

Influence of Biochar Addition to Nursery Container Media: Trace Gas Efflux, Growth, and Leachate N¹

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Abstract

Biochar is a pyrolytic product generated by heating biomass in the absence of oxygen such as during bioenergy production. Biochar can be made from various feedstocks and research into its potential use in agricultural systems has examined its effects on plant growth, trace gas emissions, and N loss. However, since a paucity of work has examined biochar use in horticultural container production systems, we investigated how biochar additions to growth media impacted trace gas efflux (CO₂, CH₄, and N₂O), plant growth, and N loss via leachate in two separate experiments: a peat-based greenhouse study using viola (*Viola cornuta* L. 'Sorbet® XP Deep Orange') and a pinebark-based outdoor study using daylily (*Hemerocallis* x 'EveryDaylily Cream PBR' L.). Biochar had little effect on viola growth, but growth inhibition was noted for daylily. Both studies clearly showed that N in leachate was reduced by biochar additions, with higher biochar rates having greater effects on reducing N loss. Reductions in N loss with biochar suggest improved N use efficiencies in agricultural systems. Biochar use also decreased N₂O and CO₂ fluxes in daylily, which suggests that biochar could help mitigate global climate change. Our results suggest that future studies should focus on testing lower rates of biochar in terms of growth and environmental impacts. The complexities of N management highlight the importance of developing biochar practices that increase N retention for the benefit of both agriculture and the environment.

Species used in this study: viola (*Viola cornuta* L. 'Sorbet® XP Deep Orange'); daylily (*Hemerocallis* x 'EveryDaylily Cream PBR' L.).

Index words: climate change, container production, greenhouse gas emissions, leachate nitrogen.

Significance to the Horticulture Industry

Ornamental plant producers may be incentivized to alter production practices to reduce greenhouse gas (GHG) emissions in response to oncoming legislation, potential tax incentives or consumer demand. Two studies investigated biochar as a substrate amendment to mitigate GHG emissions from the production of one annual [*Viola cornuta* L. 'Sorbet® XP Deep Orange')] and one perennial [daylily (*Hemerocallis* x 'EveryDaylily Cream PBR' L.)] crop. Viola growth was evaluated over 42 days in five treatments [80:20 peatmoss:perlite (PMP) amended with 0, 5, 10, 20, or 30% biochar by volume]. Treatments included (1) 100% PMP, (2) 95:5 PMP:biochar, (3) 90:10 PMP:biochar, (4) 80:20 PMP:biochar, and (5) 70:30 PMP:biochar. At study termination, no differences were observed for viola top dry weight or total plant N across treatments. Emissions of N₂O were significantly less for the 30% biochar treatment at one sampling date; no differences occurred for total emissions of CO₂, N₂O or CH₄. Daylily was evaluated over

74 days in four treatments (6:1 pinebark (PB):sand control, or PB mixed with 10, 20 or 30% biochar by volume). Treatments included (1) 6:1 PB:sand, (2) 90:10 PB:biochar, (3) 80:20 PB:biochar, and (4) 70:30 PB:biochar. In general, daylily top dry weight, root dry weight, and total plant N was less for all biochar treatments compared to the control. Most notably, results early in the study indicated that the 0 biochar control treatment had higher N₂O emissions than those with any level of biochar. Total emissions of N₂O and CO₂ declined with increasing amounts of biochar. Results from both studies suggested N in leachate was reduced by biochar use. Given the growth inhibition of daylily with higher biochar levels, future work will focus on evaluating lower biochar rates and differing incorporation strategies on growth and GHG emissions.

Introduction

Since the onset of the industrial revolution, atmospheric concentrations of carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) have increased significantly (Dlugokencky et al. 2005, IPCC 2007, Prinn et al. 2000). These trace gases are the primary greenhouse gases (GHG) thought to be driving factors in global climate change (Dlugokencky et al. 2005, Florides and Christodoulides 2008). Energy production is the largest contributor to GHG emissions in the U.S, followed by agriculture (Johnson et al. 2007). Agriculture accounts for approximately one-fifth of the annual increase in emissions of these trace gases; when one considers land use changes (e.g., land clearing, biomass burning, soil degradation), the overall radiative forcing from agriculture production accounts for approximately a third of the anthropogenic greenhouse effect (Cole et al. 1997). Thus, development of mitigation

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strategies to reducing trace gas emissions from the agricultural sector is crucial to lessen impacts of climate change.

Altering agriculture production practices to mitigate trace gas emissions has been widely investigated (Cole et al. 1997, Kroeze and Mosier 2000, Lal 2004, Paustian et al. 2000, Smith et al. 2007). Most of the work on reducing trace gas emissions has focused on row crops, forests, and animal production systems. Little emphasis has been placed on contributions from specialty crop systems such as horticulture even though it is a multi-billion-dollar industry impacting rural, suburban, and urban environments (Hall et al. 2018). For example, 7,300 nursery crop producers (top 17 states) occupied approximately one-half million acres (USDA 2007). In Alabama, this industry (nursery, greenhouse, and floriculture) is estimated at \$629.2 million annually and supports ~8,000 jobs (ACES 2013). Given the magnitude of the green industry and its contribution to national, state and local economies, it is important to understand how industry management practices can be altered to mitigate climate change.

Increased interest in bioenergy has resulted in enhanced availability of biochar, a pyrolytic byproduct generated during bioenergy production from various feedstocks. Research into potential uses of biochar in agricultural systems has examined its effects on growth, yield, soil carbon sequestration, and movement of nutrients within and out of these systems, including as trace gases (Laird 2008, Clough and Condron 2010, Agegnehu et al. 2017, Ding et al. 2017, Nguyen et al. 2017). While less is known about the effects of biochar in horticultural container production systems, it represents a mechanism for increasing C sequestration and for mitigating trace gas emissions from growth substrates used in these systems by adding a highly recalcitrant form of carbon into the landscape at planting.

Some work has evaluated plant responses in growth media amended with biochar. Álvarez et al. (2018) reported that adding biochar (up to 12%) and vermicompost (up to 30%) to peat moss enhanced petunia (*Petunia x hybrida* hort. Ex E. Vilm.) and Pelargonium [*Pelargonium peltatum* (L.) L'Hér] plant size and flower production when compared with peat moss alone. In another study of Pelargonium [*P. zonale* (L.) L'Hér] response to peat replacement with biochar, Conversa et al. (2015) reported that plant growth was enhanced by biochar concentrations up to 30% when used with fertilization; however, greater rates of biochar replacement negatively impacted growth. Tomato (*Lycopersicon esculentum* Mill.) and pepper (*Capsicum annuum* L.) plant growth and development were also shown to be significantly enhanced by biochar addition (1-5%) to a commercial media containing coconut fiber and tuff (Graber et al. 2010). They attributed these positive responses from biochar to shifts towards beneficial plant growth promoting rhizobacteria or fungi and/or low doses of biochar chemicals stimulating plant growth.

Other studies have evaluated the effects of biochar additions to growth media on nutrient leaching. An examination of a standard PP (85:15 v:v) growth medium amended with 0-10% biochar suggested that biochar addition could be effective in moderating extreme fluctuations of nitrate levels in container substrates over time (Altland and Locke

2012). These same researchers also reported that biochar type influenced macronutrient retention and leaching, with each macronutrient responding differently and each biochar type having a different impact (Altland and Locke 2013). Bradley et al. (2015) found that increasing levels of biochar (0-5%) decreased cumulative levels of total N (21-59%), nitrate (17-46%), and ammonia (46-90%) in leachate, but increased cumulative leaching of total P. Nemati et al. (2014) also showed decreased nutrient leaching (11%) from adding biochar (30%) to a peat moss growth media compared with peat moss alone; additions of biochar also increased cation-exchange capacity and pH. In a study examining runoff from a greenroof study, Beck et al. (2011) reported that adding biochar (7%) to a peat-based growth media (ProMix) increased water retention and significantly decreased total N, total P, nitrate, phosphate, and organic C in discharge. These findings suggest that biochar addition could improve downstream water quality by reducing N, P, and organic C losses, decreasing turbidity and discharge quantity.

In addition to potential reductions in nutrient loss through leaching, biochar use could be included in mitigation strategies to minimize trace gas emissions associated with current management practices. Wu et al. (2019) reported that incorporation of a biochar amendment in the presence of vermicompost significantly decreased (14.1-18.6%) cumulative N₂O emissions and that the lowest emissions of both NH₃ and N₂O were achieved using biochar in combination with a low dose of vermicompost. Reduced N₂O emissions (up to ~60%) with additions of biochar were also reported by Kammann et al. (2012), who found that biochar improved the greenhouse gas (GHG) to crop yield ratio under field-relevant conditions, which is important for growers concerned with climate change.

