Research Article

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Effects of biochar and compost addition in potting substrates on growth and volatile compounds profile of basil (*Ocimum basilicum* L.)

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Abstract

BACKGROUND: Despite the optimal characteristics of peat, more environmental-friendly materials are needed in the nursery sector, although these must guarantee specific quantitative and qualitative commercial standards. In the present study, we evaluated the influence of biochar and compost as peat surrogates on yield and essential oil profile of two different varieties of basil (*Ocimum basilicum* var. Italiano and *Ocimum basilicum* var. minimum). In two 50-day pot experiments, we checked the performances of biochar from pruning of urban trees and composted kitchen scraps, both mixed in different proportions with commercial peat (first experiment), and under different nitrogen (N) fertilization regimes (second experiment), in terms of plant growth and volatile compounds profile of basil.

RESULTS: Total or high substitution of peat with biochar (100% and 50% v.v.) or compost (100%) resulted in seedling death a few days from transplantation, probably because the pH and electrical conductivity of the growing media were too high. Substrates with lower substitution rates (10–20%) were underperforming in terms of plant growth and color compared to pure commercial peat during the first experiment, whereas better performances were obtained by the nitrogen-fertilized mixed substrates in the second experiment, at least for one variety. We identified a total of 12 and 16 aroma compounds of basil (mainly terpenes) in the two experiments. Partial replacement of peat did not affect basil volatile organic compounds content and composition, whereas N fertilization overall decreased the concentration of these compounds.

CONCLUSION: Our results support a moderate use of charred or composted materials as peat surrogates.

Supporting information may be found in the online version of this article.

Keywords: pyrogenic carbon; essential oils; pot experiment; sustainability; growing media

INTRODUCTION

Peat is the standard growing medium in nurseries and greenhouses, and a major component of potting mixtures for commercial plants production. However, environmental issues and economic reasons require switching the demand for peat to other substrates. Indeed, many European countries have agreed to reduce peat extraction and to preserve and restore peatlands,[1] which are fragile ecosystems with very important ecological and social values.[2] Furthermore, the limitation to peat extraction implies increasing prices.[3]

Biochar and compost are under investigation as alternative materials in growing media[4,5] because they have some positive characteristics in common with peat and usually derive from waste, thus favouring a circular economy approach.[4,6-8]

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Biochar is the by-product of pyrolysis (i.e. heating at high temperatures under low oxygen conditions) of a wide range of organic feedstock and is increasingly used as soil amendment.[9] Biochar's properties are both inherited from the original feedstock and acquired during pyrolysis. Therefore, each biochar shows peculiar characteristics[10] and the effects of its addition in substrates may vary much. Generally, such an addition is positive in terms of stability, porosity and water retention of the substrate.[11]

Compost comprises any organic material that has undergone thermophilic and aerobic decomposition, a process called 'composting'. Compost is largely used in horticulture and there are several studies regarding its use as growing medium, partly or fully replacing peat.[12-15] It is a cheap material that shows valuable intrinsic characteristics, such as high porosity, nutrient availability and water retention.[16] These characteristics strongly depend on both parent materials and the type of composting process.[17] Compost can also contain biochar, which has several positive effects on the composting process, such as increased aeration, reduced ammonia volatilization, pathogen inactivation and improved humification.[11,18-20] Research aiming to determine the suitability of biochar and/or compost as components of growing media, however, has provided contrasting results and the relative amounts of these materials to use must be carefully evaluated for each species, aiming to avoid negative impacts on growth and productivity.[21-23]

Sweet basil (*Ocimum basilicum* L.) is an annual aromatic plant, cultivated worldwide as an appreciated culinary herb and widely used in the food processing industry. Its high content of phytochemicals with potential beneficial health effects has further increased its demand. The essential oil extracted from basil leaves and flowers is used as a flavouring agent in foods, perfumes, cosmetics and medicines,[24] indeed. Despite its widespread cultivation in nursery, only a few studies deal with the possibility of using biochar and/or compost in basil substrates and even less look at the effects of this approach on product quality. Yet, the type of substrate has been reported to influence many basil traits, such as growth rate, color and chemical composition. Plants with deep green leaves are in demand for the fresh market, which requires paying close attention to their chlorophyll content.[25] Also pivotal for basil commercialization is the content of total phenolic compounds, carotenoids and especially essential oils.[26] The latter have antifungal and antibacterial properties and were shown to be effective against some plant pathogens.[27-29] Sweet basil contains various classes of essential oils, such as mono and sesquiterpene hydrocarbons, oxygenated mono and sesquiterpenes, aliphatic alcohols, aldehydes, esters, ketones, acids and aromatic compounds. Major aromatic compounds in basil are linalool, estragole, methyl cinnamate, eugenol and cineole.[30,31] The essential oil profile is quite distinctive of the different varieties of basil and there are various 'chemotypes', basically defined by their set of volatile organic compounds (VOCs), such as methyl chavicol, linalool or methyl eugenol.[32-34]

In the present study, we deal with two of the most widely cultivated varieties of basil, belonging to two different chemotypes, 'Italiano' (*Ocimum basilicum* L., var. Italiano) and 'Greco' (*Ocimum basilicum* L., var. minimum)used for the preparation of Italian pesto sauce and for ornamental purposes, respectively. In the Italiano basil the dominant VOCs are eugenol, methyleugenol, eucalyptol (cineole) and linalool.[35] Linalool and eugenol give the basil its peculiar taste and, together with cineole, have long been studied because they are appreciated in both the kitchen and pharmacy.[36] Greco basil, also known as 'fine-leaved' basil because of its small-tight leaves, has a slightly sweeter flavour than Italiano basil and is usually cultivated as ornamental plant in pots and gardens; its principal essential oils are linalool and methyl (E) cinnamate.[37]

The present study aims to provide insight into the possible use of biochar and compost as partial substitutes for peat in basil cultivation. Our hypothesis is that, within certain proportions, both can support the growth of basil without hampering its productivity and/or essential oils profile. For this purpose, we designed a 2-year experiment on the Italiano and Greco varieties, in which we checked the effects of different doses of biochar and compost in the growing substrates, as well as different fertilization regimes, on biomass (leaves and stems), total leaf area and color, and essential oil composition.

MATERIALS AND METHODS

Substrates preparation and characterization

For both trials, we chose commercially available biochar and compost made in the closest location to our experimental set-up. Biochar was produced in a syngas plant at 750 °C from woody residues of the pruning of urban trees by the manufacturer Econsulenze SAS (Terni, Italy). Its characteristics were declared by the producer (see Supporting information, Table S1) and account for the quality class I 'EBC-Feed', according to the European Biochar Certificate.[9] Compost was produced by All Power Labs – SLO Factory (Terni, Italy) from a mix of organic wastes. In detail, the compost parent material was: 25% kitchen green waste; 48% sawdust, wood flakes and chips; 15% exhausted coffee powder; 5% abovementioned biochar; 1.5% forest topsoil; 0.5% cane sugar; and 5% water. The mixture was prepared thanks to an insulated tumbler rotating within a barrel, designed by the company. The composting lasted 1 month, during which temperature and humidity were checked daily. Later, compost was stored for at least 3 weeks at room temperature before being used for the experiments.

