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Composts from Grapevine and Hazelnut By-Products: A Sustainable Peat Partial Replacement for the Growth of Micropropagated Hazelnut and Raspberry in Containers

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Abstract: By-products of the agri-food sector are increasingly employed in the production of new organic preparations, with biofertilizer and biostimulating functions, thus reducing the consumption of non-renewable resources and turning production chains towards circular economy and sustainability. Two composts were produced with solid digestate (85%) as a common component and different sources of recyclable materials (15%): vine pruning wood (compost A), or shells and skin of hazelnuts (compost B). The two composts were used in replacement of variable percentages of peat in nursery substrates, and their effects on various growth parameters assessed on micropropagated plants of two widely demanded species: raspberry (compost A) and hazelnut (compost B). Composts revealed microbiologically safe growth conditions, nutritional content suitable for agronomic purposes and levels of heavy metals in compliance with the European standards. The trials demonstrated the possibility for a partial replacement of peat (up to 20% in hazelnut and 40% in raspberry) in nursery conditions, without compromising, and in some cases improving, the vegetative growth and plants nutritional status. Nonetheless, the highly variable hazelnut growth responses highlighted that compost concentration should be fine-tuned on sensitive species to avoid negative effects. In the case of raspberry, the use of these substrates could also be experimented for soilless production. Because of the potential for metals accumulation, analysis on the extended applications should be made prior to considering field applications.

Keywords: circular economy; sustainability; solid digestate; *Corylus avellana*; *Rubus idaeus*

Citation: Bignami, C.; Reyes, F.; Saccaggi, M.; Pane, C.; Zaccardelli, M.; Ronga, D. Composts from Grapevine and Hazelnut By-Products: A Sustainable Peat Partial Replacement for the Growth of Micropropagated Hazelnut and Raspberry in Containers. *Horticulturae* **2023**, *9*, 481. https://doi.org/10.3390/horticulturae9040481

Academic Editor: A. K. Srivastava

Received: 30 January 2023
Revised: 6 April 2023
Accepted: 7 April 2023
Published: 12 April 2023

1. Introduction

The production of new organic preparations with biofertilizer and biostimulating functions, derived from the recovery of by-products of the agri-food sector, represents an effective tool for recycling residues and wastes, providing the possibility to reduce the use of non-renewable resources and to structure the production chains based on circular economy and sustainability criteria. The production chains of hazelnut and vine-wine have great importance worldwide and generate a significant number of residues and by-products, both in the field and in processing activities [1–3].

Hazelnut is widely cultivated in the world, with Turkey and Italy being leading countries with 75% of the global nut production, which has raised up to 1,072,308 tons in 2020 [4]. In total, 90% of the nuts are directed to the kernel market and used by the food industry as shelled nuts, thus generating a large amount of recyclable residues during production and processing: husks, shells and kernel skins. Husks compost has been suggested as an amendment for tomato crop [5] and for long term ornamental nursery

crops [6]. Shells represent a fraction of 40–50% of the whole nut, an abundant by-product for which several alternative re-uses have been explored: in addition to their use for furfural extraction [3], shells have been proposed as a biological composite filler material [7]; as raw material for biogas production [8], as a substrate for growing edible and medicinal mushrooms [9] and as a source of bio-active ingredients [10]. Indeed, hazelnut shell and kernel skin contain phenolic substances with antioxidant and antimicrobial capacity and potential positive impacts on human health [10–14]. To the author's knowledge, the use of hazelnuts shells and skins to produce compost has not been yet experimented. If on the one hand, potential excess of water-soluble phenols in the raw material may negatively affect the compost quality [15], on the other hand, the same compounds may be progressively degraded and incorporated in the humic substances during composting [15]. A good stability of the compost is therefore required to avoid potential biotoxicity.

Grapevine is cultivated in the world on almost 7 million hectares, with a production of 78 Mt of grapes [4], mainly directed to winemaking. Residues and by-products of winemaking (stalks, marc, lees, vine canes) represent a considerable source of organic matter, oil, phenolic compounds, macro- and micro-elements and are considered good resources for second-generation biorefineries to produce added-value products [1,2]. According to the current European legislation, the by-products deriving from winemaking have to be entirely or partially delivered to the distillery, or be reused in alternative destinations, including energy production, upon transfer to biogas or combustion plants, or composting for subsequent agronomic purposes. Vineyard pruning generates an average of 2 to 4 t of canes per ha, which could provide 1.1 tons of organic dry matter ha^{-1} $year^{-1}$, a valuable source of macro and microelements [16], which is instead generally burned or returned to the soil. Both the vine pruning wood and hazelnut shells have in common the prevalence of the ligno-cellulosic components, providing 32.9 to 39.8% cellulose, 5.8 to 27.0% hemicellulose and 26.7 to 46.0% lignin in case of vine pruning wood [16] and 15.4% cellulose, 22.4% hemicellulose, 25.9% lignin in case of hazelnut shells [3]. A higher protein content (8%) characterizes hazelnut shells with respect to vine pruning residues (2.0–2.7%) [3,17]. Despite their slow degradation during composting, these components could be used in combination with different matrices to produce organic amendments and fertilizers in laboratory experiments or for applications on plants [18,19]. In particular, the anaerobic digestion of grape stalks with other matrices produces a solid digestate which, composted with vineyard pruning wood, has shown characteristics suitable for growing vine [18] and blueberry [20].

The continuous increase in global and national demand for hazelnuts and raspberries, both for the fresh market and processing, made it necessary to considerably expand the land use for these crops. This led to a strong demand for nursery plants by farmers. In the case of hazelnut, the supply of plants offered by the nursery market proved immediately inadequate to meet the demand, mainly due to the use of slow and relatively inefficient traditional propagation techniques (layering, stem cuttings) [21]. This situation pushed nurseries towards faster and safer propagation systems, such as micropropagation.

Concerning raspberry, the need for specific soils, with high organic matter, a subacid reaction and a low content of active limestone, along with the risks associated to the presence of telluric pathogens and replanting [22], pushed an increasing number of growers towards soilless farming solutions. This cultivation system allows for better control of the epigeal and underground plant environment and development, offering stronger guarantees for obtaining a high-quality product. Nonetheless, despite these advantages, container cultivation can be profitable, but involves high financial and environmental costs due to the use of peat (a non-renewable, running out and expensive resource) as the main cultivation substrate.

The formulation and evaluation of substrates alternative to peat, or other expensive and depleting substrates, remains a current objective of experimentation for species such as berries and hazelnut. As the organic matter content of soil/growing media plays an important role as a source of N, reducing fertilization costs and the related environmental

risks [22], the addition of compost may be expected to have a significant and positive effect, from both an economic and an environmental perspective [23–25]. The current research investigates the effects of two different peat replacement nursery substrates, originated as by-products of the biogas, hazelnut and winemaking productions, on the growth and physiological parameters of micropropagated plants of hazelnut (*Corylus avellana* L.) and raspberry (*Rubus idaeus* L.), within a general framework of circular economy, environmental protection and sustainability.

