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Successive Harvests Affect Yield, Quality and Metabolic Profile of Sweet Basil (*Ocimum basilicum* L.)

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Abstract: Sweet basil (*Ocimum basilicum* L.) is an aromatic, tender leafy green cultivated for the fresh market and industrial processing. Like many other leafy vegetables, this species can be harvested more than once to increase productivity. Although the cut-and-come-again (CC) harvest strategy is widespread in basil, not much is known about the effect of the cut. In this work, we examined differences in biomass, physiology, nutrient use efficiency and antioxidant capacity of basil leaves from two successive harvests. Moreover, we also performed an untargeted metabolomics analysis to reveal variations in the leaf metabolite profile. The data indicated that the cut affected many of the parameters under investigation, including a modest reduction of yield (−20%), of nitrogen use efficiency (−21%) and of some quality attributes such as the antioxidant capacity (−16%) and the nitrate content in leaves (+48%). Moreover, leaves of successive cuts displayed a significant variation of the profile of bioactive compounds, characterized by an overall decrease of phenylpropanoids and an increase in terpenoids. Our data revealed the impact of CC harvesting strategy in basil, and that this strategy offers the possibility to yield leaves with different metabolomics profiles and quality attributes.

Keywords: leafy vegetables; cut-and-come-again; yield; nitrogen; antioxidants; metabolomics; secondary metabolites

1. Introduction

Ocimum is a botanical genus that includes important gastronomic, industrial and medicinal herbs, collectively referred to as basil [1]. The most valuable culinary and ornamental species is *Ocimum basilicum*, also known as sweet basil [1]. It is an herbaceous, aromatic plant popular in various regions of the world [2] that is cultivated as a fresh leafy green, for decorative purposes and to produce an essential oil [3,4]. Basil is not typically eaten in amounts that can represent a significant daily source of energy, proteins, and minerals. Its nutritional value resides in the antioxidant, antimicrobial and therapeutic properties [1,3]. The increasing awareness of the health benefits of a diet rich in leafy greens, as well as fresh fruits and other vegetables, has led to a growing appreciation and use of basil [5,6]. For its low caloric value and large supply of vitamins, basil is also considered

a valuable source of antioxidants, such as ascorbic acid (vitamin C) and carotenoids, mainly beta-carotene (provitamin A) [7,8]. Finally, sweet basil is largely employed in the Mediterranean cuisine as food garnish and preservative, also for its typical pungent and composite fragrance, both fresh (e.g., pizza "Margherita") and processed (e.g., pesto sauce) [9].

In Italy, as well as in other Mediterranean countries (e.g., Spain, Turkey) professional basil production relies largely on soilless cultivation techniques, especially hydroponics [10,11]. With the latter, plant nutrients are dissolved in water to generate the so-called Nutrient Solution (NS), which can be provided to plants in an inert medium (semi-hydroponics) or not (hydroponics) [12]. A significant advantage is controlling the availability of essential plant nutrients, because the key features of the NS (i.e., osmotic potential, electrical conductivity, pH and temperature) can be continuously monitored and regulated [13,14]. A common hydroponic system for basil is the floating raft system, because of its simplicity and low cost, as well as for the short production cycle, erect stem and low canopy weight of the plant [15]. Plants are usually arranged in small holes and supported within a floating raft (e.g., polystyrene), with roots dangling into the NS below [15]. Hydroponics are valuable for leafy vegetables to obtain both higher yield and quality, offering also the advantage of more rational, standardized and interchangeable cultural practices [13,16]. Moreover, the manipulation of mineral nutrient solutions can be exploited to enhance the profile of secondary metabolites [13].

For a range of aromatic plants and leafy vegetables (e.g., amaranthus, celery, chicory, (swiss) chard, coriander, corn salad, endive, lettuces, parsley, rocket and spinach), farmers can use two harvesting strategies—once-and-done and cut-and-come-again (CC), depending on whether the leaves are harvested with or without uprooting the plant. The CC strategy is technically different for the various plant species and in basil, plants are cut above the first pair of true leaves and let to grow again. In temperate regions of Europe and more generally, with a growing season that can span from April to September, basil is harvested at least twice. While in traditional systems the first harvest is performed when plants are flowering, nowadays in professional horticulture (e.g., in soilless cultivation and for industrial processing), basil is harvested at shorter intervals to ensure the yield of more tender leaves, of an intense bright green and with a fresher and more delicate flavor [17]. Nonetheless, the main advantage of CC-basil is the cost reduction for labor and material, along with a faster production cycle and a more effective use of hydroponics.

