** Ca, **d** Mg, and **e** Na. In all cases the initial reference condition (shoot mass, nutrient concentration, and nutrient content=100) is the peat treatment at pre-fertilizer

While vector analysis suggested possible pre-fertilizer transfer of N (biochar and vermiculite treatments) and Ca (vermiculite treatment) from the shoots to other tissues (see Fig. 6a, c), statistical analyses indicated that the levels of these nutrients were similarly lower in the cuttings and roots (not shown), and thus reflect lower levels of these nutrients for the entire plant. Such increases in the ratio of biomass produced per unit of nutrient in the plant are often interpreted as an increase in nutrient use efficiency [34]. As previous descriptions of vector analysis [26, 27, 31, 32] do not appear to address this potential outcome, we recommend the inclusion of "increased nutrient use efficiency" as a possible diagnosis for the nutritional effect of "depletion" described in Fig. 1.

Linear regression supported the results of the vector analysis, specifically, that shoot biomass productivity was largely a function of N and K uptake (in addition to initial cutting diameter, as indicated by ANOVA). This demonstrates the utility of vector analysis (in conjunction with ANOVA) for identifying the specific variable(s) responsible for treatment-related plant growth responses, which, considering the number of variables at play (e.g., concentration and content of each nutrient as well as possible interactions between them), might otherwise only be accomplished with increasingly complex statistical models. Linear regression also showed that root biomass was largely a function of shoot biomass productivity and potting mix CEC, and that cutting biomass was largely a function of shoot biomass productivity and initial cutting

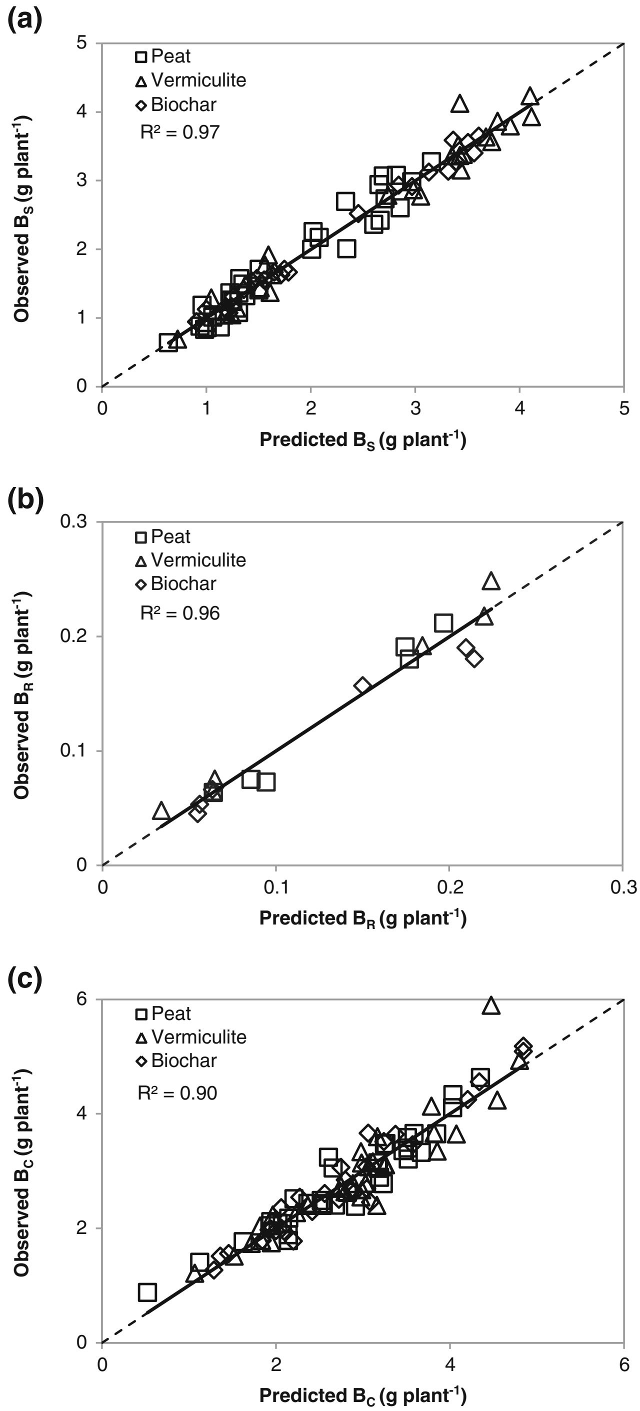


Fig. 7 Observed versus predicted values for **a** shoot biomass, B_S, **b** root biomass, B_R, and **c** cutting biomass, B_C. Individual plants were used for shoots and cuttings (n=96); for roots, plants were averaged by treatments within trays at each time (pre-fertilizer and post-fertilizer) to maintain consistency with potting mix data (n=18; see text). *Dashed lines* represent perfect 1:1 relationships

diameter. Thus, the biomass productivity of each tissue was influenced by the potting mix, whether directly via K availability (shoots) and CEC (roots) or indirectly via the influence of the potting mix on shoot biomass (roots and cuttings).