We made different growing media adding different amounts of biochar and compost to a commercial peat-based medium (hereafter simply called peat), a mix of Irish and Baltic *sphagnum*, coconut fiber and bark humus ('Cuore di Terriccio', by Vigorplant Italia SRL). In the first experiment, we tested six substrates for growing Italiano basil (percentages are on a volume basis): 25% biochar/75% peat (hereafter called Char 25); 25% compost/75% peat (Comp. 25); 50% biochar/50% peat (Char 50); 50% compost/50% peat (Comp. 50); 100% biochar (Char 100); and 100% compost (Comp. 100) (Table 1). We prepared 30 pots of 300 mL for each of these seven substrates, including the control, comprising 210 pots in total. For the second trial, we focused on lower doses of biochar with or without nitrogen fertilization (N), based on the most interesting results obtained from the first trial. The following growing media were used for growing basil Italiano and Greco varieties: 100% peat (Peat and Peat N); 20% biochar/80% peat ('Char 20' and "Char 20 N"); and 10% biochar/90% peat ('Char 10' and "Char 10 N"). We prepared 30 pots for each of these six substrates, comprising 180 pots in total (i.e. 90 pots per variety).

Available inorganic nitrogen in the unmixed peat, biochar and compost was extracted with 1 M KCl and measured by inductively coupled plasma-optical emission spectroscopy. The pH and electrical conductivity (EC) of peat, biochar, compost and their mixtures were measured in a suspension in distilled water (1:2.5) with a XS pH-meter model PC8. The bulk density was measured by drying the substrates from the pots at 70 °C until constant weight and then dividing their dry weight by the known volume.

Table 1. Nomenclature and physico-chemical characteristics of the potting substrates used in the two experiments (EC, electrical conductivity; WHC, water holding capacity)

Substrates	Composition (in volume)	pH	EC mS cm^{-1}	Bulk density g cm^{-3}	WHC%	NH_4^+ mg kg^{-1}	NO_3^- mg kg^{-1}
Peat	100% peat	6.4 ± 0.0 f	1.65 ± 0.04 g	0.20 ± 0.00 e	79 ± 4 bc	32.3	778
Char 100	100% biochar	10.3 ± 0.2 a	11.88 ± 0.02 a	0.31 ± 0.01 ab	53 ± 2 d	0.8	7.1
Char 50	50% biochar/50% peat	9.4 ± 0.2 b	3.48 ± 0.04 c	0.27 ± 0.00 c	76 ± 1 c	–	–
Char 25	25% biochar/75% peat	8.4 ± 0.3 c	2.09 ± 0.07 f	0.23 ± 0.01 d	75 ± 1 c	–	–
Comp. 100	100% compost	8.5 ± 0. 4c	5.49 ± 0.10 b	0.32 ± 0.01 a	86 ± 4 ab	25.7	55.4
Comp. 50	50% compost/50% peat	7.6 ± 0.2 d	3.17 ± 0.01 d	0.29 ± 0.01 b	90 ± 4 abc	–	–
Comp. 25	25% compost/75% peat	7.1 ± 0.1 e	2.35 ± 0.01 e	0.24 ± 0.02 d	82 ± 3 bc	–	–
Char 20	20% biochar/80% peat	8.1 ± 0.3 c	1.95 ± 0.5 f	0.22 ± 0.01 d	76 ± 2 c	–	–
Char 10	10% biochar/90% peat	7.6 ± 0.2 d	1.80 ± 0.4 g	0.20 ± 0.01 e	78 ± 2 bc	–	–

On pure substrates [i.e. Peat (control), Char 100, and Comp 100], available inorganic nitrogen (NH_4^+ and NO_3^-) was also measured.
Values are the mean ± SD of three replicates. Different lowercase letters indicate significant differences between values in the same column, according to Tukey's test ($P < 0.05$).

The water holding capacity (WHC) of the substrates was determined according to de la Rosa *et al.*[10] Briefly, 6 g of each sample, dried at 50 °C in a stove, was placed on a Whatman No. 2 filter (Cytivia, Marlborough, MA, USA) into a funnel, saturated with distilled water and left 12 h to lose water through the filter; then, the weight of the moist sample was measured and the weight difference between dry and moist sample calculated. This difference relative to the dry weight of the sample was assumed as the maximum WHC.

Experimental design and growth conditions

The experimental design of both trials was based on a randomized block scheme and consisted of three replicated blocks, each comprising 10 pots per substrate (210 pots in total, three plants per pots in the first trial; 180 pots in total, three plants per pots in the second trial). Each replicate was placed in a saucer (20 × 50 cm) and the saucers were arranged on a bench equipped with a transparent polyethylene roof, with a density of 70 pots m^{-2}. The seedlings were purchased from Cooperativa Agricola di Legnaia (Firenze, Italy) and transplanted in the substrates 2 days after, when the plants were approximately 2 cm high.

The trials were performed outdoor at the School of Agriculture of the University of Firenze. Both started on mid-June and ended at the end of July, in 2019 and 2020. During the first trial, the lowest temperature recorded was 16.9 °C and the highest 33.2 °C, with a mean of 24.7 °C, whereas, in the second trial, the same temperatures were 15.5, 32.6 and 25.7 °C, respectively. The seedlings of Italiano basil in the first trial and Italiano and Greco in the second one were irrigated every 3 days all trial long to 100% WHC. In the first experiment, a commercial NPK 20–20-20 fertilizer (Grow More, Gardena, CA, USA) was applied on two occasions, 10 and 30 days after seedling transplanting, for a total of 120 mg of nitrogen, phosphorus and potassium each pot. In the second experiment, the fertilized pots received a total of 250 mg N, in the form of ammonium nitrate, distributed equally on five occasions. Three weeks after the beginning of the experiments, plants were treated with an imidacloprid-based insecticide against cutworms.

Growth and biomass measurements

Starting on the seventh day, the following traits were measured on the tallest plant per pot: height, Soil Plant Analysis Development (SPAD) index with a leaf chlorophyll meter SPAD-502 Minolta, and three colorimetric variables, L^*, a^* and b^*, on one completely formed leaf by a portable colorimeter Minolta Chroma Meter CR-100 (Konica Minolta, Chiyoda, Japan). Such variables account for the lightness (L^*, 0 = absolute black, 100 = absolute white), the position on the red–green axis (a^*, −60 for green, +60 for red) and that on the yellow–blue axis (b^*, −60 for blue, +60 for yellow), respectively.