2. Materials and Methods

2.1. Composts Preparation and Analysis

Composting was carried out at the facilities of the Reggio Emilia headquarters of the University of Modena and Reggio Emilia. Two compost formulations were obtained, both containing solid digestate as a common component and differentiated by vine pruning wood (compost A), or shells and skin of toasted hazelnuts (compost B) (Table 1). Solid digestate was obtained from the Cooperativa Agroenergetica Territoriale (CAT, Correggio, Reggio Emilia, Italy) as a by-product of the biogas supply chain. Here, the biogas plant uses as input of the anaerobic digestion: maize (*Zea mays* L., 43%) and triticale (X Triticosecale Wittmack, 22%) silage, cow slurry (27%) and grape stalks (8%) obtained as byproducts from local vineyards. For compost A, winter pruning wood was collected in a nearby vineyard (cv. Lambrusco Salamino, Coviolo, Reggio Emilia, Italy). Concerning compost B, shells and skins of toasted nuts (cv. Tonda Gentile) were provided by the Ferrero Hazelnut Company factory (Alba, Cuneo, Italy).

Table 1. Composition of the two composts produced and of the five experimental substrates for each species.

Raspberry (*Rubus idaeus* L.) cv Himbo Top					
Compost	**Raw Material**	**% d.w.**	**Substrate**	**% Compost**	**% Peat**
A	Digestate (CAT)	83.3	T1	10	90
			T2	20	80
	Vine winter pruning	15.0	T3	40	60
			T4	0	100
	Mature compost	1.7	T5	0	100 + fertilizer
Hazelnut (*Corylus avellana* L.) cv Tonda Gentile Delle Langhe					
Compost	**Raw Material**	**% d.w.**	**Substrate**	**% Compost**	**% Peat**
B	Digestate (CAT)	83.3	T1	10	90
			T2	20	80
	Nut shells and skins	15.0	T3	40	60
			T4	0	100
	Mature compost	1.7	T5	0	100 + fertilizer

To produce compost A, the vine prunings were finely chopped (chips 1 cm in length) and composted with solid digestate at a dry weight ratio of 15.0% pruning wood and 83.3% digestate, as previously described in Bignami et al. (2022) [20]. The same ratio was used in composting the mixture of finely chopped hazelnut shells and skins with solid digestate (compost B). In both composts, in addition a proportion of 1.7% of mature compost was used in the composting process, following a protocol adopted in previous research [18,20]. Two static piles of 1 m^3 were prepared with these materials and composted for 115 days, mixing weekly to aerate the mass. The relative humidity (RH) of piles was periodically checked and maintained always higher than 50% by irrigation. The composting process was monitored by temperature measurements in different areas inside the pile using thermoresistance sensors (PT100, Gandolfi, Parma, Italy). Fifty-five days of thermophilic phase were followed by a further 2-month curing period.

The mass was thoroughly turned to achieve uniformity, then six subsamples were collected in different points and mixed to obtain a 3 kg homogenous sample from each compost. Physico-chemical analysis of the samples was then carried out by the external laboratory EST (Bergamo, Italy). Total organic carbon (C), total nitrogen (N), Mg, Fe, NaO, Ca, P_2O_5 and K_2O, CEC (Cation Exchange Capacity), water content, Pb, Zn, Cu, Cd, Ni, Hg, Cr and pH were determined. In addition, electrical conductivity (EC) was measured on the wet material (1:5 ratio), using a CRISON GLP 31 EC meter (Crison Instrument, Barcelona, Spain).

2.2. Characterization of Composts Phytotoxicity

Compost phytotoxicity was assessed in terms of a germination index (GI) following [26]: 4 mL of water compost extract (50 g L^{-1}) were applied on sterile filter paper in Petri dishes on which 20 seeds of garden cress (*Lepidium sativum* L.) were spread and incubated at 25 °C for 5 days. The procedure was replicated three times. The germination index (GI%), respectively, for roots (1) and shoots (2) was calculated following the formula [27]:

$$GI\% = 100 \times (G1/G2) \times (R1/R2) \quad (1)$$

$$GI\% = 100 \times (G1/G2) \times (S1/S2) \quad (2)$$

where G are germinated seeds, R is the mean root length (1) and S is the mean shoot length (2), while 1 and 2 refer, respectively, to composts A or B, and control.

2.3. Microbiological and Suppressiveness Characterizations

The amount of culturable filamentous fungi, total bacteria and spore-forming bacteria in the two composts was evaluated by a serial 10-fold (10^{-1} to 10^{-7}) dilution method in three replicates [28]. Fungi were counted on a potato dextrose agar (PDA, Oxoid) having pH 6 and supplemented with 150 mg L^{-1} of nalidixic acid and 150 mg L^{-1} of streptomycin. Total bacteria were counted on a selective medium composed of glucose 1 g L^{-1}, protease peptone 3 g L^{-1}, yeast extract 1 g L^{-1}, potassium phosphate buffer 1 g L^{-1} and agar 15 g L^{-1} and supplemented with 100 mg L^{-1} of actidione. Spore-forming bacteria were counted by plating 10-fold dilutions of the compost on nutrient agar after heating at 90 °C for 10 min. Population densities were expressed as colony forming units (CFU) per gram of compost dry weight. Coliform, *Escherichia coli* and *Salmonella* spp. were detected according to Cekmecelioglu et al. [29].

The two composts were also tested for suppressiveness capacity against damping off disease caused by *Rhizoctonia solani* and *Sclerotinia minor*, using garden cress as host plant. To do so, each compost was added to commercial peat at a rate of 20% by volume, mixed and used to fill 10 pots per treatment; additional pots filled with commercial peat only were used as a control [28]. Pathogen inoculations and a damping-off assessment were performed according to Pane et al. [30]. Pathogen isolates of *Sclerotinia minor* and *Rhyzoctonia solani* were obtained, respectively, from diseased lettuce (*Lactuca sativa* L.) and cabbage (*Brassica oleracea* L.). Common millet seeds were used to prepare pathogen inocula in 0.5 l flasks, saturated with potato dextrose broth solution inoculated with fungi cultured on potato dextrose agar for 7 days, and incubated for 21 days at 20 °C. The obtained powdered fungal millet inoculum was added to the peat-compost mixtures at a concentration of 1% *w*/*w*, dry weight. Non-inoculated common millet was added to the control pots. The disease incidence of *Rhyzoctonia* and of *Sclerotinia* was recorded, respectively, after 7 and 9 days. Damping-off (%DO) was calculated as:

$$\%DO = (HPc - HPi/HPc) \times 100$$

where HPc is the number of healthy plants in non-inoculated control substrate and HPi the number of healthy plants in the inoculated mixture. The bioassay was done in two replicates.