The economic and dietary interest for fresh basil as well as other leafy vegetables mainly resides in the phytochemical composition, such as the antioxidant capacity. Studies carried out also on aromatic plants indicated that multiple harvests could significantly affect various traits [18–22] but very little is known on the metabolomics changes associated with those differences. In addition, not much is known on the effects of the cut in basil in intensive production systems [23,24], especially from the agronomic point of view [10]. In this work, we studied the difference in agronomic, physiological, and quality-related traits of basil harvested in two consecutive cuts. Specifically, we analyzed the antioxidant capacity as well as the nitrate content in the edible product, as there is a consensus that leafy greens with a reduced nitrate content should be preferred to limit possible health risks associated to carcinogenic compounds from dietary precursors. Finally, we also performed an untargeted metabolomic analysis to deepen our understanding of the compositional changes in leaves.

2. Materials and Methods

2.1. Plant Material, Experimental Design and Conditions

The work was carried out on *Ocimum basilicum* L. var. *basilicum*, Genovese type, at the Azienda Agraria e Zootecnica Universitaria Torre Lama (Università degli Studi di Napoli Federico II) in Bellizzi (SA), 40° 61 N, 14° 93 E (60 m a.s.l.). Plantlets with three true leaves were transplanted on April in mesh bottom pots (10 × 10 × 15 cm) filled with 1.3 l of a (2:1 *v*/*v*) peat/perlite substrate. Plantlets were arranged in rows on an aluminum bench with a density of 23 plants per square meter in a metal glasshouse. The mean air temperature during the experiment was 26 °C (min: 16 °C; max:

33 °C) and the relative humidity was set at 57% (respectively, 80%) at day (resp., at night). Plants were fertigated from six days after transplanting with a modified Hoagland nutrient solution (NS) with a pH of 6.0 and an electric conductivity of 2 dS/m. Concentrations were—9.6 mM nitrate, 1.5 mM phosphorus, 4.5 mM potassium, 6.5 mM calcium, 2.5 mM sulphur, 2.0 mM magnesium, 20 μM iron, 9 μM manganese, 0.3 μM cupper, 1.6 μM zinc, 20 μM boron and 0.3 μM molybdenum. The experiment was performed with ten pots per experimental unit in three blocks, for a total of 30 plants. In each experimental unit, four plants were considered as guard (and cut as the others), leaving six plants for the various measurements.

2.2. Harvest and Growth Analysis

Shoots were harvested at 47 and 75 days since the transplant, at approximately the same height (around 35 cm), by cutting plants just above the first pair of opposite true leaves. For each harvest, fresh (FW) shoot material was weighed with an ExplorerR balance (OHAUS Europe, Nänikon, Switzerland). Immediately after, leaves were detached. Their weight was measured and added per plant, to provide the Fresh Leaf Weight (FLW), expressed in g per plant. Leaf area was determined with a LiCor 3100 C area meter (LI-COR Biosciences, Lincoln, Nebraska, USA) and added per plant to give the total leaf area (TLA), expressed in cm^2 per plant. The Harvest index (HI) was calculated as the ratio between the commercial products (leaf mass) and the total biomass of the shoot. Dry (DW) biomass (g) was obtained in a forced-air oven at 70 °C for 3 days until constant weight. Dry matter percentage was calculated as DM = 100 × DW/FW. Specific Leaf Area was calculated as SLA = TLA/(Total leaf DW) [25]. The Water Content (%) was calculated as WC = [(100 x (FW − DW)] x FW^{-1} and Leaf Succulence (g cm^{-2}) as LS = (FW − DW) (leaf area) $^{-1}$ [26].

2.3. Leaf Gas Exchange

Leaf gas exchange measurements were conducted between 11:00 h and 13:00 h using a portable infrared gas analyzer (LCA-4; ADC BioScientific Ltd., Hoddesdon , UK) equipped with a 6.25 cm^2 leaf chamber, on nine replicate plants per treatment (three replicates per experimental unit). Net photosynthetic carbon dioxide assimilation rate (An) (μmol m^{-2} s^{-1}) and transpiration (E) (mmol m^{-2} s^{-1}) were determined just before each harvest on fully expanded intermediate (non-apical) leaves. The climatic conditions (e.g. the photosynthetically active radiation, relative humidity, and carbon dioxide concentrations) were set at ambient temperature and the flow air rate was 400 ml s^{-1}. The instantaneous Water Use Efficiency (WUEi) (μmol CO_2 assimilated per mmol of transpired water) was calculated as the ratio between An and E.