Fifty days after transplant, the plants were harvested by collecting all the aboveground biomass. The youngest four completely formed leaves of the dominant plant were weighted and stored at −80 °C for quantitative and qualitative analysis of essential oils. Immediately after harvesting the total leaf area (nine plants per variety and substrate) was measured by scanning all leaves of each dominant plant with a LI-COR LI-3100 Area Meter (LI-COR Biosciences, Lincoln, NE, USA); the fresh biomass, leaves plus stems, was weighed and then oven-dried at 105 °C to constant weight. Specific leaf area (SLA) ($cm^2\ g^{-1}$) was calculated dividing the leaf area by the leaf dry weight. Leaf dry matter content (LDMC) and leaf area ratio (LAR) were also calculated. LDMC is the ratio of the oven-dry mass of leaves to their fresh mass (g dry mass g^{-1} fresh mass), whereas LAR is the ratio of the total leaf area to the whole dry plant biomass ($cm^2\ g^{-1}$), which accounts for the size of the photosynthetic surface relative to the respiratory mass.[38]

VOC analyses

VOCs were extracted from 0.5 g of the last four completely formed leaves, previously stored at −80 °C, using 1 mL of heptane as a solvent and tridecane (20 ppm) as an internal standard, vortexed for 5 min, sonicated for 15 min and then agitated overnight. After centrifugation at 1800 × *g* for 10 min, the heptane phase was collected for the gas chromatography mass spectrometry (GC-MS) analysis.

The GC-MS analysis was performed with an Agilent 7820 Gas Chromatograph system equipped with a 5977E MSD with EI ionization (Agilent Technologies, Palo Alto, CA, USA). One microliter of heptane phase was injected in a split/splitless injector operating in splitless mode. A MPS2 XL autosampler (Gerstel, Mülheim, Germany) equipped with liquid option was used. The chromatographic settings were: injector in splitless mode set at 260 °C

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(1 min), J&W HP Innowax column (Agilent Technologies) (30 m, 0.25 mm inner diameter, 0.5 μm film thickness); oven temperature program: initial temperature 40 °C for 1 min, then 5 °C min^{-1} until 200 °C, 10 °C min^{-1} until 220 °C, 30 °C min^{-1} until 260 °C, hold time 3 min. The mass spectrometer was operating with an electron ionization of 70 eV, in scan mode in the range *m/z* 29–330, at three scans per second.

The deconvoluted peak spectra, obtained using Masshunter software (Agilent), were matched against the NIST 11 spectral library (National Institute of Standards and Technology, Gaithersburg, MD, USA) for tentative identification. Kovats' retention indices were calculated for further compound confirmation and compared with those reported in literature for the chromatographic column used. The Kovats retention index of a compound is its retention time normalized to the retention times of adjacently eluting *n*-alkanes. To determine the content of each single VOC, a calibration curve was built injecting known concentrations of authentic standards (purchased from Sigma-Aldrich, St Louis, MO, USA) into the gas chromatograph-mass spectrometer. VOC content was expressed as mg g^{-1} dry weight. The relative content of each monoterpene was expressed as percentage of total monoterpenes, whereas the relative content of alcohols, monocyclic sesquiterpenes and phenylpropanoids was expressed as a percentage of total compounds (VOCs), assuming the latter as the sum of all identified compounds. The absolute amount of extracted VOCs was expressed in mg per plant, thus taking into account the biomass of the basil plants leaves (dry mass).

Statistical analysis

Normality of data and homogeneity of variance were checked with the Shapiro–Wilks test and Levene's test, respectively. Then, data from the first experiment underwent one-way analysis of variance (ANOVA) according to a completely randomized block design with three blocks and 10 plants per treatment in each block. In the second experiment, the design implied three blocks and 15 plants per treatment and the data were analyzed by two-way ANOVA, using N fertilization (done or not) and biochar doses as fixed factors. If there was a significant interaction effect between the two variables, a simple main effect analysis of biochar dose within fertilization treatments was conducted (i.e. the biochar dose effect was evaluated separately on fertilized and unfertilized substrates). Otherwise, the single effect of the two fixed variables was investigated. Significant differences between means for multiple comparisons were determined by Tukey's post-hoc significance test at $P < 0.05$. All the statistical analyses were performed using SPSS, version 27 (IBM Corp., Armonk, NY, USA).

RESULTS

Chemical and physical characteristics of substrates

Biochar and compost addition significantly changed the characteristics of the peat-based substrate (Table 1). Both added materials increased its pH and ECs, biochar significantly more so than compost. Compost addition implied higher WHC, opposite to biochar. The mixed substrates had BD ranging from 0.23 to 0.32 g cm^{-3}, hence being significantly higher than pure peat.

Seedling survival, growth and quality

In the first trial, all seedlings in Char 100 already showed total necrosis of leaves and stems the day after transplanting. Six days later, signs of necrosis appeared on top of all the seedlings and the youngest leaves in Comp 100 and Char 50; on day 10, necrosis affected the whole seedlings of those treatments. No other plants revealed such symptoms or other diseases throughout the trial.

No substrate allowed basil growth comparable to that on pure peat (Table 2). In general, biochar- and compost-including substrates did not show significant differences between each other in terms of total fresh aboveground biomass (FW), leaf fresh weight (LFW) and total leaf area (LA). However, plants grown on Char 25 were taller than those grown on compost mixes and showed values of percentage of fresh leaves on total fresh weight (PLW), SLA and LAR comparable to those grown on pure peat (Table 2). Plants grown on compost-bearing substrates were the smallest but showed the highest SLA and LAR.

The negative effect of biochar and compost on basil growth and quality was confirmed by SPAD and lightness (L^*) readings, which in mixed substrates differed significantly from pure peat (Table 2). Some differences between biochar- and compost-bearing substrates were also observed. Plants grown on Char 25 were commercially worse in terms of colour than those grown on Comp 25 because they had lower SPAD and greenness (a^*) and higher yellowness (b^*).

In the second trial, no necrosis was observed on top of the seedlings or the young leaves, in both Italiano and Greco varieties. In general, N addition had a significant impact on the investigated variables in all the substrates (Table 3). Indeed, the plants not only grew better, but also showed higher SPAD and a^* and lower L^* and b^*. The two types of basil, however, showed different responses with regards to biochar substitution doses and the interaction of the latter with fertilization. The Italiano variety showed a significant interaction effect between fertilization and biochar dose for some quantitative variables and for the b^* value (Table 3). N fertilization and the lowest dose of biochar (Char 10) resulted in the highest values of FW, LFW and LA with respect to Char 20 substrate, although b^* was negatively affected compared to both peat and the highest biochar dose (Char 20). The Greco variety showed the worst performances in terms of plants height when the dose of biochar was increased from 10% to 20%, whereas even the lowest dose of added biochar negatively affected FW and PLW compared to pure peat (Table 3). An opposite interaction effect between fertilization and biochar dose was found for SLA and LDMC; indeed, biochar addition at the highest dose had a positive impact on SLA, albeit just upon N fertilization, whereas it increased the LDMC at any dose in the absence of N fertilization. Finally, biochar decreased SPAD and a^* values and increased b^* proportionally to the applied dose, which, overall, means a reduction in basil quality.

VOCs

In the first trial, we clearly identified ten different monoterpenes, one alcohol (1-octen-3-ol) and one phenylpropanoid (eugenol), whereas other compounds (i.e. camphor, α-humulene and methyleugenol) were detected in trace amounts and therefore are not reported here (Table 4). Eugenol was the dominant VOC in all treatments, followed by linalool and cineole (Table 4; see also Supporting information, Table S2). The total concentration of each individual VOC did not show any significant difference between treatments. Conversely, the absolute amount of VOCs, calculated taking into account the dry mass of leaves, was significantly higher in plants obtained from peat than from the other substrates, with the latter being comparable to each other (Fig. 1).