Recent efforts at our laboratory have begun to investigate contributions of the Southeastern horticulture container industry to climate change (Marble et al. 2011, Prior et al. 2011), as well as opportunities to reduce these contributions (by decreasing GHG emissions and/or increasing C sequestration) through management. These systems primarily use a soilless PB-based potting growth substrate (Marble et al. 2011, 2016). Previous work has examined effects of container size (Marble et al. 2012a), fertilizer placement (Marble et al. 2012b) and/or irrigation (Murphy et al. 2018) on growth and GHG emissions. This work has utilized a number of varying plant types, e.g., woody or herbaceous, perennial or annuals, and sun or shade tolerant (Murphy et al. 2019). More recent work has begun to examine how use of alternative growth media (as opposed to PB or peat-based substrates) might impact growth and GHG emissions from ornamental plants (Murphy et al. 2021).

Given the potential of biochar to reduce GHG emissions and to enhance soil C sequestration, it was logical for our work to progress into examining biochar incorporation in nursery containers. While some research has examined the effects of biochar additions to peat-based growth media, no work to our knowledge has investigated biochar use in PB-based container systems. The primary objective of this research was to determine how different levels of biochar

additions to growth media impact trace gas efflux (CO_2 , CH_4 , and N_2O) in two separate experiments: a peat-based greenhouse study and a PB-based outdoor study. In addition, this work examined impacts of biochar additions on plant growth and loss of nitrogen via leachate.

Materials and Methods

Two separate biochar studies were conducted. In the first study, the Paterson Greenhouse Complex (Auburn University, AL) was utilized with viola (*Viola cornuta* L. 'Sorbet® XP Deep Orange') as the test crop. On August 31, 2018, liners [3 plugs from a 200-cell flat per pot] were transplanted into 1.33 L (1.41 qt) pots (06.00 AZ TW; Dillen Products, Middlefield, OH). Containers were filled with a peat:perlite (80:20) media. Treatments were established by adding biochar (Premium Biochar; Mother Earth®, Vancouver, WA) to the standard greenhouse growth medium [80:20 (v:v) fine professional sphagnum peatmoss: coarse horticultural perlite (PM:P) blend] to create five treatments: 1-) 0% biochar (100% 80:20 PM:P); 2-) 5% biochar (remaining 95% is 80:20 PM:P blend); 3-) 10% biochar (remaining 90% is 80:20 PM:P blend); 4-) 20% biochar (remaining 80% is 80:20 PM:P blend); and 5-) 30% biochar (remaining 70% is 80:20 PM:P blend). All substrate treatments were amended on a per cubic yard basis at mixing with: 2.3 kg (5 lb) dolomitic limestone, 0.9 kg (2 lb) of 8:2:2:10 N:P:K (8-5-12 $\text{N:P}_2\text{O}_5:\text{K}_2\text{O}$) starter nutrient charge (GreenCare Fertilizers, Kankakee, IL), and 0.45 kg (1 lb) AquaGro-G granular wetting agent (The Scotts Co., Marysville, OH).

The study used 12 replicates for each treatment; all containers were placed on greenhouse benches in a randomized complete block design. Containers were hand-watered as needed (generally, every 2-3 days). Containers were fertigated (150 ppm N 20-10-20 fertilizer; GreenCare Fertilizers, Kankakee, IL) four times over the course of the study [days after planting (DAP) 13, 17, 31, and 39].

Pour-thru leachates were collected from unused substrate mixtures at study initiation (1 DAP) using the Virginia Tech Pour-Thru technique to determine substrate pH and EC (Altland 2021, Wright 1986). Leachates were collected at three additional dates (13, 31, and 41 DAP). Given the protocol for conducting pour-thru leachates, which requires substrates to be saturated to their maximum water-holding capacity and then waiting 60 minutes to allow substrates to reach equilibrium, four separate reps were used to collect leachates at each collection date.

After leachate collection, samples were centrifuged, vacuum filtered through a 0.45 μm membrane, acidified with concentrated HCl, and then stored at 4 C (39 F) until analysis. Filtered samples were analyzed for inorganic N using the Lachat QuickChem 8500 Series 2 Flow Injection Analysis System (Hach, Loveland, CO). This method of determining N used colorimetric procedures like those described by Kovar and Pierzynski (2009).

Trace gas efflux from containerized plants in this first experiment were sampled *in situ* four times across the final 10 days of the study (DAP 32, 35, 39, and 42) using the static closed chamber method (Hutchinson and Mosier 1981, Hutchinson and Livingston 1993). Based on criteria

described in the GRACEnet protocol (Baker et al. 2003, Parkin and Kaspar 2006), we constructed custom-made gas efflux chambers designed to accommodate nursery containers. These chambers consisted of a polyvinyl chloride (PVC) cylinder base [25.4 cm (10 in) inside diameter by 38.4 cm (15.1 in) tall] that was sealed at the base. During gas efflux measurement, the containerized plant was placed inside the base cylinder; a vented efflux chamber [25.4 cm (10 in) diameter x 11.4 cm (4.5 in) height] was then placed on top of the base cylinder. The top efflux chambers were also constructed of PVC, covered with reflective tape, and contained a center sampling port. Following chamber closure, samples for CO_2 , CH_4 , and N_2O were taken at 0-, 20-, and 40-minute intervals. At each interval, the center sampling port was pierced with a polypropylene syringe and a 10 mL (0.6 in³) gas sample extracted; samples were then transferred by injection into evacuated glass vials [6 mL (0.4 in³)] fitted with butyl rubber stoppers (Parkin and Kaspar 2006) for analysis via gas chromatography.

A gas chromatograph (Shimadzu GC-2014, Columbia, MD) was used to analyze gas samples. This gas chromatograph was equipped with three detectors: a thermal conductivity detector for CO_2 , an electrical conductivity detector for N_2O , and a flame ionization detector for CH_4 . Gas standards (Air Liquide America Specialty Gases LLC, Plumsteadville, PA) were used to develop standard curves from which gas sample concentrations were determined. Gas efflux was calculated from the rate of change in trace gas concentration in the chamber headspace during the time interval when the chambers were closed (Parkin and Venterea 2010); data were expressed as mg $\text{CO}_2\text{-C}$, mg $\text{CH}_4\text{-C}$, and mg $\text{N}_2\text{O-N}$ per day. Cumulative efflux estimates of each trace gas were calculated from efflux at each sampling date integrated over time using a basic numerical integration technique (i.e., trapezoidal rule).

At study termination, all plants were harvested. Shoots were cut at the soil line and roots were separated from the growing medium using the sieve method (Bohm 1979). Shoots and roots were dried for approximately 72 hours at 55 C (130 F) in a forced-air oven and weighed. Roots and shoots were then ground separately to pass through a 0.2 mm (0.08 in) mesh sieve and C and N determined using a LECO 600-CHN analyzer (St. Joseph, MI).

The second experiment was conducted at the soil bin facilities of the USDA-ARS National Soil Dynamics Laboratory, Auburn, Alabama and utilized daylily (*Heemerocalis* x 'EveryDaylily Cream PBR' L.) as the test species. As previously described by Prior et al. (2003), the bin used for the experiment was 6 m wide x 76 m long and was modified for container studies by installation of a geomembrane liner (20 mil) and gravel drain system to ensure a good working surface and drainage for container studies.

This study used 2.5 L (#1 trade gal) nursery containers filled with a PB:sand (6:1 v:v) media as a control (0% biochar). Biochar treatments consisted of containers filled with PB:biochar at 10, 20, or 30% biochar; specific treatments included (1) 6:1 PB:sand, (2) 90:10 PB:biochar, (3) 80:20 PB:biochar, and (4) 70:30 PB:biochar. The biochar used in this study was granulated coconut char (GC 8 X

Table 1. *Viola*^z biomass data [top^y, root^x, and total^w dry weights (DW)], root:shoot ratio^v (R:S), total N^u, and carbon:nitrogen ratio^t (C:N) for the biochar treatment levels.

Biochar (%)	Top DW (g)	Root DW (g)	Total DW (g)	R:S	Total N (mg)	C:N
0	2.27a ^s	0.16b	2.43a	0.071b	87.74a	11.71b
5	2.58a	0.17b	2.75a	0.068b	94.21a	12.33ab
10	2.34a	0.17b	2.51a	0.072b	92.73a	11.43b
20	2.38a	0.18b	2.56a	0.076b	91.72a	11.66b
30	2.57a	0.28a	2.85a	0.108a	85.87a	13.61a
P value	0.125	0.025	0.612	0.014	0.891	0.018

^z*Viola cornuta* L. ‘Sorbet® XP Deep Orange’ plants were potted into 1.33L (1.41 qt) containers filled with peat:perlite (80:20) media containing five levels of biochar (0, 5, 10, 20, or 30 %), and amended on a per cubic yard basis at mixing with 2.3 kg (5 lb) dolomitic limestone, 0.9 kg (2 lb) of 8:2:2:10 N:P:K (8-5-12 N:P₂O₅:K₂O) starter nutrient charge, and 0.45 kg (1 lb) AquaGro-G granular wetting agent.