2.4. Nitrogen Determination and Nutritional Efficiency Indices

Nitrate was analyzed from an aqueous extraction of 0.25 g of dried and ground leaf material according to already published procedures [27]. The determination of total nitrogen (N) was performed on one gram of ground leaf material using the Kjeldahl method [28]. The result is expressed in mg g^{-1} DW. Total nitrogen content (TNC) was calculated as described by Sorgonà et al. [29] and expressed in mg g^{-1} DW. Total nitrogen accumulation (TNA) was calculated multiplying TNC with the total DW of the leaves [29], with the result expressed in mg N. Nitrogen Use Efficiency (NUE) was equal to the Total DW of the leaves divided by TNC and it is expressed in g^2 DW mg^{-1} of nitrogen [30]. This parameter is analogous to the Nitrogen utilization efficiency (NUtE) as defined in Siddiqi et al. [31]. The definition of NUE was developed for grain production and for leafy vegetables. In general, for biomass productivity NUE is also calculated in relation to the actual yield (for basil, fresh leaves). For this reason, we also determined the NUE-Yield as a ratio between total FW and TNC, expressed in g^2 FW mg^{-1} of nitrogen. The Nitrogen Efficient Ratio (NER) was calculated, dividing the yield (DW) per TNA and it is expressed in g DW per mg of nitrogen [32].

2.5. Antioxidant Capacity and Polyphenol Quantification

For the analysis of quality-related traits, fresh basil material was immediately frozen in liquid nitrogen and stored at −80 °C until use. Frozen tissue was lyophilized in an Alpha 1–4 LSC Basic (M. Christ, Osterode, Germany) freeze-dryer. Both hydrophilic (HAA) and lipophilic (LAA) antioxidants activities, as well as total phenols (TP), were quantified by UV–Vis spectrophotometry, according to previously reported procedures [33,34]. Solution absorbances were measured at 734, 505 and 525 nm for HAA, LAA and TP, respectively. HAA, LAA and TP are expressed in mmol eq. ascorbic acid 100 g^{-1}, mmol eq. trolox 100 g^{-1} and mg eq. gallic acid g^{-1} (DW), respectively. Total ascorbic acid (TAA) was analyzed on the fresh basil material as reported [35,36] using a Hach DR 4000 (Hach Co., Loveland, CO, USA) spectrophotometer. TAA is expressed in μg g^{-1} (FW).

2.6. Metabolomics

For the untargeted metabolomics by high resolution mass spectrometry coupled to reverse phase liquid chromatography,leaf samples (1.0 g), stored at −80 °C , were pulverized in liquid nitrogen. The extraction was performed in 10 ml of 0.1% formic acid in cold 80% aqueous methanol using a homogenizer (Ultra-Turrax Ika T-25, Staufen, Germany). Following centrifugation (12,000× g), the clear supernatant was filtered through a 0.22 μm mesh cellulose membrane. Chromatography, mass spectrometry, data acquisition and spectral pre-processing (including feature extraction) were performed as described [37]. Extreme and outlier values were filtered out considering the interquantile range (>20%) of the peak intensity. Samples were normalized by row-wise sum and scaling (i.e., mean-centered and divided by standard deviation of each variable). For the two groups' data (Cut 1 vs Cut 2), exploratory data analysis was carried out using the independent Student's t-test. The statistical significance (p value) was adjusted for multiple comparisons (False Discovery Rate based on the Benjamini-Hochberg procedure; FDR). These analyses were carried out with Metaboanalyst 4.0 [38]. The classification of the differential compounds into biochemical classes was manually curated following the information retrieved from the PubChem [39], the MetaCyc [40] and the KEGG database [41].

2.7. Statistics

Differences between cuts were evaluated with an independent Student's t-test using the Statistical Package for Social Sciences (SPSS 20) software version 20 (IBM Corp., Armonk, NY, USA) and visualized in R [42].