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Table 2. Final height (H), total fresh weight (FW), leaf fresh weight (LFW), percentage of fresh leaves weight on total fresh weight (PLW), total leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), leaf area ratio (LAR), SPAD index and color values (a^*, b^* and L^*) of basil plants grown on peat (control) or substrate mixtures of peat and biochar (25%) and compost (50 and 25%) in the first experiment

Substrates[a]	H	FW	LFW	PLW	LA	SLA	LDMC	LAR	SPAD	Color values		
	cm	g	g	%	cm^2	$cm^2\ g^{-1}$	$g\ g^{-1}$	$cm^2\ g^{-1}$	–	a^*	b^*	L^*
Peat	43.9 ± 6.0 a	18.4 ± 4.9 a	11.3 ± 2.8 a	61.9 ± 2.2 b	488.1 ± 113.4 a	482.3 ± 36.2 b	0.09 ± 0.0 1a	240.8 ± 19.3 b	30.8 ± 1.6 a	2.5 ± 2.3 a	25.0 ± 3.1 b	46.0 ± 1.8 b
Char 25	35.6 ± 5.0 b	13.3 ± 3.9 b	8.5 ± 2.3 b	64.3 ± 4.1 ab	328.6 ± 89.4 b	505.7 ± 57.5 b	0.08 ± 0.01 b	256.7 ± 35.6 b	27.7 ± 2.1 c	−2.4 ± 4.1 c	28.6 ± 3.1 a	49.4 ± 2.3 a
Comp. 50	26.8 ± 3.3 d	10.6 ± 1.4 b	7.0 ± 0.9 b	66.0 ± 1.7 a	270.4 ± 32.2 b	563.0 ± 15.7 a	0.07 ± 0.00 c	308.4 ± 19.0 a	28.5 ± 1.6 bc	1.8 ± 2.7 bc	26.2 ± 1.8 b	48.9 ± 2.4 a
Comp. 25	29.9 ± 4.0 c	11.0 ± 1.2 b	7.2 ± 0.8 b	65.5 ± 1.6 a	292.1 ± 33.9 b	587.2 ± 38.2 a	0.07 ± 0.01 bc	318.3 ± 26.7 a	29.1 ± 1.8 b	−0.6 ± 2.4 b	26.1 ± 2.0 b	48.6 ± 2.4 a

Values are the mean ± SE of 30 replicates for H, SPAD and color values and 9 replicates for the other variables. Different lowercase letters indicate significant differences between values in the same column, according to Tukey's test ($P < 0.05$).
[a] Plants grown on Char 100, Char 50 and Comp. 100 substrates were not analyzed because of plants death or generalized necrosis during the trial.

The VOCs extracted and identified in the second trial in the Italiano basil slightly differed from those found in the same variety in the first trial, in terms of both number and type of compounds. The compounds found in both varieties were: ten monoterpenes, one monocyclic sesquiterpene (humulene) and two phenylpropanoids (eugenol and methyl eugenol) (Table 5). Consistent with the results of the first experiment, biochar substitution at the low doses (10% and 20%) did not affect total concentration of VOCs, whereas N fertilization caused a significant and generalized decrease in the concentration of all isolated compounds but camphor in the Italiano leaves. The lowest dose of biochar (10%) resulted in the lowest differences between the N-treated and untreated plants regarding the concentration of all isolated compounds (with a decrease of 34%, 55% and 48% in Char 10, Char 20 and Peat substrates, respectively). By contrast, the total amount of extracted VOCs was not affected neither by biochar addition, nor by N fertilization (Fig. 2). In all treatments, linalool was the dominant VOC, followed by cineole and eugenol (Table 5; see also Supporting information, Table S3). Biochar and N showed an interaction effect on two of the main compounds detected, linalool and eugenol, because their concentration was significantly lowered by biochar at 20% compared to peat, albeit just upon N addition.

In the Greco basil, eugenol and methyleugenol were the dominant VOCs in all treatments, followed by cineole and linalool (Table 5; see also Supporting information, Table S3). Biochar and N fertilization showed an interaction effect on most of the extracted compounds. In the unfertilized treatments, biochar application resulted in a significant increase of VOC's concentration, except for methyleugenol, which did not change compared to peat substrate. Conversely, N fertilization affected all extractable VOCs, but with different effects on the biochar-bearing substrates compared to pure peat. In the substrates with biochar, N addition caused a general decrease in VOC concentration in the basil leaves (41% and 44% in Char 10 and Char 20, respectively). Biochar and N showed a negative interaction effect ($P = 0.36$) also on the total amount of VOCs (Fig. 2). By contrast, in the plants grown on pure peat, N addition caused just a slight (3%) decrease in the total concentration of VOCs, but an increase of the absolute amount of extracted VOCs as N fertilization promoted the growing of basil leaf biomass.

DISCUSSION

Chemical and physical characteristics of substrates

Apart from peat, none of the experimental substrates fell within the optimal pH range for most vegetable seedlings (i.e. 5.8–6.8),[39] nor within the optimal pH for basil's substrate (i.e. 5.5–6.0), whereas pH values beyond 7.0 are increasingly inhibitory to plant growth[40] (Table 1). Concerning EC, which refers to the soluble salts concentration, only pure peat showed a value in the range 0.5–1.6 mS cm^{-1} defined as optimal for basil seedlings,[41] although other studies report that ECs up to 4.0 mS cm^{-1} do not depress significantly basil growth.[42-46] Biochar-based substrates, which have been often reported to decrease plant growth because of the osmotic stress caused by the high EC,[47] in our trials showed overall high but not extreme EC values, except only for pure biochar. The latter also showed very low WHC, in contrast to other studies, such as by Nieto *et al.*,[43] which could be a result of the (transient) hydrophobic nature of fresh biochar.[11]

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Table 3. Final height (H), total fresh weight (FW), leaf fresh weight (LFW), percentage of fresh leaves weight on total fresh weight (PLW), total leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), leaf area ratio (LAR), SPAD index and color values (*a**, *b** and *L**) of 'Italiano' and 'Greco' varieties grown on peat (control) or mixtures of peat and biochar (20% and 10%), with and without N fertilization in the second experiment