^yTop dry weights (g) determined by drying the above-substrate portion of the plant in a 55 C (130 F) forced air oven for 72 hours.

^xRoot dry weights (g) were determined by removing the substrate from root interface and drying the within-substrate portion of the plant in a 55 C (130 F) forced air oven for 72 hours.

^wTotal dry weight equaled top dry weight plus root dry weight.

^vRoot:Shoot equaled root dry weight divided by top dry weight.

^uTotal N was determined by adding nitrogen found in tops and roots; tissues were ground separately to pass a 0.2 mm (0.08 in) mesh sieve prior to N concentration determination (LECO 600-CHN analyzer) and multiplying N concentration by each dry weight portion.

^tCarbon:Nitrogen equaled whole plant carbon divided by whole plant nitrogen determined using a LECO 600-CHN analyzer.

^sWithin a column, means followed by the same letter are not significantly different ($p < 0.05$) according to the LSMeans statement under the Proc Mixed Procedure of SAS.

30S; General Carbon Corp., Patterson, NJ). The growth media was amended with 6.9 g (0.015 lb) lime and 27 g (0.059 lb) fertilizer (16-5-10 Osmocote – 12-month release with micronutrients) per container. Rooted cuttings of day-lily were potted into treatments on May 18, 2020. The study was conducted as a randomized complete block design of the four biochar treatments with six blocks.

To collect 100% of the leachate from containers, the 3 L containers were retrofitted with a collar constructed from another 3 L container. Each collar was made from the upper ~10 cm (~4 in) of the other container. This 10 cm was cut off, turned upside down, and slid onto an intact 3 L container from the bottom such that the collar was flush with the bottom of the intact container. The collar and the intact container were secured using silicone to insure a watertight seal. This retrofitted container was then snugly placed over a 15.2 cm (6 in) standard nursery pot (drain holes sealed with silicone) to act as a leachate collection vessel. These retrofitted container/collection vessels were placed into modified standard wooden pallets to hold them in an upright and stable fashion.

Leachate was collected (from irrigation and rainfall events) and held in 3.8 L (1 gal) jugs. At the end of each week, total leachate volume was determined using graduated cylinders and a 50 ml subsample was collected for leachate N analyses. Leachate N was analyzed using the methods described for the first study above. From the volume and N analyses data we calculated N concentration, N content, and total N lost in leachate.

Trace gases were sampled weekly on the same 13 dates on which leachate was collected. Trace gases were measured using the same custom-made gas efflux chambers described for the first study. The methodologies used to collect, analyze, and manipulate the trace gas data in the *viola* study were used in this daylily experiment. Further, daylily plants were harvested, processed, and analyzed at

study termination in the same manner described for the *viola* plants in the first study.

For both studies, data analyses were conducted using the Mixed Models Procedure (Proc Mixed) of the Statistical Analysis System (Littell et al., 1996). Means separations were performed using the LSMeans statement under Proc Mixed. In both studies, a significance level of ($p \leq 0.05$) was established *a priori*.

Results and Discussion

Biomass (*viola* greenhouse study). There was no effect of biochar on *viola* top dry weight (Table 1). However, in the highest biochar level (30%), *viola* had significantly greater root dry weight compared to all other treatments. Given that shoots dominated dry weight, total dry weight also did not differ among biochar treatments. The overall total dry weight averaged 2.62 g per plant.

As seen with root dry weight, root-to-shoot ratio (R:S) was greatest at the highest biochar level (30%) and was significantly higher than all other treatments. Although total plant N was not affected by biochar level, the carbon-to-nitrogen ratio (C:N) was impacted by biochar treatments. The C:N was highest at 30% biochar and was significantly higher than all treatments except for 5% biochar (Table 1).

Trace gas efflux (*viola* greenhouse study). There were no significant differences among biochar levels for *viola* daily trace gas efflux (CO₂, CH₄, and N₂O) at any of the four measurement dates (Fig. 1). However, on DAP 39 there was a trend ($p = 0.096$) for N₂O to be lowest at highest level of biochar (Fig. 1). The average daily efflux across the sampling periods was 46.75 mg CO₂-C-d⁻¹, 0.0064 mg CH₄-C-d⁻¹, and 0.0056 mg N₂O-N-d⁻¹ and were not significantly affected by biochar level (Table 2). Cumulative efflux of all three trace gasses were also not significantly affected by biochar level and

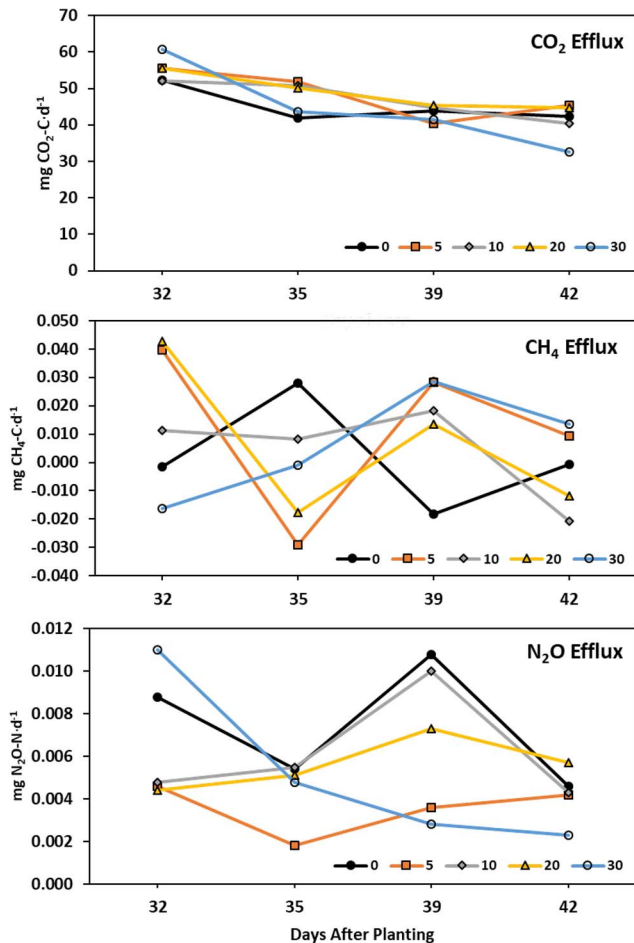


Fig. 1. Daily trace efflux (CO₂, CH₄, and N₂O) at five levels of biochar (0, 5, 10, 20, and 30%) for the viola greenhouse study.

averaged 462.06 g CO₂-C, 0.0630 g CH₄-C, and 0.0599 g N₂O-N (Table 2).

Leachate nitrogen (viola greenhouse study). In general, NO₃ concentration in leachate was numerically lowest at the higher biochar levels (20 and 30%) and numerically

highest at lower biochar levels (5 and 10%) for the first three sampling dates (Table 3). A similar response pattern was observed for NH₄ concentration; thus, this overall pattern held true for total N concentration. Biochar-driven differences in both N species and total N became negligible at the last leachate sample date. When averaged across sampling dates, total N was significantly lower at 30% biochar than treatments with 0, 5, and 10% biochar (Table 3). Average leachate N for the 30% biochar treatment was numerically lower than that of 20% biochar, but statistically similar. In general, higher levels of biochar released less N from containers via leachate.

Biomass (daylily outdoor study). Compared to the control (0 biochar), all biochar levels decreased top dry weight (Table 4). In general, top dry weight decreased with increasing biochar added to growth media. Root dry weight also showed decreases with biochar compared to the control; however, there were no significant differences among biochar addition rates (i.e., 10, 20, and 30%). Given that root dry weights were larger than top dry weights, total dry weight followed the same pattern seen with roots. As expected, R:S showed a reverse pattern, in which plants grown with 30% biochar had significantly greater R:S than all other treatments. Plant crowns (the small white cores located between the leaves and the roots) and flowers showed a general pattern of decreasing numbers at higher biochar levels (Table 4).

Total plant N content was significantly greater in controls (0 biochar) compared to all levels of added biochar (i.e., 10, 20, and 30%). In general, plant C:N increased as more biochar was added to the containers (Table 4).

Trace gas efflux (daylily outdoor study). In general, CO₂ efflux tended to decline with increasing biochar rates, but this decline was often not statistically significant, except for a few dates (Fig. 2). On DAP 1, CO₂ efflux was higher ($p=0.005$) for the control (0 biochar) than for all levels of added biochar (10, 20, and 30%). On DAP 5, CO₂ efflux was greater ($p=0.040$) at 10% biochar compared with 0 biochar or 30% added biochar. On DAP 12, CO₂ efflux was greater ($p=0.023$)

Table 2. Viola^z average daily and cumulative trace gas efflux for the biochar treatment levels.