3. Results

3.1. Analysis of Agronomic and Physiological Parameters

The basil yield was significantly affected by the Cut factor (Figure 1). The fresh yield, which for leafy vegetables and many horticultural species is mainly determined by the water content of the harvestable product, was higher at the first cut (CT1), regardless of the higher number of leaves produced at Cut2 (CT2). This is because the average leaf size was significantly higher at the first cut, leading to a larger total leaf area per plant. The dry weight of the shoot biomass (i.e., leaves and stem) was not different between the two harvests ($p > 0.05$; t-test). Leaves at the second cut had a higher percentage of dry biomass, although the water content (as percentage) was not significantly affected. For this reason, leaves at CT2 had a lower tissue density (e.g., they were more succulent). As indicated by the differences in the Harvest Index, plants at the first cut had a higher capacity to allocate biomass for leaf production. Although total DW biomass production was higher at CT1 ($p < 0.05$; t-test), the fraction of dry biomass partitioned to the leaves was higher at CT2. As indicated by the SLA, the growth output in terms of return on captured resources at the first cut was also higher because plants were able to grow larger leaves per dry matter.

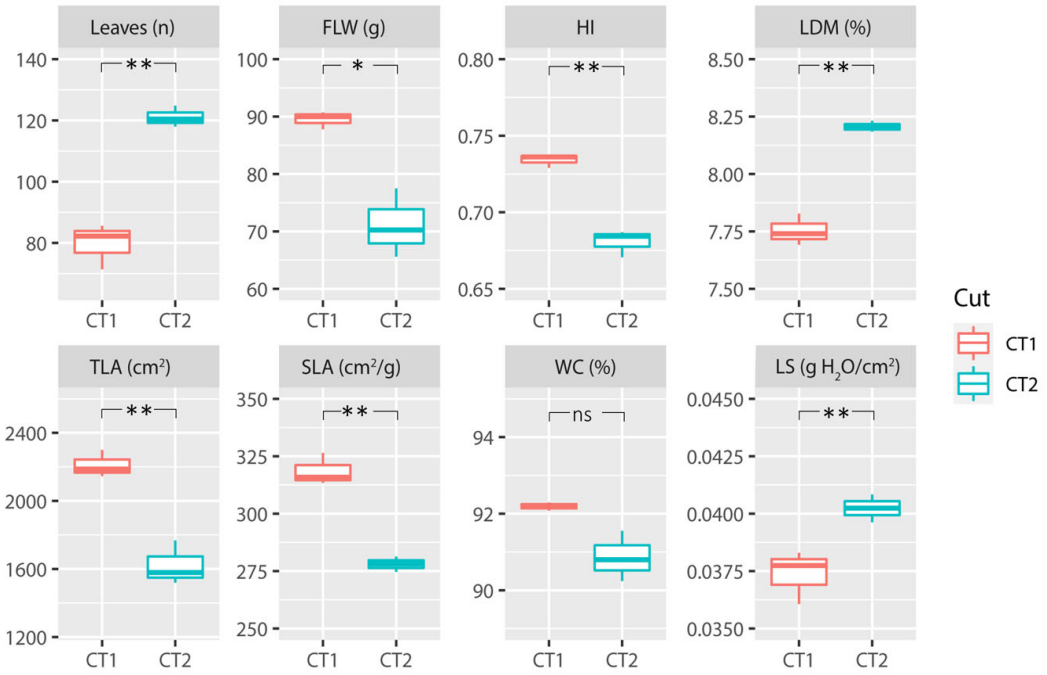

Figure 1. Box and whisker plots of morphological parameters. Leaves: number (*n*) of leaves per plant; FLW: fresh leaf weight; HI: harvest index; LDM: leaf dry matter; TLA: total leaf area; SLA: specific leaf area; WC: water content; LS: leaf succulence. CT1: first cut; CT2: second cut. Asterisks denote a statistically significant difference (**: $p < 0.01$; *: $p < 0.05$; ns: not significant) according to the Student's t-test.

When the environmental factor is fixed, plant growth (in terms of dry biomass) is mainly driven by the photosynthetic activity. The leaves at the first Cut had a significantly higher net photosynthetic rate (Figure 2). Even if a full linear relationship between photosynthetic activity and total leaf area does not necessarily hold true, the difference in the photosynthetic ability between the cuts should be considered relevant because plants at CT1 had a larger leaf area. Plants at the second cut had a higher transpiration rate. However, a significant difference of the overall plant transpiration ($p > 0.05$; t-test) was not present considering the higher TLA at CT1. The instantaneous water use efficiency of leaves at the first cut was significantly higher (more than double) than leaves at the second cut.