Previous research by Graber et al. [15] showed that pepper and tomato plant growth were significantly enhanced by the addition of biochar to their potting mix. They concluded that this was not due to improved nutrient availability (based on a lack of significant differences in leaf nutrient concentrations), and hypothesized instead that the biochar may have stimulated beneficial soil microbes and/or contained non-nutrient chemicals that directly stimulated plant growth. However, their fertilizer regime (fertigation applied 2–3 times daily throughout the experiment) may have supplied sufficient nutrients to the plants directly, negating any differences in the ability of the growing media to supply nutrients. Our study, on the other hand, purposefully induced suboptimal nutrient conditions to test for differences in nutrient availability and uptake by the plants. As such, our study demonstrates that biochar may enhance plant growth via improved nutrient availability under suboptimal nutrient conditions.

Oxygen availability is also an important consideration in potting mixes, as limited oxygen has been shown to decrease shoot and root growth in poplars [35]. Though we did not evaluate oxygen availability in this study, we did observe that the bulk density of the biochar mix was approximately 50 % greater than that of the other mixes, which we expect would decrease oxygen availability. Indeed, previous research has shown that pelleted biochar mixed with peat at the same ratio used in our study (25 % biochar and 75 % peat by volume) reduced air-filled porosity from 47 to 38 % and lowered relative oxygen diffusivity by approximately half compared to peat alone [14]. Thus, additional research on the effects of biochar on oxygen availability (particularly over longer time periods) is recommended.

Finally, it has been established that biochars derived from different feedstocks and under different pyrolysis conditions have unique physical and chemical properties [36, 37]. As such, additional testing with a variety of biochars is needed to compare how the selection of feedstocks and processes affect the ability of different biochars to serve as a renewable substitute for vermiculite. The costs associated with different feedstocks and processes will also be important in determining the most economical substitute for vermiculite, which in the greenhouse industry commands a price of US\$135 to 155 m^{-3} (approximately US\$1,500 Mg^{-1}) based on supplier catalog pricing (BFG Supply Co., Burton, OH).

Acknowledgments The authors would like to thank the US Forest Service Northern Research Station Institute for Applied Ecosystem Studies (IAES) for supporting this project. They would also like to thank Ronald Zalesny, Jr., Adam Wiese, Ed Bauer, and Bruce Birr from IAES for producing the mini-cuttings and conducting the nutrient analyses and the Iowa State University Center for Sustainable Environmental Technologies for providing the biochar. One of the authors (CB) would like to acknowledge support from a National Science Foundation Graduate Research Fellowship.