	H	FW	LFW	PLW	LA	SLA	LDMC	LAR	SPAD	Color values		
Substrates	cm	g	g	%	cm^2	$cm^2\ g^{-1}$	$g\ g^{-1}$	$cm^2\ g^{-1}$	–	*a**	*b**	*L**
'Italiano'												
Peat	26.6 ± 5.3	17.9 ± 4.8 a	11.9 ± 3.6 a	65.9 ± 2.9 a	335.5 ± 88.9 a	326.3 ± 19.6	0.09 ± 0.00	190.4 ± 11.7	23.8 ± 3.3	−11.8 ± 7.0	44.7 ± 8.5a	66.0 ± 11.0
Peat N	31.3 ± 4.6	32.0 ± 8.4 A	22.0 ± 5.3 A	69.1 ± 2.0	599.0 ± 138.6 A	338.4 ± 30.9	0.08 ± 0.01	227.6 ± 19.7	33.5 ± 4.1	1.9 ± 3.5	21.3 ± 5.1A	53.6 ± 7.8
Char 20	25.4 ± 3.3	14.3 ± 3.5 a	9.6 ± 2.5 a	67.0 ± 1.7 a	266.1 ± 58.9 a	319.9 ± 19.5	0.09 ± 0.01	189.1 ± 14.0	21.7 ± 2.3	−14.3 ± 7.4	42.1 ± 7.2a	71.2 ± 3.8
Char 20 N	30.2 ± 4.1	24.1 ± 6.5 B	16.5 ± 4.5 B	68.2 ± 1.6	447.6 ± 108.3 B	347.0 ± 21.5	0.08 ± 0.00	222.3 ± 14.1	32.4 ± 2.4	−2.2 ± 2.6	26.7 ± 1.9A	61.0 ± 2.1
Char 10	25.3 ± 3.2	13.9 ± 6.1 a	9.2 ± 4.4 a	64.1 ± 5.3 a	260.6 ± 111.6 a	347.6 ± 33.1	0.08 ± 0.01	194.9 ± 13.3	25.4 ± 2.3	−11.3 ± 5.8	40.0 ± 5.7a	68.9 ± 4.1
Char 10 N	30.8 ± 4.6	37.0 ± 10.5 A	24.9 ± 7.5 A	67.0 ± 1.9	701.9 ± 204.2 A	351.4 ± 23.2	0.08 ± 0.01	225.4 ± 30.7	35.5 ± 1.8	−3.1 ± 7.0	29.4 ± 5.1B	62.1 ± 3.1
Statistics[a]												
Biochar dose	$P = 0.526$	$\mathbf{P = 0.017}$	$\mathbf{P = 0.027}$	$P = 0.069$	$\mathbf{P = 0.009}$	$P = 0.085$	$P = 0.668$	$P = 0.758$	$\mathbf{P < 0.001}$	$P = 0.245$	$P = 0.655$	$\mathbf{P = 0.006}$
Fertilization	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P = 0.004}$	$\mathbf{P < 0.001}$	$\mathbf{P = 0.042}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$
Biochar dose* Fertilization	$P = 0.925$	$\mathbf{P = 0.021}$	$\mathbf{P = 0.029}$	$P = 0.541$	$\mathbf{P = 0.011}$	$P = 0.384$	$P = 0.490$	$P = 0.490$	$P = 0.860$	$P = 0.355$	$\mathbf{P = 0.008}$	$P = 0.390$
'Greco'												
Peat	23.1 ± 2.6	16.5 ± 5.7	8.3 ± 3.0	49.6 ± 4.1	265.4 ± 88.9	420.1 ± 35.9 a	0.08 ± 0.01 a	185.4 ± 30.8	24.4 ± 3.0	−8.7 ± 3.9	38.5 ± 5.8	71.4 ± 3.8
Peat N	24.4 ± 2.1	21.1 ± 5.8	10.8 ± 2.8	51.8 ± 4.7	379.9 ± 109.3	444.3 ± 46.2 A	0.08 ± 0.01 A	239.7 ± 39.4	33.2 ± 2.9	3.5 ± 4.2	26.4 ± 5.0	59.5 ± 4.0
Char 20	20.1 ± 2.1	11.4 ± 2.4	6.3 ± 1.3	55.5 ± 3.5	197.8 ± 53.7	368.8 ± 35.2 b	0.08 ± 0.00 b	180.0 ± 18.6	22.4 ± 4.1	−13.1 ± 4.8	42.5 ± 6.3	73.8 ± 5.7
Char 20 N	21.4 ± 1.8	17.9 ± 4.2	10.1 ± 2.3	56.4 ± 2.4	331.0 ± 82.5	493.7 ± 59.2 B	0.07 ± 0.01 B	255.0 ± 24.9	29.9 ± 3.2	−4.4 ± 3.2	32.8 ± 3.5	65.1 ± 2.3
Char 10	22.5 ± 2.3	11.8 ± 3.4	6.5 ± 1.9	54.7 ± 3.5	222.0 ± 68.4	388.1 ± 46.2 ab	0.09 ± 0.01 b	183.3 ± 25.3	25.4 ± 2.7	−7.1 ± 4.3	35.2 ± 5.4	63.2 ± 9.3
Char 10 N	22.9 ± 1.5	16.4 ± 2.1	9.5 ± 1.2	58.3 ± 3.0	328.3 ± 43.6	450.5 ± 32.4 A	0.08 ± 0.00 A	253.1 ± 8.4	30.9 ± 2.5	−1.3 ± 2.0	29.2 ± 3.0	58.9 ± 5.0
Statistics[a]												
Biochar dose	$\mathbf{P < 0.001}$	$\mathbf{P = 0.003}$	$P = 0.084$	$\mathbf{P < 0.001}$	$P = 0.066$	$P = 0.615$	$\mathbf{P = 0.001}$	$P = 0.784$	$\mathbf{P = 0.004}$	$\mathbf{P < 0.001}$	$\mathbf{P = 0.002}$	$\mathbf{P < 0.001}$
Fertilization	$\mathbf{P < 0.021}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P = 0.028}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$
Biochar dose* Fertilization	$P = 0.627$	$P = 0.742$	$P = 0.739$	$P = 0.534$	$P = 0.868$	$\mathbf{P = 0.004}$	$\mathbf{P < 0.001}$	$P = 0.478$	$P = 0.134$	$P = 0.052$	$P = 0.068$	$P = 0.130$

Values are the mean ± SE of 15 replicates for H and SPAD and color values and 9 replicates for the other variables.
[a] The results of the two-way analysis of variance using fertilization and biochar doses as fixed factors are shown. Values in bold highlight level of significance of P<0.05. In case of a significant interaction effect, different lowercase and uppercase letters indicate significant differences between not fertilized and fertilized treatments, respectively, in the same column.

Table 4. Concentration of VOCs (mg g^{-1}, on a dry weight basis), extracted from basil plants grown on peat (control) or mixtures of peat and biochar (25%) and compost (50 and 25%), and total concentration (per plant) of isolated compounds (mg), calculated on a dry mass of leaves basis (mg) in the first experiment

Substrates[a]	α-pinene	β-pinene	Sabinene	Myrcene	Limonene	Cineole	β-*cis*-ocimene	1-octen-3-ol	Linalool	Terpinen-4-ol	α-terpineol	Eugenol	Total concentration
Peat	0.02 ± 0.00 a	0.02 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.54 ± 0.23 a	0.03 ± 0.02 a	0.14 ± 0.04 a	2.55 ± 0.84 a	0.13 ± 0.08 a	0.10 ± 0.03 a	7.91 ± 2.27 a	11.47 ± 3.48 a
Char 25	0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.01 ± 0.01 a	0.73 ± 0.35 a	0.03 ± 0.03 a	0.14 ± 0.07 a	2.73 ± 1.15 a	0.17 ± 0.12 a	0.12 ± 0.05 a	8.91 ± 3.33 a	12.92 ± 4.94 a
Comp. 50	0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.01 ± 0.01 a	0.68 ± 0.29 a	0.03 ± 0.03 a	0.14 ± 0.06 a	2.81 ± 1.12 a	0.15 ± 0.12 a	0.11 ± 0.05 a	8.43 ± 3.11 a	12.44 ± 4.76 a
Comp. 25	0.02 ± 0.01 a	0.02 ± 0.02 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.51 ± 0.17 a	0.02 ± 0.02 a	0.11 ± 0.04 a	2.20 ± 0.98 a	0.10 ± 0.06 a	0.09 ± 0.03 a	7.05 ± 2.24 a	10.15 ± 3.46 a

Values are the mean ± SE of 14 replicates. Different lowercase letters indicate significant differences between values in the same column, based on Tukey's test ($P < 0.05$).
[a] Plants grown on Char 100, Char 50 and Comp. 100 substrates were not analyzed because of plants death or necrosis during the trial.