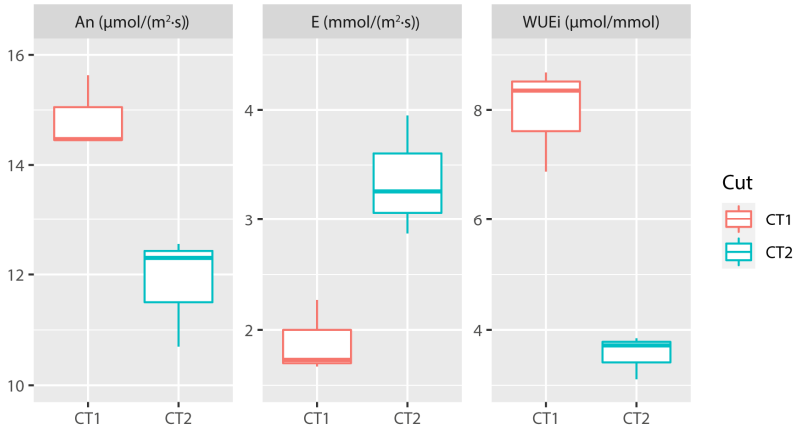

Figure 2. Box and whisker plots of physiological parameters. An: net photosynthetic carbon dioxide assimilation rate: E: transpiration; WUEi: instantaneous Water Use Efficiency. CT1: first cut; CT2: second cut. Asterisks denote a statistically significant difference (**: $p < 0.01$; *: $p < 0.05$) according to the Student's t-test.

Overall, the CC strategy affected both the morphology and the physiological of basil and plants at the second harvest had a higher number of smaller and more succulent leaves, less efficient in terms of photosynthesis and transpiration.

3.2. Nitrogen Accumulation

In leafy vegetables, the relation between harvested product and plant nutrients is mainly analyzed with respect to nitrogen. Leaves at the second cut had a higher content of nitrogen and nitrate (Figure 3). Nonetheless, the total nitrogen accumulation in leaves was not significantly different, mainly because plants of the first cut had larger leaves. The definition of NUE is different depending on whether vegetative biomass productivity or grain production is the harvest product [30]. With reference to the dry weight of the basil leaves, plants at the first cut displayed a significant higher efficiency in the use of nitrogen, considering that the hydroponically grown basil plants were supplied with equal and constant amount of nitrogen fertilization. The difference in nitrogen use was also present considering the fresh weight of the leaves, the parameter that defines the commercial yield of the plant. As indicated by the NER, the unit of dry mass that is produced relative to the unit of nitrogen present in leaves is approximately 20% higher for plants at the first cut.