2.4. Plant Materials and Nursery Trial

Five different substrates were prepared for each of the two species considered in the experiment, hazelnut (*Corylus avellana* L.) and raspberry (*Rubus idaeus* L): three made of commercial peat and different ratios (T1: 10%; T2: 20%; T3: 40%) of compost, and two reference substrates consisting of unfertilized (T4) and fertilized peat (T5). Compost A (vine added) was used for raspberry and compost B (hazelnut added) for hazelnut. Peat fertilization consisted of 0.3 g of Osmocote top dress (22-5-6 + 2MgO) per pot, applied at the end of May. The commercial peat-based substrate (TERCOMPOSTI Professional Extra Quality, Tercomposti SpA, Calvisano-BS) contained acid peat, expanded perlite, pumice and AVSNC simple non-composted vegetable amendment (coconut), and was characterized by pH 6, CE 0.3 dS m^{-1}, bulk density 172 kg m^{-3} and total porosity 91%.

Micropropagated and acclimatized raspberry (primocane cultivar Himbo Top) and hazelnut (cultivar Tonda Gentile delle Langhe) plants were provided by a nearby nursery (Battistini Nursery, Cesena, Italy) and transplanted at the beginning of April in 1.5 L plastic containers filled with the five experimental substrates previously described. At the beginning of the trial, the micropropagated raspberry plants consisted of a short cane with a length ranging from 26 to 29 cm, with no significant differences between theses (data not shown). An average of 1.7 suckers were formed at the base of each shoot, the most developed of which was preserved with pruning and its growth was followed during the spring and summer seasons.

Containers were then placed in a greenhouse, under conditions of natural photoperiod and solar radiation, with temperature kept between 28 and 19 °C (day/night) and air relative humidity between 50 and 70%. At the beginning of July, plants were transplanted into 5 L containers, keeping the same substrate types as before, and moved out of the greenhouse under shade nets, while maintaining the water content of the substrate at field capacity by means of nightly sprinkler irrigation. The microclimate was characterized by a mean air of 26.1 °C in August (with a mean maximum monthly temperature of 30.0 °C), 20.7 °C in September, 16.6 °C in October and 10.1 °C in November. Rainfall during the August-November period was 380.0 mm, of which 19.2 mm fell in August.

2.5. Data Collection and Analysis

Pots were arranged in a fully randomized design with twelve replicates. The height of raspberry and hazelnut plants was periodically measured during the growing season, on six dates (day of the year—DOY 113, 144, 173, 196, 225, 266). On 15 July (196 DOY), the total leaf area per plant was nondestructively estimated based on a leaf number count and on the measure of leaf blade length and width of a sample of 10 leaves per treatment. The total leaf area of hazelnut and raspberry plants was then calculated according to the following equations obtained for the hazelnut cultivar *Tonda Gentile (=Tonda delle Langhe)* by Cristofori et al. [31] and for different cultivar of raspberry by Fallovo et al. [32]:

$$\text{Hazelnut: Leaf area} = (2.59 + 0.74 \times L \times W) \times n,$$

where L = Length of leaf blade; W = Width of leaf blade; n = number of leaves.

$$\text{Raspberry: Leaf area} = (0.03 + 0.71 \times L \times W) \times n,$$

where L = length of leaf blade; W = width of leaf blade; n = number of leaves.

Indices correlated to the content of leaf chlorophyll (CHL), flavonoids (FLAV) and anthocyanins (ANT), and the nitrogen balance index (NBI) (the ratio between CHL and FLAV), which expresses the nitrogen balance in the leaf, were non-destructively measured on five plants per treatment in each of five dates (DOY 131, 185, 206, 225, 255). Measurements were performed by means of a Dualex 4 Scientific (Dx4, FORCE-A, Orsay, France) in order to evaluate the nutritional and physiological conditions of the plants [33].

Concerning raspberry, the partitioning of wet and dry biomass in the roots and the aerial part of the plants were measured in raspberry by the end of the trial (327 DOY). The

roots were extracted from the pots and carefully cleared. Plants were separated into the aerial part (A) and roots (R), then their fresh weight (AFW and RFW) was measured, and total fresh weight of the plant (TFW) was calculated. Successively, each part was oven-dried at 70 °C until a constant weight, and the dry weight of the aboveground part (ADW) and root (RTDW) was weighed; total dry weight (TDW) was calculated and the fractions of total dry weight allocated to the above ground part of the plant (FDWA) and to the roots (FDWR) were calculated.

The mean species responses to the different treatments were compared by means of one-way analysis of variance (ANOVA) followed by post-hoc Duncan test (at $p < 0.05$) using the R statistical software ver. 4.2 (R Core Team, 2022, Vienna, Austria).

3. Results and Discussion

3.1. Compost Characteristics

The differences in substrate composition (15% in dry mass) had a moderate impact on the variables analyzed. Both composts revealed a good content of the main macronutrients required for plant growth, in particular N, P, K and Mg (Table 2). The C/N ratio was lower in compost A (12.9) than in compost B (15.2), nonetheless indicating in both cases a good level of compost maturity [16]. According to Jiménez et al. [34], CEC values may also give information about compost stabilization. The values of 54.3 meq/100 g obtained for compost A and of 59.5 for compost B are very close to the necessary minimal values (60 meq/100 g) assuring an acceptable maturity for compost obtained from urban wastes.

Table 2. Chemical characteristics of the compost from digestate and grapevine pruning wood (A) or digestate and hazelnut shells and seed skin (B). All values are on a dry weight basis.

Compost Characteristics	Compost A	Compost B
pH	7.03	7.44
EC (dS m^{-1})	4.15	3.18
Moisture (%)	69.5	60.9
CEC (meq/100 g)	54.3	59.5
Organic carbon (%)	34.5	38.3
Total nitrogen (% N)	2.67	2.52
Organic nitrogen (% N tot)	99.45	99.45
Humic and fulvic carbon (%)	16.4f	17.1
Cr VI (mg kg^{-1})	<0.50	<0.50
Pb (mg kg^{-1})	11.7	11.3
Zn (mg kg^{-1})	191.1	202,9
Cu (mg kg^{-1})	48.2	44.9
Hg (mg kg^{-1})	<0.20	<0.20
Cd (mg kg^{-1})	0.5	0.5
Ni (mg kg^{-1})	9.8	8.9
C/N Ratio	12.9	15.2
P (P_2O_5 %)	1.7	2.6
K (K_2O %)	1.9	2.1
Al (mg kg^{-1})	4597.0	4283.0
Mg (MgO %)	0.90	0.98
Fe (mg/kg)	6253.0	5810.0
Ca (CaO %)	4.19	4.71
Na (mg kg^{-1})	607.1	659.9
S (%)	0.469	0.379
Mn (mg kg^{-1})	215.0	259.3

Both composts presented relatively high Na concentrations (compost A = 607.1 mg kg^{-1}; B = 659.9 mg kg^{-1}), contributing to determine the relatively high EC levels of slightly saline substrates. The grapevine pruning wood conferred a slightly lower pH (7.03) and higher EC (4.15 dS m^{-1}) to compost A, with respect to the hazelnut shells and seed skins of compost B (pH = 7.44, EC = 3.18 dS m^{-1}). In both cases, composts presented a pH value slightly

higher than the need of the species (between 5.6 and 6.5 for cane berries [35]; between 6.0 and 7.0 for hazelnut [36]) on which these composts were to be tested, excesses that might be mitigated by mixing with different percentages of a commercial peat-based substrate.