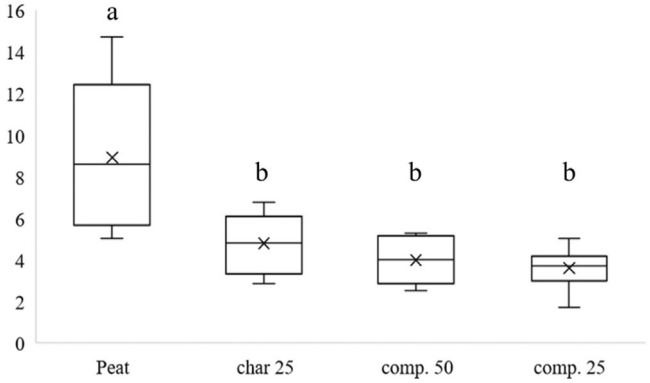

Figure 1. Boxplot of absolute amount of VOCs (mg) in the leaves (dry mass) of basil plants grown on peat (control) or mixtures of peat and biochar (25%) and compost (50 and 25%) in the first experiment. Values are the mean ± SE of 14 replicates. Different lowercase letters indicate significant differences between treatments according to Tukey's test ($P < 0.05$).

Seedling survival and basil growth and quality

The sudden death of the seedlings or the fast necrosis of plants in pure biochar and compost was most likely caused by the combination of too high pH and EC. Nocentini *et al.*[5] reached the same conclusion when working with tomato seedlings, whereas Huang *et al.*[39] found basil seedlings to die after a few weeks in a potted growing medium made of 90% in volume hardwood biochar, 5% chicken manure compost and 5% commercial substrate, showing a pH of around 8.5. On the other hand, Yu *et al.*[48] found that, on substrates made with up to 50% of hardwood, biochar basil seedlings grew as well or even better than those grown on commercial peat. Our results substantially confirm these findings, but suggest that it is worth lowering further (up to 10%) the substitution rate of peat with biochar to achieve growth rates comparable to those obtained on pure peat (Table 2). In the case of the Italiano variety, the combined effect of N fertilization and biochar in the substrate led to even higher values of FW, LFW and LA compared to peat (Table 3). The Greco basil, instead, was positively affected by biochar just in terms of PLW and SLA, the latter with 20% of biochar substitution rate, which, conversely, resulted in lower LDMC values. Indeed, SLA and LDMC, which are associated with important aspects of plant growth,[49] are generally inversely related to each other.[50,51] On the other hand, SLA and its relationship with photosynthesis are the result of trade-offs between different functions of the leaf;[52] this may explain some of the contrasting results from different substrates and basil varieties in the present study.

There are few and contrasting results in the literature about the effect of compost addition to peat for growing basil. Hewidy *et al.*[53] observed that growing media comprising up to 30% compost from organic green wastes collected from a farmyard, enhanced some plant variables, such as height, dry mass and essential oil content compared to pure peat moss, whereas DeKalb *et al.*[54] found negative effects on basil height and weight of more than 20% for a yard waste derived compost in the substrate. In the present study, the replacement of 25% or 50% of peat with compost had generally negative effects in terms of basil growth, being even worse than those obtained with 25% of biochar for some variables (e.g. Height, SLA and LAR).

SPAD and related colorimeter values are used as indicators of the commercial quality of green foliage[55] and are strictly correlated to plant nutritional status.[56] In particular, the higher the

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Table 5. Concentration of VOCs (mg g^{-1}, on a dry weight basis) extracted from basil plants of 'Italiano' and 'Greco' varieties grown in the second experiment on peat (control) or mixtures of peat and biochar (20% and 10%), with and without N fertilization, and total concentration (per plant) of isolated compounds (mg), calculated on a dry mass of leaves basis (g)

	'Italiano'						Statistics[a]		
Substrates	Peat	Peat N	Char 20	Char 20 N	Char 10	Char 10 N	Biochar dose	Fertilization	Biochar dose* Fertilization
α-pinene	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	**P < 0.001**	**P < 0.001**	P = 0.700
β-pinene	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	**P < 0.001**	**P < 0.001**	P = 0.912
Sabinene	0.03 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	**P < 0.001**	**P < 0.001**	P = 0.928
Myrcene	0.05 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.04 ± 0.02	**P < 0.001**	**P < 0.001**	P = 0.895
Limonene	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	**P < 0.001**	**P < 0.001**	P = 0.894
Cineole	1.32 ± 0.11	0.66 ± 0.24	1.69 ± 0.34	0.71 ± 0.26	1.53 ± 0.49	0.88 ± 0.35	P = 0.081	**P < 0.001**	P = 0.226
Ocimene	0.08 ± 0.02	0.05 ± 0.02	0.07 ± 0.01	0.05 ± 0.03	0.08 ± 0.02	0.08 ± 0.03	**P < 0.050**	**P < 0.036**	P = 0.126
Linalool	4.85 ± 0.39 ab	2.49 ± 0.65 A	5.32 ± 0.86 a	2.36 ± 0.58 A	4.28 ± 1.53 b	2.82 ± 0.86 A	P = 0.611	**P < 0.001**	**P < 0.048**
Camphor	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.01	0.01 ± 0.00	P = 0.169	P = 0.381	P = 0.594
α-humulene	0.03 ± 0.01 a	0.01 ± 0.00 A	0.01 ± 0.00b	0.01 ± 0.00 A	0.02 ± 0.01 a	0.01 ± 0.00 A	**P < 0.001**	**P < 0.001**	**P < 0.026**
α-terpineol	0.18 ± 0.03	0.09 ± 0.03	0.20 ± 0.05	0.09 ± 0.03	0.20 ± 0.06	0.11 ± 0.05	P = 0.379	**P < 0.001**	P = 0.677
Methyleugenol	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	P = 0.445	**P < 0.001**	P = 0.081
Eugenol	0.75 ± 0.10 a	0.39 ± 0.05 A	0.66 ± 0.11 b	0.35 ± 0.02 A	0.53 ± 0.11 c	0.41 ± 0.06 A	**P = 0.002**	**P < 0.001**	**P < 0.001**
Total concentration	7.39 ± 0.45	3.82 ± 0.97	8.15 ± 1.35	3.70 ± 0.93	6.87 ± 2.16	4.49 ± 1.35	P = 0.445	**P < 0.001**	P = 0.445