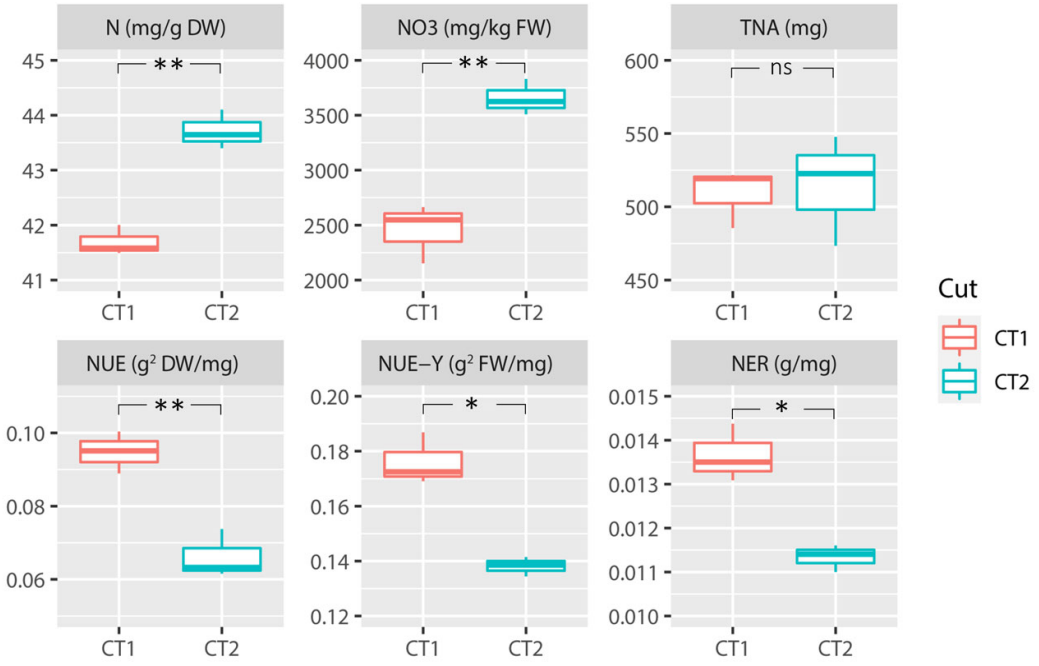

Figure 3. Box and whisker plots of nutritional parameters. N: total nitrogen; NO3: nitrate content; TNA: total nitrogen amount; NUE: nitrogen use efficiency (relative to the dry weight, DW); NUE-Y: nitrogen use efficiency (relative to the yield's fresh weight, FW); NER Nitrogen Efficient Ratio. CT1: first cut; CT2: second cut. Asterisks denote a statistically significant difference (**: $p < 0.01$; *: $p < 0.05$; ns: not significant) according to the Student's t-test.

3.3. Antioxidants

We analyzed possible differences in the antioxidant capacity of the leaves because fresh basil represents an affordable, rich source of various phytochemicals with antioxidant properties [43]. Both the lipid-soluble and water-soluble antioxidant activity of the leaves from the first cut were higher (approx. 15%) than of the leaves of the second cut (Figure 4). A significant difference was not present considering the total ascorbic acid, one of the major contributors of the water-soluble antioxidant capacity. Finally, the concentration of phenolic compounds did not display differences between cuts.

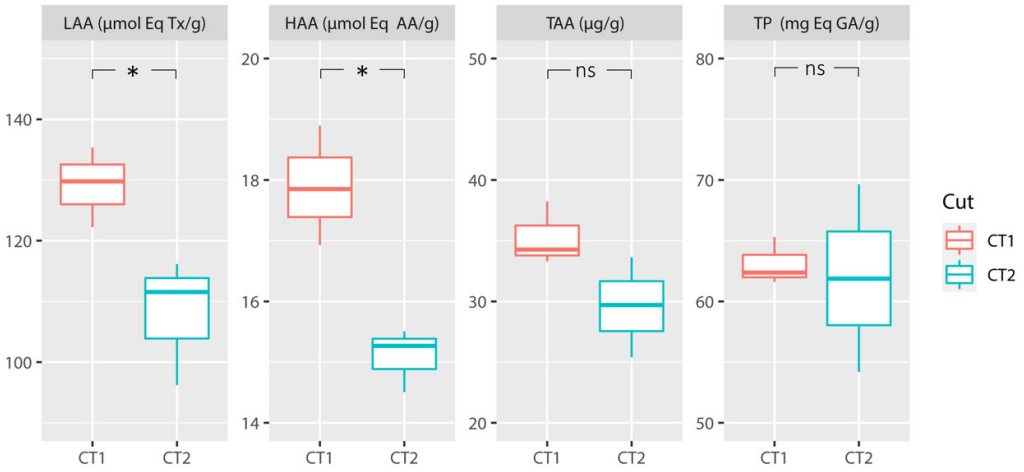

Figure 4. Box and whisker plots of antioxidants properties. LAA: lipid soluble antioxidant activity; HAA: water soluble antioxidant activity; TAA: total ascorbic acid: TP: total polyphenols. CT1: first cut; CT2: second cut. Asterisks denote a statistically significant difference (*: $p < 0.05$; ns: not significant) according to the Student's t-test.