When coming to phytotoxicity, their content in heavy metals did not exceed the limits fixed by the European Regulation for commercial amendment (CE 2003/2003). The use of different percentages of compost also made it possible to reduce the concentrations of some microelements, such as Al, which could cause phytotoxic effects, e. g. in raspberries [37].

The germination assays on 'garden cress' resulted in germination indices higher than the phytotoxicity threshold (50%) [27] for both raspberry and hazelnut, with higher values for shoots (raspberry: 136%; hazelnut: 127%) than for roots (raspberry: 60%; hazelnut: 91%) (Table 3), indicating no major impact due to the use of the experimental composts.

Table 3. Phytotoxicity assessment, and microbiological and suppressiveness characterization of Compost A and B. * = statistically significant difference at p-value < 0.05; CFU = colony forming unit.

Variable	Compost-A	Compost-B
GI root (%)	60 *	91
GI shoot (%)	136 *	127 *
Pseudomonas (CFU g^{-1})	2.3×10^6	6.6×10^6
Bacillus (CFU g^{-1})	6.9×10^5	8.2×10^5
Fungi (CFU g^{-1})	5.0×10^4	5.5×10^5
Bacteria (CFU g^{-1})	1.0×10^7	3.3×10^7
Total Coliform bacteria (CFU g^{-1})	Absent	Absent
Faecal Coliform (CFU g^{-1})	Absent	Absent
Yeasts (CFU g^{-1})	Absent	Absent
Streptococci (CFU g^{-1})	Absent	Absent
Escher coli (CFU g^{-1})	Absent	Absent
Salmonella spp. (CFU g^{-1})	Absent	Absent
Clostridia spp. (CFU g^{-1})	Absent	Absent
Rhizoctonia solani damping-off (%)	87.6 *	90.9 *
Sclerotinia minor damping-off (%)	40.3	47.0

Composts disease-suppressiveness have long been known and mainly attributed to the activities of antagonistic micro-organisms [38]. However, the potentially beneficial effects on the cultivated species and the underlying mechanism of action differ depending on the matrix, the microbiota population and the species needs [38]. Gaining knowledge about this variability is essential to assess the compost quality as an appropriate component of a growing substrate. In our assay, the population densities of fungi and bacteria were respectively of 5.0×10^4 and 1×10^7 CFU g^{-1} in compost A, and of 5.5×10^5 and 3.3×10^7 CFU g^{-1} in compost B, with coliform bacteria, *Escherichia coli*, *Clostridia* spp. and *Salmonella* spp. completely absent in both composts (Table 3), thus eligible for the Community eco-label on soil amendments and cultivation substrates, based on the European legislation (*Decision 2001/688/CE*). The results of suppressive bioassays indicate a moderate yet significant reduction of garden cress damping-off in samples of both tested composts inoculated with *R. solani*, while a reduction higher than 50% in samples inoculated with *S. minor* (Table 3).

Quality and safety are of paramount importance when producing and using a new compost, starting from its raw materials. The digestate added to the composting process, especially when manure or slurry are among the ingestates, may be a carrier not only of pathogens, but also of organic pollutants that can reach the soil/substrate and damage it [39]. Specific regulatory instruments at the EU level or at the individual country level exist for heavy metals and other pollutants [40]. Although their use in nursery substrates poses fewer environmental problems than the direct use in the field, certainly the limited scientific knowledge and the current legislative shortcomings regarding emerging pollutants cannot be underestimated. In this trial, as far as digestates used in the two composts are concerned, the safety conditions are ensured at the origin, since currently biogas plants do not accept

livestock waste with heavy metals, as well as emerging pollutants beyond the legal limits, both because they would produce problems to the anaerobic digestion and because the use of the produced digestates would be impeded.

3.2. Vegetative Growth and Physiological Indices in Raspberry

Raspberry plants mean initial height was comprised between 7.9 cm and 11.0 cm, rapidly increased until late June, and then continued increasing at lower rates, to start approximating an asymptote starting from mid-August, with heights comprised between 109.3 cm and 113.9 cm. No significant differences were found between theses at any measurement date (Figure 1, Supplementary Table S1). Conversely, leaf area per plant, estimated in mid-July, was significantly affected by the growing substrate (Figure 2, Supplementary Table S1). Plants grown on T2 and T3 substrates, containing 20% and 40% compost, respectively, presented a significantly greater leaf surface than the ones grown on unfertilized peat (T4), while intermediate results were found for the substrate containing 10% compost (T1) and for the fertilized peat (T5).

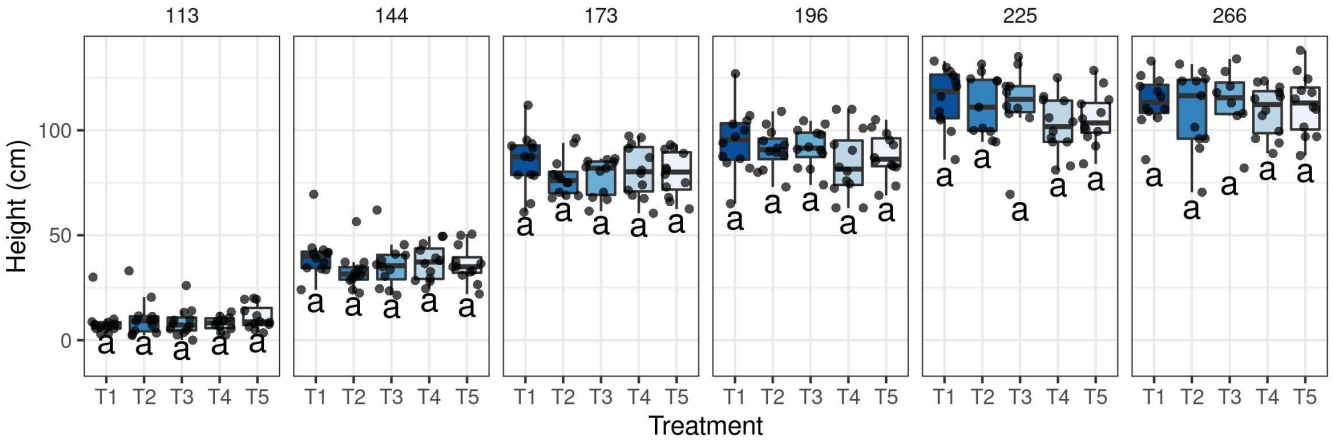

Figure 1. Boxplot of raspberry Himbo Top plant height depending on substrate and DOY. T1 = Commercial Peat (P) 90% + Compost A (CA) 10%; T2 = P 80% + CA 20%; T3 = P 60% + CA 40%; T4 = Commercial Peat (P) 100%; T5 = P Commercial Peat 100% + Osmocote. Different letters indicate statistically significant differences after ANOVA and Duncan post-hoc test at p-value < 0.05.