References

1. Brown RC (2003) Biorenewable resources: engineering new products from agriculture. Iowa State Press, Ames, 286 p
2. Sohi SP, Krull E, Lopez-Capel E, Bol R (2010) A review of biochar and its use and function in soil. Adv Agron 105:47–82
3. Atkinson CJ, Fitzgerald JD, Hipps NA (2010) Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant Soil 337:1–18
4. Laird DA, Fleming P, Davis DD, Horton R, Wang B, Karlen DL (2010) Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. Geoderma 158:443–449
5. Peng X, Ye LL, Wang CH, Zhou H, Sun B (2011) Temperature- and duration-dependent rice straw derived biochar: characteristics and its effects on soil properties of an ultisol in southern China. Soil Tillage Res 112:159–166
6. Jeffery S, Verheijen FGA, van der Velde M, Bastos AC (2011) A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agric Ecosyst Environ 144:175–187
7. Karhu K, Mattila T, Bergstrom I, Regina K (2011) Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity – results from a short-term pilot field study. Agric Ecosyst Environ 140:309–313
8. Yuan JH, Xu RK (2011) The amelioration effects of low temperature biochar generated from nine crop residues on an acidic ultisol. Soil Use Manag 27:110–115
9. Major J, Rondon M, Molina D, Riha S, Lehmann J (2010) Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant Soil 333:117–128
10. Unger R, Killorn R (2011) Effect of three different qualities of biochar on selected soil properties. Comm Soil Sci Plant Anal 42:2274–2283
11. Laird D, Fleming P, Wang B, Horton R, Karlen D (2010) Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158:436–442
12. Taghizadeh-Toosi A, Clough TJ, Sherlock RR, Condron LM (2012) A wood based low-temperature biochar captures NH_3-N generated from ruminant urine-N, retaining its bioavailability. Plant Soil 353:73–84
13. Zhang A, Liu Y, Pan G, Hussain Q, Li L, Zheng J, Zhang X (2012) Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from central China plain. Plant Soil 351:263–275
14. Dumroese RK, Heiskanen J, Englund K, Tervahauta A (2011) Pelleted biochar: chemical and physical properties show potential use as a substrate in container nurseries. Biomass Bioenergy 35:2018–2027
15. Graber ER, Harel YM, Kolton M, Cytryn E, Silber A, David DR, Tsechansky L, Borenshtein M, Elad Y (2010) Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. Plant Soil 337:481–496
16. Graber ER, Tsechansky L, Gerstl Z, Lew B (2012) High surface area biochar negatively impacts herbicide efficacy. Plant Soil 353:95–106
17. Nag SK, Kookana R, Smith L, Krull E, Macdonald LM, Gill G (2011) Poor efficacy of herbicides in biochar-amended soils as affected by their chemistry and mode of action. Chemosphere 84:1572–1577
18. Sun K, Keiluweit M, Kleber M, Pan Z, Xing B (2011) Sorption of fluorinated herbicides to plant biomass-derived biochars as a function of molecular structure. Bioresour Technol 102:9897–9903
19. Nguyen BT, Lehmann J (2009) Black carbon decomposition under varying water regimes. Org Geochem 40:846–853
20. Singh BP, Hatton BJ, Singh B, Cowie AL, Kathuria A (2010) Influence of biochars on nitrous oxide emissions and nitrogen leaching from two contrasting soils. J Environ Qual 39:1224–1235
21. Goerndt ME, Mize C (2008) Short-rotation woody biomass as a crop on marginal lands in Iowa. North J Appl For 25:82–86
22. Zalesny RS Jr, Hall RB, Zalesny JA, McMahon BG, Berguson WE, Stanosz GR (2009) Biomass and genotype × environment interactions of *Populus* energy crops in the Midwestern United States. BioEnerg Res 2:106–122
23. Zalesny RS Jr, Riemenschneider DE, Hall RB (2005) Early rooting of dormant hardwood cuttings of *Populus*: analysis of quantitative genetics and genotype × environment interactions. Can J For Res 35:918–929
24. Bradshaw HD Jr, Ceulemans R, Davis J, Stettler R (2000) Emerging model systems in plant biology: poplar (*Populus*) as a model forest tree. J Plant Growth Regul 19:306–313
25. Jansson S, Douglas CJ (2007) *Populus*: a model system for plant biology. Annu Rev Plant Biol 58:435–458
26. Timmer VR (1985) Response of a hybrid poplar clone to soil acidification and liming. Can J Soil Sci 65:727–735
27. Lteif A, Whalen JK, Bradley RL, Camire C (2008) Diagnostic tools to evaluate the foliar nutrition and growth of hybrid poplars. Can J For Res 38:2138–2147
28. Ciesielski H, Sterckeman T (1997) A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils. Agronomie 17:9–16
29. Dickmann D, Phipps H, Netzer D (1980) Cutting diameter influences early survival and growth of several *Populus* clones. USDA Forest Service North Central Forest Experiment Station, Research Note NC-261. 4 p
30. Coyle DR, Coleman MD (2005) Forest production responses to irrigation and fertilization are not explained by shifts in allocation. For Ecol Manag 208:137–152
31. Haase DL, Rose R (1995) Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. For Sci 41:54–66
32. Imo M, Timmer VR (1997) Vector diagnosis of nutrient dynamics in mesquite seedlings. For Sci 43:268–173
33. Glynn C, Herms DA, Egawa M, Hansen R, Mattson WJ (2003) Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. Oikos 101:385–397
34. Baligar VC, Fageria NK, He ZL (2001) Nutrient use efficiency in plants. Comm Soil Sci Plant Anal 32:921–950
35. Smit B, Stachowiak M, Van Volkenburgh E (1989) Cellular processes limiting leaf growth in plants under hypoxic root stress. J Exp Bot 40:89–94
36. Brewer CE, Schmidt-Rohr K, Satrio JA, Brown RC (2009) Characterization of biochar from fast pyrolysis and gasification systems. Environ Prog Sustain Energy 28:386–396
37. Brewer CE, Unger R, Schmidt-Rohr K, Brown RC (2011) Criteria to select biochars for field studies based on biochar chemical properties. Bioenerg Res 4:312–323