Biochar (%)	Average Daily Trace Gas Efflux ^y			Cumulative Trace Gas Efflux ^x		
	CO ₂ -C (mg·d ⁻¹)	CH ₄ -C (mg·d ⁻¹)	N ₂ O-N (mg·d ⁻¹)	CO ₂ -C (mg)	CH ₄ -C (mg)	N ₂ O-N (mg)
0	45.09a ^w	0.0019a	0.0074a	442.18a	0.0313a	0.0769a
5	48.23a	0.0121a	0.0036a	473.62a	0.0712a	0.0498a
10	46.95a	0.0043a	0.0061a	472.68a	0.0783a	0.0678a
20	48.89a	0.0075a	0.0056a	484.19a	0.0422a	0.0584a
30	44.57a	0.0062a	0.0052a	437.61a	0.0922a	0.0464a
P value	0.690	0.974	0.133	0.705	0.997	0.544

^zViola cornuta L. 'Sorbet® XP Deep Orange' plants were potted into 1.33L (1.41 qt) containers filled with peat:perlite (80:20) media containing five levels of biochar (0, 5, 10, 20, or 30 %), and amended on a per cubic yard basis at mixing with 2.3 kg (5 lb) dolomitic limestone, 0.9 kg (2 lb) of 8:2:2:10 N:P:K (8-5-12 N:P₂O₅:K₂O) starter nutrient charge, and 0.45 kg (1 lb) AquaGro-G granular wetting agent.

^yAverage daily trace gas efflux was the average of four measurements made during the final 10 days of the study using the static closed chamber method (Hutchinson and Mosier 1981, Hutchinson and Livingston 1993) with data expressed as mg CO₂-C, mg CH₄-C, and mg N₂O-N.

^xCumulative trace gas efflux was the total efflux across the four measurements during the final 10 days of the study calculated using the trapezoid rule with data expressed as mg CO₂-C, mg CH₄-C, and mg N₂O-N.

^wWithin a column, means followed by the same letter are not significantly different ($p\leq 0.05$) according to the LSMeans statement under the Proc Mixed Procedure of SAS.

Table 3. Leachate^z N concentration^y (NO₃, NH₄, and Total N in mg·L⁻¹) for the viola^x greenhouse study by sample date (DAP = days after planting) for the biochar treatment levels.

Nitrogen	Biochar (%)	DAP 1	DAP 13	DAP 31	DAP 41	Average
NO ₃	0	94.06bc ^w	117.57bc	59.81ab	39.49a	77.73ab
	5	112.94a	144.03ab	70.37a	41.22a	92.04ab
	10	109.35ab	183.34a	80.69a	46.27a	104.91a
	20	84.77c	98.33bc	40.21b	44.44a	66.94bc
	30	56.01d	62.36c	34.51b	34.52a	46.85c
	P value	<0.001	0.007	0.025	0.258	0.002
NH ₄	0	34.24b	44.49ab	27.96a	12.80a	29.87ab
	5	41.80a	46.28ab	23.53ab	12.97a	31.14ab
	10	41.92a	61.28a	32.49a	10.56a	36.56a
	20	31.99bc	37.07b	13.98bc	12.80a	23.96bc
	30	26.66c	29.02b	11.96c	9.70a	19.34c
	P value	0.001	0.033	0.006	0.207	0.020
Total N	0	128.30b	162.06bc	87.77ab	52.29a	107.60ab
	5	154.33a	190.31ab	93.90a	54.18a	123.18ab
	10	151.27a	244.62a	113.18a	56.83a	141.48a
	20	116.76b	135.40bc	54.19bc	57.24a	90.90bc
	30	82.67c	91.38c	46.47c	44.23a	66.19c
	P value	<0.001	0.010	0.016	0.342	0.003

^zLeachate collected using the Virginia Tech pour-through method (Wright 1986).

^yNitrogen concentrations (NO₃, NH₄, and Total N = NO₃ + NH₄) in leachate were determined using the Lachat QuickChem 8500 Series 2 Flow Injection Analysis System using procedures described by Kovar and Pierzynski (2009).

^x*Viola cornuta* L. 'Sorbet® XP Deep Orange' plants were potted into 1.33L (1.41 qt) containers filled with peat:perlite (80:20) media containing five levels of biochar (0, 5, 10, 20, or 30 %), and amended on a per cubic yard basis at mixing with 2.3 kg (5 lb) dolomitic limestone, 0.9 kg (2 lb) of 8:2:2:10 N:P:K (8-5-12 N:P₂O₅:K₂O) starter nutrient charge, and 0.45 kg (1 lb) AquaGro-G granular wetting agent.

^wWithin a column, means followed by the same letter are not significantly different ($p \leq 0.05$) according to the LSMeans statement under the Proc Mixed Procedure of SAS.

with 0 biochar and 10% added biochar than at 30% added biochar. On DAP 75, there was a trend ($p=0.059$) for the control (0 biochar) to have higher CO₂ efflux than 20 and 30% added biochar. It was expected that CO₂ flux would decrease as biochar percentages increased, which was the general pattern observed. This was expected for two reasons: 1-) more biochar = less organic substrate to be degraded by microbes leading to less CO₂ flux; and 2-) biochar, particularly at the highest rate, reduced plant growth; smaller plants would be expected to respire less. It should also be noted that trace

gas efflux data tend to be highly variable and don't always follow the expected pattern.

Daily CH₄ efflux was not significantly affected by biochar rates and all rates resulted in containers being very small net sinks or sources of CH₄ (Fig. 2). On DAP 68 and 75, trends ($p=0.089$ and $p=0.102$, respectively), were noted for 30% added biochar to have higher efflux than most other biochar rates. It is possible that at this highest tested rate of biochar, soil remained wet longer leading to greater anaerobic respiration and greater CH₄ flux.

Table 4. Daylily^z crown and flower numbers^y, biomass data [top^x, root^w, and total^v dry weights (DW)], root:shoot ratio^u (R:S), total N^t, and carbon:nitrogen ratio^s (C:N) for the biochar treatment levels.

Biochar (%)	Crown (no.)	Flower (no.)	Top DW (g)	Root DW (g)	Total DW (g)	R:S	Total N (mg)	C:N
0	5.67a ^t	5.33a	4.25a	12.64a	16.89a	2.88b	531.58a	13.25c
10	5.17a	5.17a	2.12b	4.98b	7.10b	2.39b	217.19b	13.76bc
20	4.17ab	3.00b	1.30bc	3.68b	4.98b	2.84b	127.79b	15.86ab
30	3.00b	0.17c	0.40c	2.80b	3.20b	7.35a	94.50b	16.58a
P value	0.048	<0.001	<0.001	0.004	0.001	0.004	<0.001	0.044

^zDaylily (*Hemerocallis x 'EveryDaylily Cream PBR' L.*) plants were potted into 2.5 L (#1 trade gal) containers filled with pinebark:sand (6:1 v:v) media containing four levels of biochar (0, 10, 20, or 30 %), and amended with 6.9 g (0.015 lb) lime and 27 g (0.059 lb) fertilizer (16-5-10 Osmocote – 12-month release with micronutrients) per container.

^yCrown and flower numbers determined by manual counts.

^xTop dry weights (g) determined by drying the above-substrate portion of the plant in a 55 C (130 F) forced air oven for 72 hours.

^wRoot dry weights (g) were determined by removing the substrate from root interface and drying the within-substrate portion of the plant in a 55 C (130 F) forced air oven for 72 hours.

^vTotal dry weight equaled top dry weight plus root dry weight.

^uRoot:Shoot equaled root dry weight divided by top dry weight.

^tTotal N was determined by adding nitrogen found in tops and roots; tissues were ground separately to pass a 0.2 mm (0.08 in) mesh sieve prior to N concentration determination (LECO 600-CHN analyzer) and multiplying N concentration by each dry weight portion.

^sCarbon:Nitrogen equaled whole plant carbon divided by whole plant nitrogen determined using a LECO 600-CHN analyzer.

^tWithin a column, means followed by the same letter are not significantly different ($p < 0.05$) according to the LSMeans statement under the Proc Mixed Procedure of SAS.