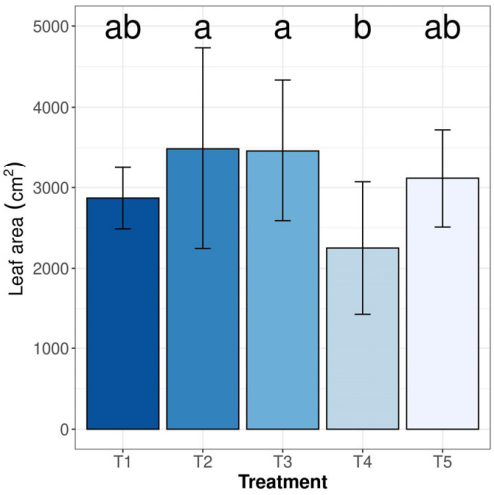

Figure 2. Leaf area of raspberry Himbo Top on day of year 196 depending on substrate. T1 = Commercial Peat (P) 90% + Compost A (CA) 10%; T2 = P 80% + CA 20%; T3 = P 60% + CA 40%; T4 = Commercial Peat (P) 100%; T5 = P Commercial Peat 100% + Osmocote. Different letters indicate statistically significant differences after ANOVA and Duncan post-hoc test at p-value < 0.05.

Depending on treatment, compost had positive or no effects on leaf composition (Figure 3, Supplementary Table S2). The chlorophyll index of raspberry leaves, indicative of the nitrogen status of the plants, differed between theses in the early measurement dates (Figure 3). Unfertilized peat (T4) had values significantly lower with respect to substrates with 20% compost (T2) in May, and with respect to substrates with 20% and 40% compost (respectively, T2 and T3) or with fertilized peat (T5) in early July. This positive effect can be attributed to a better nutritional availability due to compost in T2 and T3 and fertilizer application in T5. Conversely, the compost rate in T1 was not sufficient to determine significant effects with respect to the use of only peat. No significant differences emerged between theses in the following dates, up to the end of the vegetative growth.

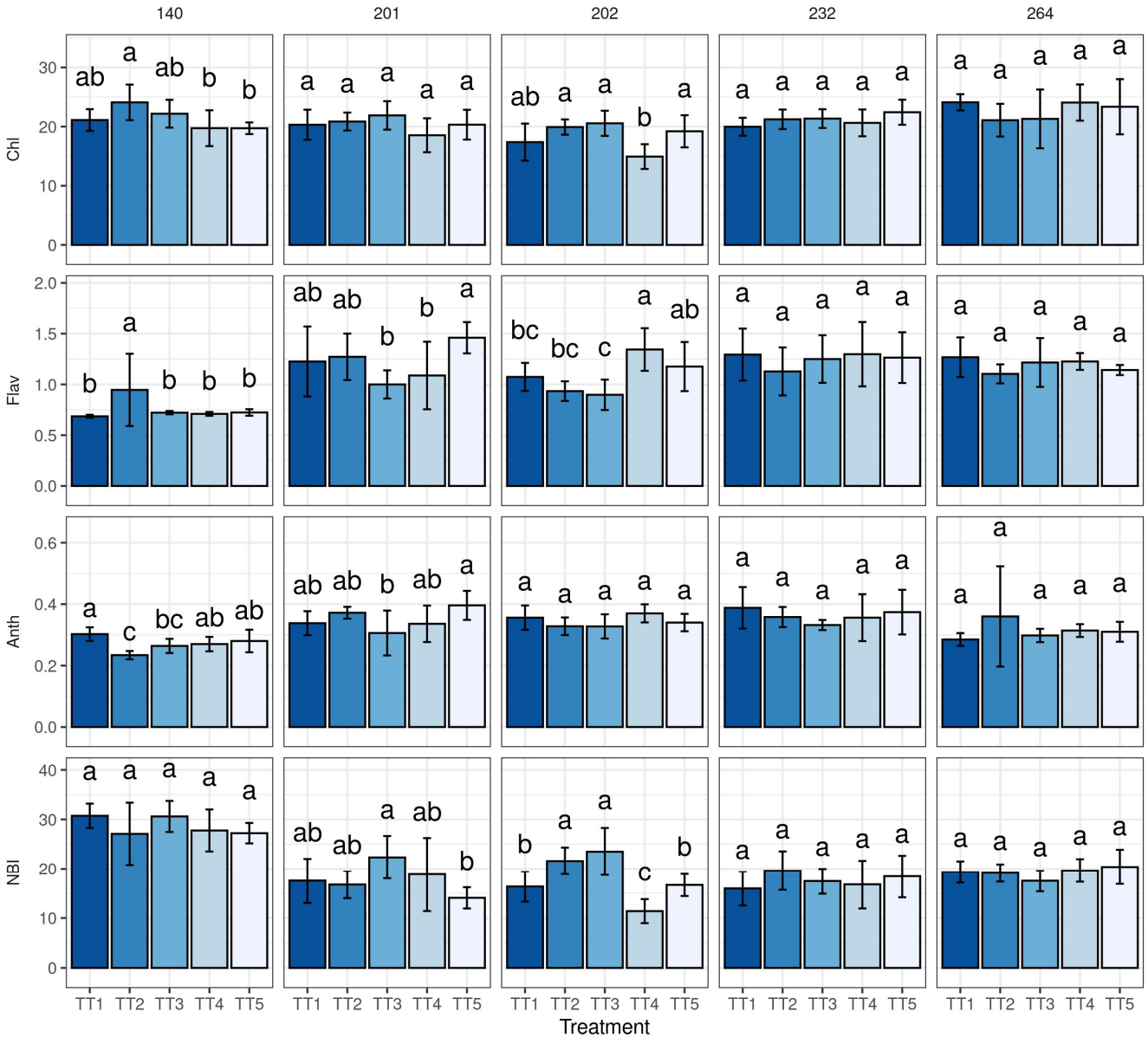

Figure 3. Barplot of raspberry Himbo Top mean chlorophyll (CHL), flavonoids (FLA) and anthocyanins (ANTH) index, and nitrogen balance index (NBI) depending on DOY and depending on the substrate. TT1 = Commercial Peat (P) 90% + Compost A(CA) 10%; TT2 = P 80% + CA 20%; TT3 = P 60% + CA 40%; TT4 = Commercial Peat (P) 100%; TT5 = P Commercial Peat 100% + Osmocote. Error bars indicate standard deviations around the mean. Different letters indicate statistically significant differences after ANOVA and Duncan post-hoc test at p-value < 0.05.