	'Greco'						Statistics[a]		
Substrates	Peat	Peat N	Char 20	Char 20 N	Char 10	Char 10 N	Biochar dose	Fertilization	Biochar dose* Fertilization
α-pinene	0.01 ± 0.00 a	0.01 ± 0.00 A	0.02 ± 0.01 ab	0.01 ± 0.00 A	0.02 ± 0.01 b	0.01 ± 0.00 A	P = 0.524	**P = 0.004**	**P = 0.048**
β-pinene	0.02 ± 0.01 a	0.03 ± 0.01 A	0.04 ± 0.01 b	0.02 ± 0.01 A	0.04 ± 0.01 b	0.03 ± 0.01 A	P = 0.319	**P = 0.005**	**P = 0.010**
Sabinene	0.02 ± 0.01 a	0.02 ± 0.01 A	0.03 ± 0.01 b	0.02 ± 0.00 A	0.03 ± 0.01 b	0.02 ± 0.01 A	P = 0.228	**P = 0.016**	**P = 0.003**
Myrcene	0.02 ± 0.01 a	0.02 ± 0.01 A	0.03 ± 0.01 b	0.02 ± 0.00 A	0.03 ± 0.02 b	0.02 ± 0.01 A	P = 0.088	**P = 0.004**	**P = 0.007**
Limonene	0.02 ± 0.00 a	0.02 ± 0.00 A	0.02 ± 0.01 b	0.01 ± 0.00 A	0.03 ± 0.01 b	0.02 ± 0.01 A	P = 0.063	**P < 0.001**	**P = 0.009**
Cineole	0.67 ± 0.27 a	0.71 ± 0.22 A	1.27 ± 0.43 b	0.62 ± 0.15 A	1.45 ± 0.49 b	0.76 ± 0.20 A	**P = 0.001**	**P < 0.001**	**P = 0.002**
Ocimene	0.01 ± 0.00 a	0.02 ± 0.01 A	0.02 ± 0.01 b	0.01 ± 0.00 A	0.03 ± 0.01 b	0.01 ± 0.01 A	P = 0.052	**P = 0.003**	**P = 0.001**
Linalool	0.38 ± 0.20 a	0.48 ± 0.36 A	0.74 ± 0.48 ab	0.47 ± 0.24 A	1.02 ± 0.64 b	0.60 ± 0.2 A	P = 0.611	**P < 0.001**	**P = 0.048**
Camphor	0.10 ± 0.02 a	0.09 ± 0.03 A	0.16 ± 0.04 b	0.08 ± 0.02 A	0.19 ± 0.04 b	0.10 ± 0.02 A	**P < 0.001**	**P < 0.001**	**P < 0.001**
α-humulene	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	P = 0.691	**P < 0.001**	P = 0.105
α-terpineol	0.12 ± 0.04 a	0.10 ± 0.03 A	0.19 ± 0.06 b	0.09 ± 0.02 A	0.22 ± 0.07 b	0.11 ± 0.02 A	**P < 0.001**	**P < 0.001**	**P = 0.004**
Methyleugenol	2.29 ± 0.83	1.85 ± 1.38	2.89 ± 1.15	1.29 ± 0.80	2.21 ± 1.18	1.39 ± 0.56	P = 0.637	**P = 0.001**	P = 0.228
Eugenol	2.67 ± 1.29 a	2.56 ± 0.94 A	4.11 ± 1.30 b	2.71 ± 0.52 A	5.30 ± 1.81 c	3.10 ± 0.79 A	**P < 0.001**	**P < 0.001**	**P = 0.036**
Total concentration	6.33 ± 1.73 a	6.12 ± 1.35 A	9.52 ± 2.38 b	5.35 ± 0.89 A	10.56 ± 2.22 b	6.19 ± 1.17 A	**P = 0.001**	**P < 0.001**	**P = 0.001**

Values are the mean ± SE of 9 replicates.
[a] The results of the two-way analysis of variance using fertilization and biochar doses as fixed factors are shown. Values in bold highlight level of significance of P<0.05. In case of a significant interaction effect, different superscript lowercase and uppercase letters indicate significant differences between not fertilized and fertilized treatments, respectively, in the same row.

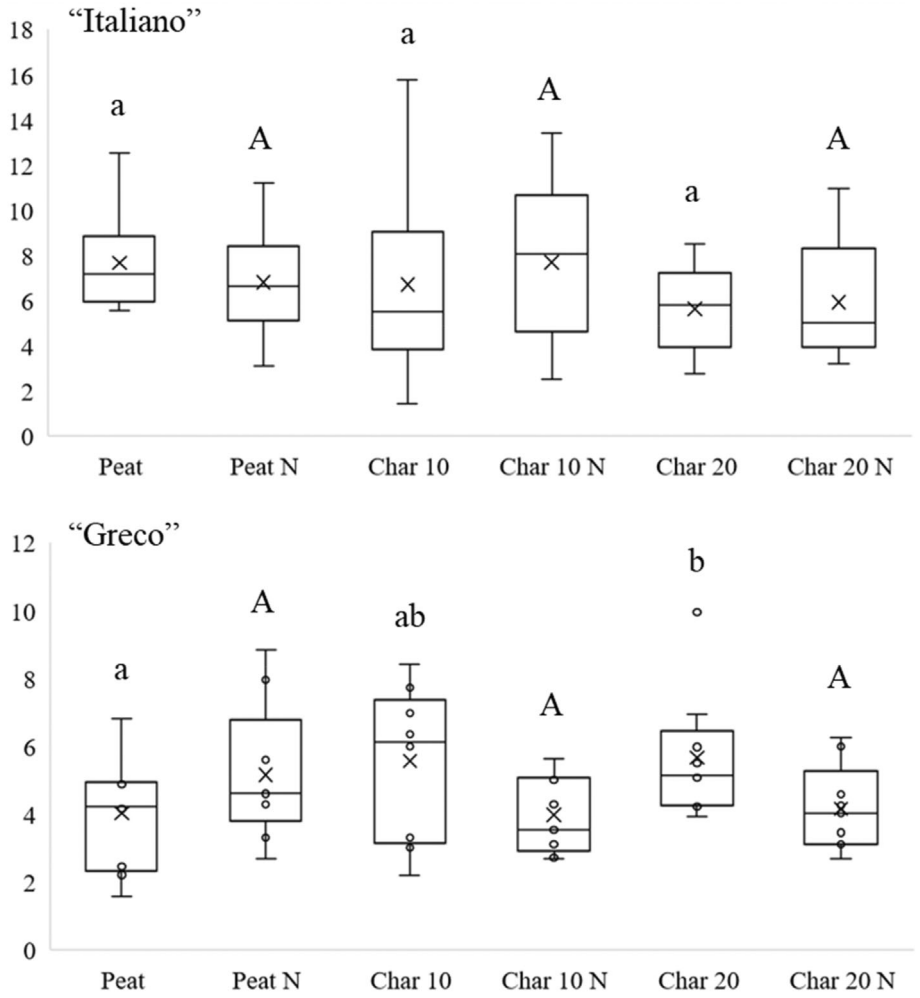

Figure 2. Boxplot of absolute amount of VOCs (mg) in the leaves (dry mass) of basil plants, 'Italiano' (above) and 'Greco' (below) varieties, grown on mixtures of peat (control) and biochar (20% and 10%), with and without N fertilization, in the second experiment. Different lowercase and uppercase letters indicate significant differences between fertilized and unfertilized treatments, respectively.