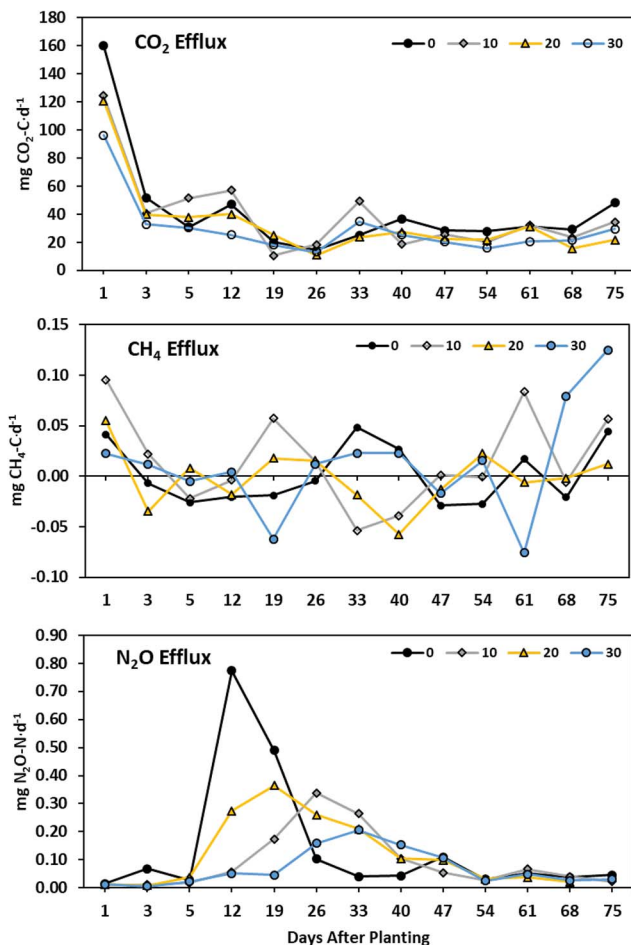


Fig. 2. Daily trace efflux (CO₂, CH₄, and N₂O) at four levels of biochar (0, 10, 20, and 30%) for the daylily outdoor study.

Early in the study, N₂O efflux showed significant differences among biochar levels (Fig. 2). On DAP 3, the 0 biochar control had higher N₂O efflux ($p < 0.001$) than all levels of added biochar. On DAP 12 the control also showed higher N₂O efflux ($p < 0.001$) than all biochar additions; on this date 20% added biochar had higher N₂O efflux than 10 and 30% biochar. On DAP 19, 0 and 20%

biochar had higher N₂O efflux ($p = 0.001$) than 10 and 30% added biochar. On DAP 26, 10% added biochar had higher N₂O efflux ($p = 0.0001$) compared to 0 and 30% biochar; further, 20% biochar had higher N₂O efflux than the 0 biochar control. On DAP 33, the control (0 biochar) had lower N₂O efflux ($p = 0.004$) than all levels of added biochar. On all other dates, N₂O efflux was not affected by biochar rate. As with the CO₂ data, it was expected that - if biochar is binding N (as the leachate data suggest) - N₂O flux should decrease as percentages of biochar increases. While this general pattern was observed in some of the data, the high variability in trace gas data, noted in the discussion of the CO₂ results, impacted results for N₂O flux as well.

The average daily CO₂ efflux across sampling periods declined with increasing amount of biochar (Table 5). Average daily CH₄ effluxes were low and showed no differences among biochar treatments. Average daily N₂O efflux was highest for the control (0 biochar) and lowest at the highest biochar level (30%), with the other treatments falling between these extremes. Cumulative efflux of the three trace gases followed patterns like those seen for average daily efflux for each trace gas (Table 5).

Leachate nitrogen (daylily outdoor study). In general, daily leachate NO₃, NH₄, and total N concentrations tended to decrease as biochar level increased (Table 6). The control (0 biochar) tended to have the highest leachate N concentrations and it was usually higher than all biochar added treatments. The highest biochar treatment (30%) usually had the lowest leachate N concentrations, but differences among the three biochar levels varied across sampling dates and with N type. Average leachate N concentrations also tended to decrease as biochar level increased (Table 6).

Leachate N content tended to follow the same general pattern as leachate N concentrations although fewer significant differences among the three levels of added biochar were noted (Table 7). Further, effects of biochar rate on leachate NO₃ content became non-significant toward the end of the study, while NH₄ and total N content in treatments with 30% biochar remained significantly lower than the other treatments throughout the study period. Again,

Table 5. Daylily^z average daily and cumulative trace gas efflux for the biochar treatment levels.

Biochar (%)	Average Daily Trace Gas Efflux ^y			Cumulative Trace Gas Efflux ^x		
	CO ₂ -C (mg·d ⁻¹)	CH ₄ -C (mg·d ⁻¹)	N ₂ O-N (mg·d ⁻¹)	CO ₂ -C (mg)	CH ₄ -C (mg)	N ₂ O-N (mg)
0	42.31a ^w	0.0018a	0.1414a	2387.32a	-0.1360a	12.2056a
10	38.88ab	0.0157a	0.0916b	2343.36ab	0.6028a	8.0920bc
20	33.62bc	-0.0015a	0.1147ab	1963.41bc	-0.3651a	10.1143ab
30	29.46c	0.0120a	0.0688b	1763.78c	0.5048a	5.9920c
P value	0.020	0.528	<0.001	0.049	0.708	<0.001

^zDaylily (*Heemerocallis* x 'EveryDaylily Cream PBR' L.) plants were potted into 2.5 L (#1 trade gal) containers filled with pinebark:sand (6:1 v:v) media containing four levels of biochar (0, 10, 20, or 30 %), and amended with 6.9 g (0.015 lb) lime and 27 g (0.059 lb) fertilizer (16-5-10 Osmocote - 12-month release with micronutrients) per container.

^yAverage daily trace gas efflux was the average of four measurements made during the final 10 days of the study using the static closed chamber method with data expressed as mg CO₂-C, mg CH₄-C, and mg N₂O-N.

^xCumulative trace gas efflux was the total efflux across the four measurements during the final 10 days of the study calculated using the trapezoid rule with data expressed as mg CO₂-C, mg CH₄-C, and mg N₂O-N.

^wWithin a column, means followed by the same letter are not significantly different ($p \leq 0.05$) according to the LSMeans statement under the Proc Mixed Procedure of SAS.

Table 6. Leachate^z N concentration^y (NO₃, NH₄, and Total N in mg·L⁻¹) for the daylily^x outdoor study by sample date (DAP = days after planting) for the biochar treatment levels.

Nitrogen	Biochar (%)	DAP 1	DAP 3	DAP 5	DAP 12	DAP 19	DAP 26	DAP 33	DAP 40	DAP 47	DAP 54	DAP 61	DAP 68	DAP 75	Average
NO ₃	0	51.99a ^w	34.32a	95.07a	117.91a	72.10a	179.19a	113.58a	162.71a	54.68a	116.91a	133.98a	92.61a	97.36a	101.78a
	10	27.00b	22.33ab	30.60c	95.96b	32.06c	77.06b	64.91b	104.47b	42.13a	75.46b	86.83b	73.26b	72.35b	61.88b
	20	24.30b	27.26a	53.39b	86.46bc	46.14b	75.65b	64.49b	88.95c	45.21a	69.62b	69.72bc	55.54c	61.01c	59.06b
	30	18.13c	10.89b	30.63c	68.04c	40.55bc	64.88b	68.88b	80.19c	42.96a	64.79b	54.94c	47.91c	47.13d	49.22c
	P value	<0.001	0.022	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.366	0.004	<0.001	<0.001	<0.001	<0.001
NH ₄	0	24.40a	23.77a	37.22a	43.51a	22.78a	28.36a	14.76a	27.41a	7.95a	24.53a	40.88a	33.07a	28.71a	27.49a
	10	15.42b	10.34b	14.10b	24.76b	13.02b	18.78ab	3.07b	3.67b	0.05b	4.04b	9.07b	10.64b	9.60b	10.50b
	20	11.24bc	7.43b	6.30c	9.64c	6.15c	8.24bc	4.57b	2.25b	0.89b	1.65b	1.99b	4.00bc	4.62b	5.31c
	30	7.53c	11.11b	8.30c	13.15bc	0.20d	5.09c	1.24b	0.22b	0.02b	1.60b	0.03b	0.03c	3.36b	4.00c
	P value	<0.001	<0.001	<0.001	0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Total N	0	76.39a	58.09a	132.29a	161.43a	94.88a	208.27a	128.34a	190.11a	62.63a	144.43a	174.86a	125.68a	162.08a	129.27a
	10	42.42b	32.66b	44.70b	120.72b	45.08bc	95.84b	67.98b	108.14b	42.17a	79.50b	95.90b	83.90b	81.94b	72.38b
	20	35.54bc	34.69b	59.70b	96.10bc	52.29b	83.90bc	69.06b	91.19c	46.09a	71.27b	71.71bc	59.54c	65.63c	64.36c
	30	25.65c	22.06b	28.93b	81.19c	40.76c	69.97c	70.13b	80.41c	42.99a	66.39b	54.97c	47.84c	50.50c	53.21d
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.092	0.001	<0.001	<0.001	<0.001	<0.001

^zLeachate was collected (from irrigation and rainfall events), held in 3.8 L (1 gal) jugs, measured weekly for total volume using graduated cylinders, and a 50 ml subsample collected for leachate N analyses.^yNitrogen concentrations (NO₃, NH₄, and Total N = NO₃ + NH₄) in leachate were determined using the Lachat QuickChem 8500 Series 2 Flow Injection Analysis System using procedures described by Kovar and Pietrynski (2009).^xDaylily (*Heimerocallis* x 'EveryDaylily Cream PBR' L.) plants were potted into 2.5 L (#1 trade gal) containers filled with pinebark:sand (6:1 v:v) media containing four levels of biochar (0, 10, 20, or 30 %), and amended with 6.9 g (0.015 lb) lime and 27 g (0.059 lb) fertilizer (16-5-10 Osmocote – 12-month release with micronutrients) per container.^wWithin a column, means followed by the same letter are not significantly different ($p \leq 0.05$) according to the LSMeans statement under the Proc Mixed Procedure of SAS.

average leachate N contents tended to decrease with increasing amount of biochar. Cumulative leachate N losses (NO₃, NH₄, and total N) were all highest with no added biochar (control) and tended to decline as biochar level increased with the lowest N content being at the highest biochar rate (30%; Table 8).