The flavonoid index generally increased from May to late July, possibly related to different times of leaf growth and maturity. Plants grown on compost added substrates (T1–3) showed lower values of this index with respect to the controls in early July, while T3 and T4 presented lower values than the other thesis in late July (Figure 3). As a result of the reciprocal adjustments over time of the chlorophyll and flavonoid indices, by the beginning of July the NBI index was significantly higher in T2 (20% compost) and T3 (40% compost) than in all other theses, and significantly lower in T4 (unfertilized peat) than in all the other theses. By the end of July, T3 plants still had significantly higher NBI values than T5 plants, suggesting a strong orientation towards primary metabolism and protein synthesis. Conversely, plants grown on substrates with a lower compost content (T1 and T2) showed an intermediate behavior.

Nitrogen is the key nutrient for raspberry vegetative growth. In field experiments, the supply of different N fertilizer rates revealed no significant effects on vegetative growth and yield in a soil with high organic matter content [23]. This lack of response highlighted the importance of organic matter mineralization and plant reserves, which may be able to meet plants nutritional needs, making the effect of external contributions less evident. As the raspberries of this trial were micropropagated, they were small, and in the previous in vitro phase under conditions of heterotrophy, they did not have the possibility to accumulate substantial reserves. On this basis, the positive effect of T2 and T3 on chlorophyll and NBI indices is presumably due to an appropriate rate of compost in the substrate, allowing for a good availability of organic matter and N.

The destructive surveys carried out at the end of the year made it possible to evaluate the effects of the different substrates on the overall production of biomass and its allocation to different parts of the plant (Table 4). In terms of total fresh weight (TFW) (but not dry weight), T2 had higher productivity with respect to T1 and T4, while T3 and T5 did not significantly differ from the other treatments. Considering resource allocation, the ratio between the weight of the roots and the total weight (FDWR) of the plant, indicates that the greatest amount of biomass, fresh and dry, had been allocated to the root system in all theses, with the highest ratio observed on the unfertilized peat. In particular, similar FDWR values were obtained in compost added soils, suggesting that these compost rates were high enough to assure comparable resources availability with respect to fertilized peat. As such carbon allocation seems not to be influenced towards the root system, at the expense of the aerial growth, neither by the lack of resource availability nor by an excessive content in heavy metals [41].

Table 4. Allocation of fresh and dry weight to the aerial and epigeal parts of raspberry plants cv Himbo Top grown on substrates with various ratios of peat and compost obtained from solid digestate and pruning woods. Means followed by the same letter do not significantly differ at p-value < 0.05. T1, Commercial Peat (P) 90% + Compost A (CA) 10%; T2, P 80% + CA 20%; T3, P 60% + CA 40%; T4, Commercial Peat (P) 100%; T5, P 100% + Osmocote. AFW, fresh weight of the aerial part of the plant; RFW, root fresh weight; TFW, total fresh weight; ADW, shoot dry weight; RDW, root dry weight; TDW, total dry weight; FDWR, fraction dry weight to root.

Treatment	AFW (g Plant^{-1})	RFW (g Plant^{-1})	TFW (g Plant^{-1})	ADW (g Plant^{-1})	RDW (g Plant^{-1})	TDW (g Plant^{-1})	FDWR (%)
T1	19.8 ab	33.1 a	52.9 b	9.7 ab	10.62 a	20.38 a	51 b
T2	26.6 ab	47.6 a	74.2 a	11.4 a	17.58 a	28.94 a	61 b
T3	21.9 ab	39.1 a	61.0 ab	9.7 ab	14.72 a	24.59 a	60 b
T4	14.3 b	37.4 a	51.7 b	5.6 b	12.95 a	18.48 a	70 a
T5	29.7 a	39.1 a	68.8 ab	10.1 ab	15.00 a	25.17 a	59 b

Considering resource allocation to the aerial part of the plant, similar results were obtained for fresh (AFW) and dry (ADW) weights. The fresh weight of the sucker growing on the innovative substrates T1, T2 and T3 was similar to the one obtained on fertilized peat (T5) which, in turn, was significantly higher than the one obtained on unfertilized peat (T4).

Similarly, in terms of dry weight, no significant differences were found between innovative substrates and the fertilized control. Conversely, T2 obtained a fresh aerial biomass twice as high and significantly different than unfertilized peat (T5).

3.3. Vegetative Growth and Physiological Indices of Hazelnut Tonda Gentile Delle Langhe

Hazelnut plants survival strongly differed among treatments. All plants on T1 (10% compost), T4 and T5 (controls) were alive and in good health by the end of the trial. Conversely, 15% and 80% of the plants were lost during the trial in T2 (20% of compost) and T3 (40% of compost), highlighting the importance of fine tuning the concentration of the experimental compost for this species, without exceeding 20% of total mass and possibly maintaining lower values to avoid plants losses.

Plants mean initial height was not significantly different among treatments and was comprised between 11.5 cm (T1) and 12.3 cm (T2) (Figure 4, Supplementary Table S3). Heights rapidly increased until mid-July, when only some individuals continued growing until no later than mid-August. Significant differences among treatments rapidly appeared (in no more than one month) and got accentuated until the end of the growing season, when T5 and T1 obtained the highest growth (respectively, 50.2 cm and 49.8 cm), while the other treatments reached significantly lower values, comprised between 37.0 (T2) and 26.0 cm (T3).