SPAD, the more available some macro- and micro-nutrients involved in the biosynthesis of chlorophyll.[57] Mininni *et al.*[14] found that basil seedlings grown on pure peat had higher fresh weight, dry weight and leaf area, but also a lower SPAD than those grown on peat mixed at different rates with compost from green urban wastes. Conversely, in our first trial, as well as in the second one, but just for the Greco variety upon N fertilization, we found the highest SPAD in plants grown on pure peat. There is a close relationship between N concentration in leaves and their greenness;[58-60] usually, low SPAD readings indicate low concentrations of both chlorophyll and N in leaves.[61] The lower SPAD values we found in basil grown on substrates with added compost or biochar, therefore, might be related to shortage in N availability. Indeed, compost and especially biochar had less available N than peat (Table 1) and these materials are even able to immobilize N, further reducing its availability to plants.[11,39,54] Compost and biochar may also reduce Fe availability through their high pH, which finally implies a lower SPAD.[61]

Overall, we found that leaf color, which is a crucial variable for marketed fresh basil, was affected by substrate composition and that leaves from plants grown on peat and on nitrogen fertilized substrates showed a more intense green color. Indeed, in the first trial we found a negative and significant correlation between SPAD and b^* ($R = -0.383$, $P < 0.05$) or L^* ($R = -0.409$, $P < 0.05$), whereas a positive and significant correlation was found between SPAD and a^* ($R = 0.495$, $P < 0.01$), overall supporting that the dark green color of leaves is the result of high chlorophyll content. These results were also confirmed by the second trial, where a negative and significant correlation was found for both varieties between SPAD and b^* ($R = -0.688$, $P < 0.01$ for the Italiano variety and $R = -0.695$, $P < 0.01$ for the Greco variety), as well as between SPAD and L^* ($R = -0.529$, $P < 0.01$ for Italiano and $R = -0.657$, $P < 0.01$ for Greco), whereas a positive and significant correlation was found between SPAD and a^* ($R = 0.634$, $P < 0.01$ for Italiano and $R = 0.725$, $P < 0.01$ for Greco).

The relatively worse results we obtained for basil plants grown on mixed substrates compared to pure peat are consistent with those of Bekhradi *et al.*,[62] who reported lower basil biomass and chlorophyll content with increasing salinity of the growing media. Indeed, salinity can depress plant growth and the content of pigments,[47] with obvious effects on leaf color.[63] LAR values are also reported to decrease because of salinity, as a strategy to reduce water loss[38]; nonetheless, we found the highest LAR values in the basil grown on compost-added substrates.

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VOCs

The factors that mostly affect the set of essential oils in basil are genetic variability, the phenological stage, the specific part of the plant, and environmental factors such as light radiation and soil/substrate characteristics, including pH and water availability.[64,65] However, in both our experiments, the addition of compost or biochar at any dose did not significantly influence the oil profile of the Italiano basil (Tables 4 and 5), although the yield of essential oil was considerably reduced by a relatively high rate of peat substitution (Fig. 1). In basil grown hydroponically and in the open field, Bernstein *et al.*[63] and Ekren *et al.*,[66] respectively, observed that the production of essential oil increased because of salinity or water stress, in both cases associated with a decrease in plant biomass. Yet, we did not find any significant correlations between essential oil concentration and differences in EC or WHC between the substrates. Even, the Greco variety showed the highest values of total VOC concentration in the non-fertilized biochar-added substrates, which could be explained by a stimulation induced by some mild abiotic stresses[67] such as pH, EC or the presence of some toxic compounds formed during the pyrolysis, including pyrogenic volatile organic compounds, polycyclic aromatic hydrocarbons and persistent free radicals.[68]

In the second trial, the N-fertilized plants of both varieties had higher biomass than the unfertilized ones but lower total VOCs concentration, which further supports the positive role of moderate stress on the production of these substances. Nonetheless, N fertilization did not affect the absolute amount of VOCs in the Italiano variety on all the substrates and even increased the yield of essential oils in the Greco variety grown on pure peat (Fig. 2).

The terpene profile of plants (i.e. the relative contents of volatile terpenes) is under strong genetic control and usually is little affected by abiotic factors.[69,70] Indeed, the terpene profile is largely used as biochemical marker to characterize plant species, provenance and clones in chemosystematic studies.[69,71] In our second trial, in both basil varieties, various compounds showed different trends in response to biochar addition and nitrogen fertilization. However, it should be noted that the basil aroma (i.e. the relative contents of aromatic compounds) is mainly the result of a few compounds (e.g. linalool and cineole) and any differences in the concentration of the least expressed compounds is substantially insignificant.[72]

Compounds that typically worsen basil flavor, such as estragole, camphor and thymol,[24] were absent or present in trace amounts in the leaves of the Italiano variety collected in both trials, whereas camphor was detected in significant amounts in the Greco variety. Previous studies have demonstrated that the content of another undesired compound in basil, methyleugenol, depends on the degree of development of the plant, such that plants smaller than 10 cm are relatively rich in methyleugenol, which, however, decreases as the plant grows, whereas, inversely, the content of eugenol increases.[73,74] No methyleugenol was found in the leaves of the Italiano variety from the first trial and just trace amounts were detected in the second trial. Methyleugenol has been hypothesized to be toxic to humans when taken in large doses,[75] however much larger than those taken with moderate consumption of 'pesto' sauce, which is typically prepared from young basil plants.[74] The leaves of the Greco variety in the second trial were rich in methyleugenol, as previously resported,[76] but this variety is usually not used to produce pesto, at most as a seasoning spice (and therefore in low doses).

CONCLUSIONS

Biochar and compost were evaluated as potting substrates for basil, alone or together with commercial peat, under different fertilization regimes. Pure biochar or compost were detrimental, but lower substitution rates of biochar (25%) and compost (25% or 50%) combined with peat were tolerated by the plants, although they were not able to guarantee the commercial standards for basil in terms of fresh mass and leaf color. Most probably, high proportions of biochar or compost raised pH and electrical conductivity up to levels incompatible with adequate basil growth and flavor production. High rates of peat substitution caused a substantial decrease in essential oil yield for the Italiano basil variety, the one required by the food industry, although its typical oil composition was unaltered. Conversely, the substitution of peat with just 20% or 10% of biochar did not significantly depress either the plant height or the essential oil concentration in the Italiano variety. Accordingly, peat can be replaced by 20% or less using biochar in the cultivation of basil, without negative consequences and, if anything, with slight positive implications in terms of concentration and yield of essential oils. Lastly, nitrogen fertilization in general implied a decrease in the essential oil concentration of basil, whatever the growing substrate, although not necessarily a decrease in the yield of essential oil because of the consequent increase in leaf biomass. The peat replacement rates that we found to be the best in our experiments are purely indicative of general guidelines because there are many possible types of both biochar and compost and corrective actions that can be carried out on these matrices to mitigate some negative aspects for plant growth, such as high pH and electrical conductivity.

ACKNOWLEDGMENTS

We are grateful to Silvia Sandri and ALL power Labs company for providing the compost used in the first trial.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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