Biochar amendment to agricultural systems could potentially be a means of improving plant performance and soil conditions (Agegnehu et al. 2017). In our viola greenhouse study, biochar additions did not positively affect aboveground and total plant dry weights. However, root dry weight was observed to be significantly increased at the highest biochar addition level (30%). This may have been a result of nitrogen being tied up by biochar and unavailable to the plant, thereby leading to a proliferation of roots searching for nitrogen. Supporting this contention, we noted that top N concentration (data not shown) was lowest at this highest level of biochar despite having the largest root biomass. Plants grown with 30% biochar also had higher total C:N (as seen in Table 1), which was noted with top and root C:N values as well (data not shown).

Biochar amendment did not significantly impact trace gas efflux from viola at any of the four measurement dates. Average daily and cumulative efflux values were also not affected by biochar addition. However, we did note a trend for biochar to reduce N₂O emissions on one date. It is important to note that technical problems prevented earlier assessments of trace gas efflux during the first month of the experiment. Thus, it is possible that we missed a biochar effect which might have occurred earlier in the growth cycle. To date, little work has examined effects of biochar on trace gas emissions in containerized systems. Kammann et al. (2012) reported reduced N₂O emissions with biochar, but no significant effect on CH₄ emissions. Wu et al. (2019) also found that incorporation of biochar (combined with vermicompost) reduced N₂O emissions.

As opposed to trace gas findings, biochar addition did impact N leaching from viola in the greenhouse study. Higher levels of biochar reduced leachate NO₃ and NH₄ concentrations showing that biochar use resulted in less N loss from containers via leachate. Similarly, Beck et al (2011) showed that adding biochar (up to 7%) reduced N and P discharge from greenroof trays. Others have reported that biochar additions to a peat- and perlite-based media reduced nutrient leaching and those different types of biochar reacted differently for different nutrients (Altland and Locke 2012, 2013).

Unlike viola, biochar additions led to reductions in daylily dry weights. This was reflected in both top and root dry weight; plant crown and flower numbers also showed a pattern of decrease with higher biochar levels. In general, plant growth was lowest at the highest biochar level (30%). In terms of plant N, total content was reduced by biochar addition and was reflected by shifts in plant C:N. Conversa et al. (2015) reported no negative effects of biochar addition on plant growth if these additions did not exceed 30%. Graber et al. (2010) found that biochar enhanced development and productivity of pepper and tomato plants, but only tested biochar levels of 1-

Table 7. Leachate^z N content^y (NO₃, NH₄, and Total N in mg) for the daylily^x outdoor study by sample date (DAP = days after planting) for the biochar treatment levels.

Nitrogen	Biochar (%)	DAP 1	DAP 3	DAP 5	DAP 12	DAP 19	DAP 26	DAP 33	DAP 40	DAP 47	DAP 54	DAP 61	DAP 68	DAP 75	Average
NO ₃	0	25.76a ^w	24.01a	57.77a	123.13a	45.36a	126.73a	34.89a	231.55a	16.12b	56.13a	81.73a	81.06a	76.03a	75.38a
	10	15.93b	16.07ab	19.33b	100.85ab	22.74c	62.20b	31.58a	160.09b	24.15ab	42.70a	71.41a	90.39a	73.24a	56.21b
	20	13.37b	18.13a	31.97b	92.57bc	33.39b	62.36b	38.06a	142.74c	28.26a	46.60a	68.43a	75.59a	66.30a	55.23b
	30	9.34c	7.41b	19.94b	70.86c	29.24bc	56.04b	40.30a	130.72c	31.17a	47.59a	60.02a	74.69a	57.96a	48.87c
	P value	<0.001	0.020	<0.001	0.002	0.001	<0.001	0.261	<0.001	0.013	0.100	0.136	0.244	0.138	<0.001
NH ₄	0	12.04a	17.39a	22.78a	45.25a	13.99a	19.86a	4.40a	38.92a	2.38a	11.56a	25.44a	29.82a	23.40a	20.55a
	10	9.12ab	7.47b	8.95b	26.06b	9.31b	15.08a	1.38b	5.67b	0.03b	2.27b	6.96b	13.30b	9.54b	8.86b
	20	6.17bc	4.87b	3.72c	10.25c	4.36c	6.71b	2.64ab	3.62b	0.63b	1.17b	2.09b	5.65bc	5.19b	4.39c
	30	3.88c	8.13b	5.52c	13.82bc	0.14d	4.40b	0.76b	0.35b	0.02b	0.90b	0.03b	0.05c	4.20b	3.25c
	P value	0.002	<0.001	<0.001	0.001	<0.001	0.002	0.090	<0.001	0.001	<0.001	<0.001	<0.001	0.001	<0.001
Total N	0	37.80a	41.40a	80.55a	168.38a	59.35a	146.29a	39.29a	270.46a	18.50a	67.69a	107.17a	110.88a	99.44a	95.94a
	10	25.05b	23.54b	28.28b	126.91b	32.05b	77.28b	32.96a	165.76b	24.18a	44.97b	78.36b	103.69ab	82.78ab	65.06b
	20	19.55bc	23.18b	35.69b	102.82bc	37.75b	69.07bc	40.69a	146.36c	28.89a	47.77b	70.52b	81.24bc	71.49b	59.62b
	30	13.22c	15.54b	25.46b	84.68c	29.38b	60.44c	41.06a	131.07c	31.19a	48.50b	60.05b	74.73c	62.16b	52.11c
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.273	<0.001	0.061	0.008	0.003	0.015	0.017	<0.001

^zLeachate was collected (from irrigation and rainfall events), held in 3.8 L (1 gal) jugs, measured weekly for total volume using graduated cylinders, and a 50 ml subsample collected for leachate N analyses.^yNitrogen content (NO₃, NH₄, and Total N = NO₃ + NH₄) in leachate was calculated by multiplying N concentrations by total leachate volume.^xDaylily (*Heimerocallis* x 'EveryDaylily Cream PBR' L.) plants were potted into 2.5 L (#1 trade gal) containers filled with pinebark:sand (6:1 v:v) media containing four levels of biochar (0, 10, 20, or 30 %), and amended with 6.9 g (0.015 lb) lime and 27 g (0.059 lb) fertilizer (16-5-10 Osmocote – 12-month release with micronutrients) per container.^wWithin a column, means followed by the same letter are not significantly different ($p \leq 0.05$) according to the LSMeans statement under the Proc Mixed Procedure of SAS.

5%. Álvarez et al. (2018) noted that biochar additions to a peat/vermicompost growth media up to 12% (maximum tested) improved plant size and flower production compared to peat alone. In another containerized study, Kammann et al. (2012) reported that biochar addition to a soil/compost media significantly increased plant biomass compared to a soil control. Dumroese et al. (2011) reported that mixing peat growth media with biochar/wood flour pellets up to 25% demonstrated good properties for plant growth (e.g., hydraulic conductivity, water availability), but addition levels of 50% or higher would not be beneficial to plants. Collectively, the literature indicates that there may be a maximum level of biochar addition that can improve plant growth. This level may vary depending on plant species, growth media, and the makeup of the biochar. Specifically, biochars are diverse materials that have different properties depending on the original feedstock properties, pyrolysis conditions (e.g., temperature, processing, grinding, final particle size, etc.), and storage or other postproduction processes (Spokas et al. 2011), which can affect plant growth through a variety of mechanisms (e.g., pH, CEC, nutrient retention and availability, etc.). Thus, the use of different biochars might help explain some of the different responses noted between the viola and daylily studies.

Unlike the viola greenhouse study, biochar additions in the daylily outdoor study did have significant effects on lowering CO₂ and N₂O efflux with higher volumetric additions of biochar. The low, nonsignificant CH₄ emissions noted here and in the viola study were not surprising since we have observed a similar response pattern in previous container studies (Marble et al. 2012a, b, Murphy et al. 2018, 2019, 2021). Methane emissions are generally small in non-saturated soils (Robertson et al. 2000); given that containerized media are often well drained, they do not have the anaerobic conditions needed for CH₄ production and do not significantly contribute to total trace gas emissions from container-grown nursery crops.