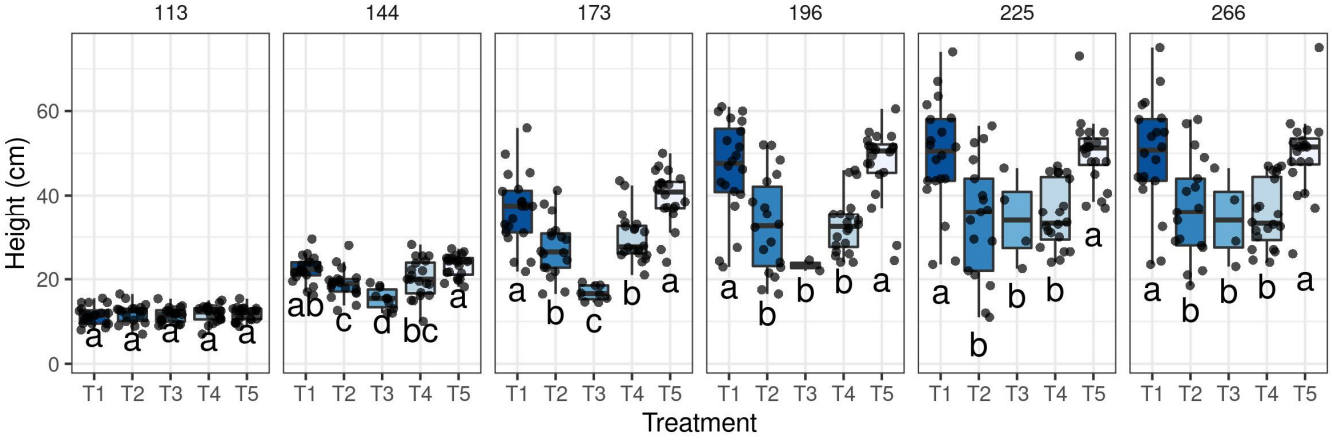

Figure 4. Boxplot of hazelnut Tonda Gentile plant height depending on substrate and DOY. T1 = Commercial Peat (P) 90% + Compost B(CB) 10%; T2 = P 80% + CB 20%; T3 = P 60% + CB 40%; T4 = Commercial Peat (P) 100%; T5 = P Commercial Peat 100% + Osmocote. Different letters indicate statistically significant differences after ANOVA and Duncan post-hoc test at p-value < 0.05. Individuals lost during the trial were removed from the analysis.

Interestingly, despite the similar mean heights, plants on the compost added substrate (T1) had a much more variable growth with respect to the fertilized control (T5), with the distribution spread towards both higher and lower heights. Such a result highlights the potential opportunity inherent to the use of an experimental compost such as the one proposed but invites for further investigations concerning methods to reduce the variability in plant responses, which may entail, among others, reducing the heterogeneity of the substrate (e.g., via better mixing).

When coming to the mean leaf area (Figure 5) assessed in mid-July, this was significantly higher: in T1 (2195 cm^2) with respect to the controls (T4 = 1515 cm^2; T5 = 1614 cm^2), respectively, with increases by 45% and 36% with respect to T4 and T5; and in the controls with respect to substrates with higher compost content (T2 = 1029 cm2; T3 = 518 cm^2). Leaf area responses highlighted that moderate shifts in compost concentration (from 10 to 20% in the substrate) may turn a positive plant response to a detrimental effect, highlighting the importance of fine tuning the concentration of this compost type in the substrate to obtain

non-negative or even growth promoting effects. This result, as well as the high mortality found at highest compost fractions, requires an in-depth analysis of the causes.

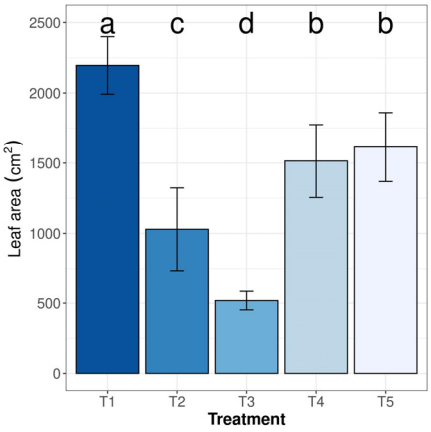

Figure 5. Leaf area of hazelnut Tonda Gentile on day of year 196 depending on substrate. T1 = Commercial Peat (P) 90% + Compost B (CB) 10%; T2 = P 80% + CB 20%; T3 = P 60% + CB 40%; T4 = Commercial Peat (P) 100%; T5 = P Commercial Peat 100% + Osmocote. Different letters indicate statistically significant differences after ANOVA and Duncan post-hoc test at p-value < 0.05.

Among the possible causes of the reduced growth occurring with compost concentrations above 10%, there is the relatively high sodium concentration of the experimental compost (Table 2). Excessive Na contents are known to induce reduced growth and leaf development [42] at concentrations that depend on the plant species and is a frequent quality issue possibly arising in composting [43]. In addition, the effect of excess levels of phenols in substrates containing more than 10% of compost cannot be excluded and may require further investigation.

Both the chlorophyll and flavonoid indices generally increased between early May and September, but with substantial differences among treatments (Figure 6). The chlorophyll index was significantly higher in T1–2 plants with respect to those growing both on the unfertilized and fertilized peat (T4–5) throughout the growing season (except on the first measurement date). Even T3 plants presented chlorophyll indices significantly higher than in T4 starting from late July, and T5 starting early August. About the opposite pattern was observed for the flavonoid index, with values significantly higher in control plants throughout the growing season with respect to the compost added substrates (except a few cases: T4 with respect to T1 in early May, and with respect to T2–3 in September; T5 with respect to T2 in late July). Finally, the NBI index closely followed the variations over time and significant differences among treatments highlighted for the chlorophyll index. NBI is considered a good indicator of leaf nitrogen concentration (32). The significantly higher values of plants grown on compost added substrates with respect to control plants testify their good nutritional status.

A recent evaluation of substrates supplemented with high fractions of compost from liquid digestate (30% and 45%) performed by Calisti 2023 [44] revealed strongly negative effects on hazelnut seedlings growth parameters (lower fresh and dry weight of leaves, stems and roots compared to the control without compost at the end of the season), outlining the need for further research and testing. Our study tested a wider range of compost ratios, ranging from 10 to 40%. Even considering the differences in plant material (micropropagated cultivar) and composted matrices (solid digestate, hazelnut shells and skins) our results confirm the sensitivity of this species to high fractions of compost in the substrate, showing that concentrations of about 10% may provide the most satisfactory results. Nonetheless, the high differences in plant responses obtained when shifting the compost concentration in the substrate by only 10% (from T1 = 10% to T2 = 20%) suggests the potential usefulness of further testing along an even finer gradient of compost concentrations.