Generally, adding biochar to the PB media reduced CO₂ efflux, which tended to decline with increasing biochar rates. This effect for biochar to reduce CO₂ emissions tended to be more apparent towards the beginning, rather than the end, of the daylily study. Similarly, N₂O efflux was reduced by biochar in the first half of the study. Average daily and cumulative efflux values of these three trace gases followed patterns similar to those seen for daily efflux noted above. These findings support the contention that biochar use can reduce N₂O emissions (Kammann et al. 2012, Wu et al. 2019) and further show that reduced CO₂ efflux is another advantage of using biochar as a tool to reduce overall GHG emissions from contain production systems, which could help mitigate climate change.

As seen in the viola study, daily leachate NO₃, NH₄, and total N concentrations showed a pattern of decreasing with increasing levels of biochar. It was clear that the control with no biochar addition had the highest leachate N concentrations, and the highest biochar treatment (30%) usually had the lowest N levels. Likewise, this was reflected in the leachate N content values over

Table 8. Cumulative leachate^z N content^y (NO₃, NH₄, and Total N in mg) for the daylily^x outdoor study for the biochar treatment levels.

Biochar (%)	NO ₃	NH ₄	Total N
0	979.99a ^w	267.22a	1247.21a
10	730.70b	115.12b	845.82b
20	717.96bc	57.06c	775.02bc
30	635.29c	42.19c	677.48c
P value	<0.001	<0.001	<0.001

^zLeachate was collected (from irrigation and rainfall events), held in 3.8 L (1 gal) jugs, measured weekly for total volume using graduated cylinders, and a 50 ml subsample collected for leachate N analyses.

^yCumulative N content (NO₃, NH₄, and Total N = NO₃ + NH₄) in leachate was calculated by adding N contents across all sample dates.

^xDaylily (*Heemerocallis x 'EveryDaylily Cream PBR' L.*) plants were potted into 2.5 L (#1 trade gal) containers filled with pinebark:sand (6:1 v:v) media containing four levels of biochar (0, 10, 20, or 30 %), and amended with 6.9 g (0.015 lb) lime and 27 g (0.059 lb) fertilizer (16-5-10 Osmocote – 12-month release with micronutrients) per container.

^wWithin a column, means followed by the same letter are not significantly different ($p \leq 0.05$) according to the LSMeans statement under the Proc Mixed Procedure of SAS.

the course of the study such that cumulative leachate N losses (NO₃, NH₄, and total N) were all highest with no added biochar (control) and showed a pattern of decline as biochar level increased. Nitrogen lost via surface water runoff can contribute to eutrophication issues in the landscape. Further, excess NO₃ leaching to groundwater can have negative health effects for humans and livestock (Carpenter et al., 1998). The leachate content data clearly show that use of biochar reduced N loss and indicates that biochar can be used as a tool to mitigate N losses to the environment.

In conclusion, we found that biochar additions to a peat-based media had little effect on overall viola growth but growth inhibition in the PB-based media was noted for daylily. Given this was the first work with biochar use in a PB-based container system, we tested high biochar rates to identify a range of response for both growth and N losses from this system. Based on these results, future studies will focus on testing lower rates of biochar and impacts on growth and environmental responses. Further, since biochar is a highly variable product (based on feedstock material and pyrolysis methods), more extensive research is required to maximize environmental and agricultural benefits of using biochar in nursery production systems.

Results from both studies clearly showed that N in leachate was reduced by biochar additions, with higher biochar rates having greater effects on reducing N loss. In addition, decreased N₂O and CO₂ fluxes with increasing biochar rates was noted in the daylily study. Collectively, decreases in these trace gases could be beneficial in helping mitigate global climate change. Further, reductions in N loss from biochar use are significant since worldwide estimates of agricultural N use efficiency are low (~30-50%) with the excess being lost to gaseous efflux, leaching, and/or runoff. Difficulties in N management highlight the importance of adopting practices that increase N

retention (such as biochar additions) for the benefit of both agriculture and the environment.

Literature Cited

- Agegehu, G., A.K. Srivastava, and M.I. Bird. 2017. The role of biochar and biochar-compost in improving soil quality and crop performance: A review. *Appl. Soil Ecol.* 119:156-170. doi.org/10.1016/j.apsoil.2017.06.008.
- Alabama Cooperative Extension System (ACES). 2013. Economic impacts of Alabama's agricultural, forestry and related industries. Combined ANR-2012, ANR-2013, ANR-2014, ANR-2015, ANR-2016, ANR-2017, and ANR-2018. Alabama Cooperative Extension System, Auburn University, AL. <https://www.madeinalabama.com/assets/2013/01/ECON-IMPACTS-AG.pdf>. Accessed September 8, 2022.
- Álvarez, J.M., C. Pasian, R. Lal, R. López, M.J. Díaz, and M. Fernández. 2018. Morpho-physiological plant quality when biochar and vermicompost are used as growing media replacement in urban horticulture. *Urban For. And Urban Green.* 34:175-180. doi.org/10.1016/j.ufug.2018.06.021.
- Altland, J.E. 2021. The pour-through procedure for monitoring container substrate chemical properties: A review. *Horticulturae* 7:536. doi.org/10.3390/horticulturae7120536.
- Altland, J.E. and J.C. Locke. 2012. Biochar affects macronutrient leaching from a soilless substrate. *HortScience* 47:1136–1140. doi.org/10.21273/HORTSCI.47.8.1136.
- Altland, J.E. and J.C. Locke. 2013. Effect of biochar type on macronutrient retention and release from soilless substrate. *HortScience* 48:1397–1402. doi.org/10.21273/HORTSCI.48.11.1397.
- Baker J., G. Doyle, G. McCarthy, A. Mosier, T. Parkin, D. Reicosky, J. Smith, and R. Venterea. 2003. GRACEnet chamber-based trace gas flux measurement protocol. Trace Gas Protocol Development Committee, March 14, p. 1-18.
- Beck, D.A., G.R. Johnson, and G.A. Spolek. 2011. Amending green-roof soil with biochar to affect runoff water quantity and quality. *Environ. Pollut.* 159:2111-2118. doi.org/10.1016/j.envpol.2011.01.022.
- Bohm, W. 1979. *Methods of Studying Root Systems*, 188 p. New York, NY: Springer-Verlag.
- Bradley, A., R.A. Larson, and T. Runge. 2015. Effect of wood biochar in manure-applied sand columns on leachate quality. *J. Environ. Qual.* 44:1720-1728. doi:10.2134/jeq2015.04.0196.
- Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* 8:559–568. doi:10.1890/1051-0761(1998)008[0559:NPOSWW]2.0.CO;2.
- Clough, T.J. and L.M. Condron. 2010. Biochar and the nitrogen cycle: Introduction. *J. Environ. Qual.* 39:1218–1223. doi:10.2134/jeq2010.0204.
- Cole, C.V., J. Duxbury, J. Freney, O. Heinemeyer, K. Minami, A. Mosier, K. Paustian, N. Rosenburg, N. Sampson, D. Sauerbeck, and Q. Zhao. 1997. Global estimates of potential mitigation of greenhouse gas emissions by agriculture. *Nutr. Cycl. Agroecosyst.* 49:221-228.
- Conversa, G., A. Bonasia, C. Lazzizzera, and A. Elia. 2015. Influence of biochar, mycorrhizal inoculation, and fertilizer rate on growth and flowering of *Pelargonium (Pelargonium zonale L.)* plants. *Front. Plant Sci.* 6:429. doi:10.3389/fpls.2015.00429.
- Ding, Y., Y. Liu, S. Liu, X. Huang, Z. Li, X. Tan, G. Zeng, and L. Zhou. 2017. Potential benefits of biochar in agricultural soils: A review. *Pedosphere* 27:645-661. doi.org/10.1016/S1002-0160(17)60375-8.
- Glugokencky, E.J., R.C. Myers, P.M. Lang, K.A. Masarie, A.M. Crotwell, K.W. Thoning, B.D. Hall, J.W. Elkins, and L.P. Steele. 2005. Conversion of NOAA atmospheric dry air CH₄ mole fractions to a gravimetrically prepared standard scale. *J. Geophys. Res.* 110:18306.
- Dumroese, R.K., J. Heiskanen, K. Englund, and A. Terbahauta. 2011. Pelleted biochar: chemical and physical properties show potential use as a

substrate in container nurseries. *Biomass and Bioenergy* 35:2018-2027. doi:10.1016/j.biombioe.2011.01.053.

Florides, G.A. and P. Christodoulides. 2008. Global warming and carbon dioxide through sciences. *J. Environ. Int.* 35:390-401.

Graber, E.R., Y. Meller Harel, M. Kolton, E. Cytryn, A. Silber, D.R. David, L. Tsechansky, M. Borenshtein, and Y. Ylad. 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. *Plant Soil* 337:481-496. doi.org/10.1007/s11104-010-0544-6.

Hall, C.R., A.W. Hodges, H. Khachatryan, and M.A. Palma. 2018. Economic contributions of the green industry in the United States in 2018. *J. Environ. Hort.* 38:73-79.

Hutchinson, G.L. and A.R. Mosier. 1981. Improved soil cover method for field measurements of nitrous oxide fluxes. *Soil Sci. Soc. Am. J.* 45:311-316. doi.org/10.2136/sssaj1981.03615995004500020017x.

Hutchinson, G.L. and G.P. Livingston. 1993. Use of chamber systems to measure trace gas fluxes. p. 63-78 *In*: L.A. Harper, A.R. Moiser, J.M. Duxbury, D.E. Rolston (eds.). *Agricultural Ecosystem Effects on Trace Gas and Global Climate Change*. ASA Spec. Publ. 55 ASA, Madison WI.

IPCC. 2007. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, p. 987 *In*: M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden and C.E. Hanson (eds.). Cambridge University Press, Cambridge, UK.

Johnson, J.M., A.J. Franzleubbers, S.L. Weyers, and D.C. Reicosky. 2007. Agriculture opportunities to mitigate greenhouse gas emissions. *Environ. Pollut.* 150:107-124.

Kammann, C., S. Ratering, C. Eckhard, and C. Müller. 2012. Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. *J. Environ. Qual.* 41:1052-1066. doi:10.2134/jeq2011.0132.

Kovar, J.L. and G.M. Pierzynski. 2009. Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters; Southern Cooperative Series Bull. 408; Virginia Tech University: Blacksburg, VA, USA. <https://sera17dotorg.files.wordpress.com/2015/02/sera-17-methods-for-p-2009.pdf>. Accessed July 15, 2022.

Kroeze, C. and A.R. Mosier. 2000. New estimates for emissions of nitrous oxide. p. 45-64 *In* J.E.A. van Ham (Ed.). *Non-CO₂ Greenhouse Gases: Scientific Understanding, Control and Implementation*. Kluwer Academic Publishers, Netherlands.

Laird, D.A. 2008. The charcoal vision: a win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agron. J.* 100:178-181. doi.org/10.2134/agronj2007.0161

Lal, R. 2004. Soil carbon sequestration to mitigate climate change. *Geoderma* 123:1-22.

Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS System for Mixed Models. SAS Institute, Inc., Cary, NC.

Marble, S.C., S.A. Prior, G.B. Runion, H.A. Torbert, C.H. Gilliam, and G.B. Fain. 2011. The importance of determining carbon sequestration and greenhouse gas mitigation potential in ornamental horticulture. *HortScience* 46:240-244. doi.org/10.21273/HORTSCI.46.2.240

Marble, S.C., S.A. Prior, G.B. Runion, H.A. Torbert, C.H. Gilliam, G. B. Fain, J.L. Sibley, and P.R. Knight. 2012a. Determining trace gas efflux from container production of woody nursery crops. *J. Environ. Hort.* 30:118-124. doi.org/10.24266/0738-2898.30.3.118.

Marble, S.C., S.A. Prior, G.B. Runion, H.A. Torbert, C.H. Gilliam, G. B. Fain, J.L. Sibley, and P.R. Knight. 2012b. Effects of fertilizer placement on trace gas emissions from nursery container production. *HortScience* 47:1056-1062. doi.org/10.21273/HORTSCI.47.8.1056.

Marble, S.C., S.A. Prior, G.B. Runion, H.A. Torbert, C.H. Gilliam, G. B. Fain, J.L. Sibley, and P.R. Knight. 2016. Species and media effects on soil carbon dynamics in the landscape. *Scientific Reports*. 6:25210. doi:10.1038/srep25210.

Murphy, A.-M., G.B. Runion, S.A. Prior, H.A. Torbert, J.L. Sibley, and C. H. Gilliam. 2018. Greenhouse gas emissions from an ornamental crop as impacted by two best management practices: Irrigation delivery and fertilizer placement. *J. Environ. Hort.* 36:58-65. doi.org/10.24266/0738-2898-36.2.58.

Murphy, A.-M., G.B. Runion, S.A. Prior, H.A. Torbert, J.L. Sibley, G. B. Fain, and J.M. Pickens. 2019. Effects of fertilizer placement on greenhouse gas emissions from a sun and shade grown ornamental crop. *J. Environ. Hort.* 37:74-80. doi.org/10.24266/0738-2898-37.3.74.

Murphy, A.-M., G.B. Runion, S.A. Prior, H.A. Torbert, J.L. Sibley, G. B. Fain, and J.M. Pickens. 2021. Effects of growth substrate on greenhouse gas emissions from three annual species. *J. Environ. Hort.* 39:53-61. doi.org/10.24266/0738-2898-39.2.53.

Nemati, M.R., F. Simard, J.-P. Fortin, and J. Beaudoin. 2014. Potential use of biochar in growing media. *Vadose Zone J.* 14(6):1-8. doi:10.2136/vzj2014.06.0074.

Nguyen, T.T.N., C.-Y. Xu, I. Tahmasbian, R. Che, Z. Xu, X. Zhou, H. M. Wallace, and S.H. Bai. 2017. Effects of biochar on soil available inorganic nitrogen: A review and meta-analysis. *Geoderma* 288:79-96. doi.org/10.1016/j.geoderma.2016.11.004.

Parkin, T.B. and R.T. Venterea. 2010. Sampling Protocols. Chapter 3. Chamber-based trace gas flux measurements, p. 3-1-3 to 39 *In*: R.F. Follet (ed.). *Sampling Protocols*. <https://www.ars.usda.gov/ARSUserFiles/np212/chapter%203.gracenet%20Trace%20Gas%20Sampling%20protocols.pdf>. Accessed August 9, 2022.

Parkin, T.B. and T.C. Kaspar. 2006. Nitrous oxide emissions from corn-soybean systems in the Midwest. *J. Environ. Qual.* 35:1496-1506. doi: 10.2134/jeq2005.0183.

Paustian, K., J. Six, E.T. Elliot, and H.W. Hunt. 2000. Management options for reducing carbon dioxide emissions from agricultural soils. *Biogeochem. J.* 48:147-163.

Prinn, R.G., R.F. Weiss, P.J. Fraser, P.G. Simmonds, D.M. Cunnold, F. N. Alyea, S. O'Doherty, P. Salameh, B.R. Miller, J. Huang, R.H.J. Wang, D.E. Hartley, C. Harth, L.P. Steele, G. Sturrock, P.M. Midgely, and A. McCulloch. 2000. A history of chemically and radiatively important gases in air deduced from ALE/GAGE/AGAGE. *J. Geophys. Res.* 105:17751-17792.

Prior, S.A., H.H. Rogers, G.L. Mullins, and G.B. Runion. 2003. The effects of elevated atmospheric CO₂ and soil P placement on cotton root deployment. *Plant Soil* 255:179-187. doi.org/10.1023/A:1026143410238.

Prior, S.A., G.B. Runion, S.C. Marble, H.H. Rogers, C.H. Gilliam, and H.A. Torbert. 2011. A review of elevated atmospheric CO₂ effects on plant growth and water relations: Implications for horticulture. *HortScience* 46:158-162. doi.org/10.21273/HORTSCI.46.2.158.

Robertson, G.P., E.A. Paul, and R.R. Harwood. 2000. Greenhouse gases in intensive agriculture: Contributions of individual gases to the radiative forcing of the atmosphere. *Science* 289:1922-1925.

Smith, K.A., I.P. McTaggart, and H. Tsuruta. 2007. Emissions of N₂O and NO associated with nitrogen fertilization in intensive agriculture and the potential for mitigation. *Soil Use Manag.* 13:296-304.

Spokas, K.A., K.B. Cantrell, J.M. Novak, D.W. Archer, J.A. Ippolito, H.P. Collins, A.A. Boateng, I.M. Lima, M.C. Lamb, A.J. McAloon, R.D. Lentz and, K.A. Nichols. 2012. Biochar: a synthesis of its agronomic impact beyond carbon sequestration. *J Environ Qual.* 41:973-89. doi: 10.2134/jeq2011.0069.

USDA. 2007. U.S. Nursery crops 2006 summary. Publ. No. Sp Cr 6-3. U.S. Department of Agriculture, National Agriculture Statistics Service.

Wright, R.D. 1986. The pour-through nutrient extraction procedure. *HortScience* 21:227-229.

Wu, D., Y. Feng, L. Xue, M. Liu, B. Yang, F. Hu, and L. Yang. 2019. Biochar combined with vermicompost increases crop production while reducing ammonia and nitrous oxide emissions from a paddy soil. *Pedosphere*. 29:82-94. doi:10.1016/S1002-0160(18)60050-5.