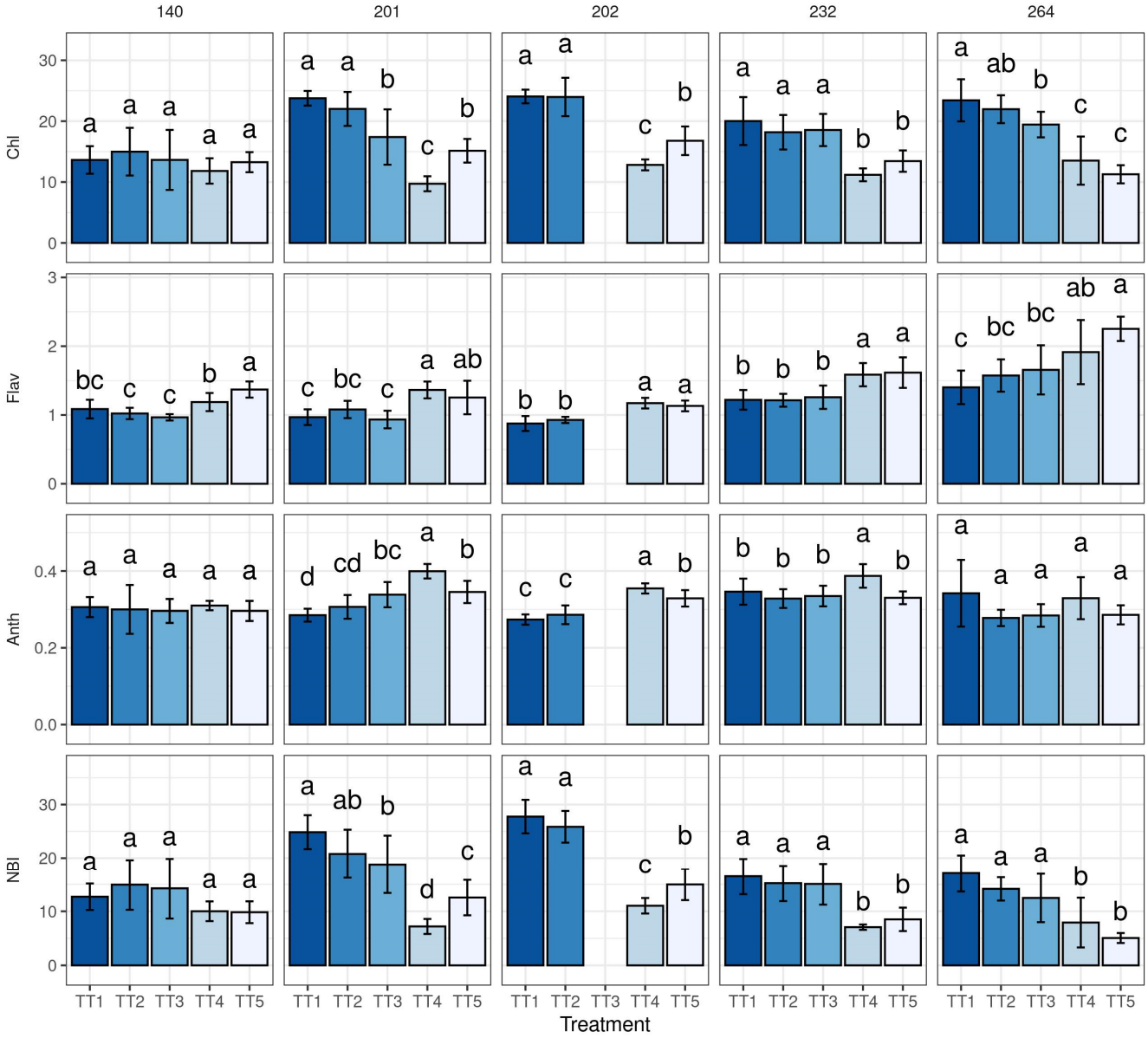

Figure 6. Barplot of hazelnut Tonda Gentile mean chlorophyll (CHL), flavonoids (FLA) and anthocyanins (ANTH) index, and nitrogen balance index (NBI) at depending on DOYs and the substrate. TT1 = Commercial Peat (P) 90% + Compost B(CB) 10%; TT2 = P 80% + CB 20%; TT3 = P 60% + CB 40%; TT4 = Commercial Peat (P) 100%; TT5 = P Commercial Peat 100% + Osmocote. Error bars indicate standard deviations around the mean. Different letters indicate statistically significant differences after ANOVA and Duncan post-hoc test at p-value < 0.05.

4. Conclusions

The results of this study highlight the possibility of consistently reducing the use of peat in the nursery production of hazelnut and raspberry plants using composts obtained by digestate and agri-food residues of the hazelnut and vine-wine production chains. This solution may replace the common practice of burning hazelnut shells and vineyard prunings in the field, which implies the waste of resources and the emission of pollutants.

However, the level of replacement of peat with new composts must be assessed on a case-by-case basis, according to the species-compost binomial. Our trials have in fact highlighted substantial differences between the two species in response to the different fractions of compost in the substrates. The vitality and the performance of vegetative growth and nutritional status of raspberry were not compromised, and even improved in

some cases, across the whole range of compost used. Positive effects in the phase of active growth have been observed on chlorophyll content and nutritional status of raspberry up to 40% of compost A. Conversely, only up to 20% of compost B could be used without a strong impact on the growth of hazelnut. Furthermore, the reduction of height and leaf area, already observed at concentrations of 20%, leads prudently to keep the ratio of compost for this species not over the 10% level.

Overall, results from this study are promising as they can help to consistently reduce the consumption of non-renewable resources in the nursery sector, and the related economic and environmental costs. Nonetheless, further investigations are needed to understand the limiting factors in compost causing the negative responses of hazelnut plants at relatively low compost concentrations. In the case of raspberry, the use of these substrates could also be experimented for soilless production. Considering the generality of our results: our study concerned potted plants and its results should not be extended to larger scale, possibly repeated, field applications without prior analysis of the potential accumulation of metals (including heavy metals) in the soil and consequent salinity and toxicity issues that may arise.

Furthermore, this research also highlights that results of this kind may be attained on plants obtained by micropropagation, a system offering various advantages for users both in the nursery and in the agronomic field and that is increasingly used for the two species evaluated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9040481/s1, Table S1: Effects of substrates with different compost ratio (0-10-20-40%) on raspberry Himbo Top plant height during the growth season and on leaf area on DOY 196; Table S2: Chlorophyll (CHL), flavonoids (FLA) and anthocyanins (ANTH) index, and Nitrogen Balance Index (NBI) of raspberry Himbo Top as affected by different compost ratio (0-10-20-40%) at different times during the growing season; Table S3: Effects of substrates with different compost ratio (0-10-20-40%) on hazelnut Tonda Gentile plant height during the growth season and on leaf area on DOY 196; Table S4: Chlorophyll (CHL), flavonoids (FLA) and anthocyanins (ANTH) index, and Nitrogen Balance Index (NBI) of hazelnut Tonda Gentile as affected by different compost ratio (0-10-20-40%) at different times during the growing season.

Author Contributions: Conceptualization, C.B. and D.R.; data curation, C.B. and F.R.; funding acquisition, C.B.; investigation, C.B., M.S., C.P., M.Z. and D.R.; methodology, C.B. and D.R.; project administration, C.B.; resources, C.B.; supervision, C.B., F.R. and D.R.; writing—original draft, C.B., F.R. and M.Z.; writing—review and editing, C.B., F.R., C.P., M.Z. and D.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fondi di Ateneo per la Ricerca di UNIMORE, FAR 2017 Interdisciplinary Projects. "La valorizzazione degli scarti agroindustriali tra diritto e scienza: processi innovativi dalla sperimentazione all'industrializzazione nel contesto legale". Grant number E91118000210005.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to agreement with the company providing the materials.

Acknowledgments: The authors acknowledge and are grateful to Giovanni Grazzi (Foliae) for some of the materials used in this study. The authors want to thank Azienda agraria IIS Antonio Zanelli for providing the vineyard prunings, Massimo Zaghi (CAT) for providing digestate and Enrico Bartolucci (Ferrero) for providing shells and pellicle of toasted hazelnuts.

Conflicts of Interest: The authors declare no conflict of interest.

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