3.4. Metabolomics

To investigate the changes in secondary metabolites between leaves of the first and second harvest, we performed an untargeted metabolomics analysis.. We detected 1820 features that were present in both conditions, which returned 661 compounds after pre-processing (filtering and normalization). Significant differences between the two conditions were evaluated with a Student's t-test. Differentially accumulating compounds were identified by the following conditions—$|\log2FC| > 1$ and an FDR < 0.05 (Figure S1). In total, 87 (respectively, 119) compounds were classified as accumulated in higher (resp. lower) quantities in the leaves of the second cut. These compounds were classified in seven categories, namely "Amino acids and derivatives", "Fatty acids", "Phenylpropanoids" (including flavonoids), "Plant hormones", "Secondary metabolites" not included in the other categories (such as alkaloids and secondary alcohol metabolites), "Terpenoids", and "Others" (which also includes primary metabolism and not classified metabolites) (Tables S1 and S2). Based on the absolute number of differentially accumulating compounds, the largest difference between leaves of the two cuts was in the specialized metabolites (Figure 5). Compared to the first cut, leaves of the second harvest showed a significant reduction of a variety of phenylpropanoids (such as simple phenylpropanoids, flavonoids and lignans) and other secondary metabolites (e.g., alkaloids), accompanied by an increase of terpenoids. Metabolomics indicated that fatty acids were only down represented at CT2. According to the ontology of the compounds, the most significantly down regulated pathway was the phenylpropanoid biosynthesis. For instance, both important blocks (e.g., coumarates, hydroxy-cinnamates) and key intermediates (e.g., caffeic acid and its derivatives) were present in lower abundance at CT2. Although below our threshold, also the amount of eugenol and isoeugenol, among main components of the basil aroma, was significantly lower at the second cut (eugenol: log2FC = −0.98, p value < 0.001; isoeugenol: log2FC = −0.98, p value < 0.001). At the second cut, gibberellins (i.e., six compounds, such as GA24, GA37, GA44 and GA97) were the only down represented plant hormones, while cytokinins (i.e., three compounds such as dihydrozeatin-7-N-glucose and dihydrozeatin-9-N-glucoside), along with one auxin and one gibberellin, were present in higher quantity. Leaves at CT2 had a higher amount of terpenoids, especially those included in the large class of diterpenoids.

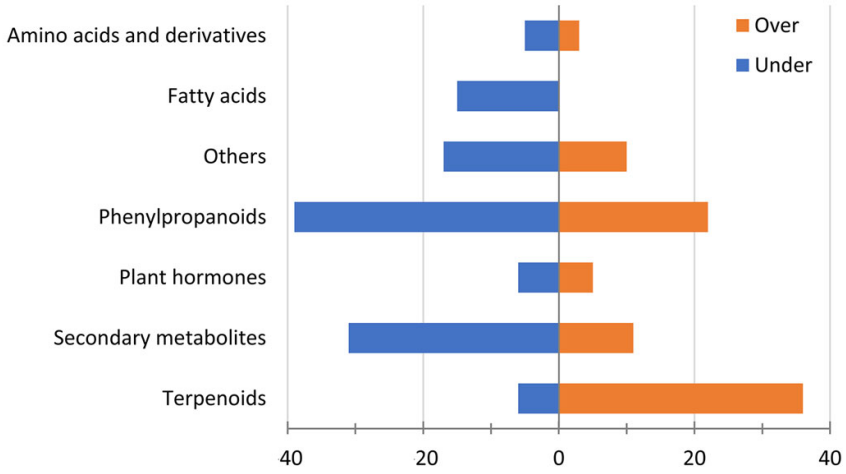

Figure 5. Distribution of the differentially accumulating metabolites between leaves of the two successive harvests. For each class of chemical compounds, the bars display the number of statistically different compounds that are present in higher ("Over" orange color) and lower ("Under" blue color) amount in the leaves of the second harvest. See text for the criteria of inclusion of the metabolites in each class. Metabolites and their classification are reported in Supplementary Tables S1 and S2.

Overall, metabolomics indicated a distinct modification of the secondary metabolite profiles in leaves that is consistent with current models of metabolic flux redirection in basil [44].

4. Discussion

Consumer demand for fresh, aromatic, functional, low-calorie food garnishes has allowed sweet basil to emerge as a growing market segment worldwide [2]. This species has become a high-income niche crop that for its fast production cycle can boost farm's cash flow as well as the profitability of hydroponics. Although multiple cuts are feasible, basil is usually harvested two to three times when cultivated between plantings of main crops. Our study indicated that various parameters differ between the leaves of the first two consecutive harvests. We observed a yield reduction [45], as also reported for other leafy vegetables [20,22,46], which in our case was not significantly related to changes in water content. Briefly, after the first harvest, plants produced a higher number of smaller leaves. Plants were harvested at approximately the same height, resulting in a lower harvest index at the second cut. The lower yield associated to a modest reduction of some quality attributes, such as the antioxidant capacity and nitrate content, while the effect on the metabolic profile was more evident, with a switch towards an increased production of terpenoids.

In basil, the CC technique is different from other leafy vegetables because not only the leaves but also the stem, with its apical meristem, is removed. During the first phase of growing, basil plants were more efficient in allocating dry matter for yield and this higher productivity is evident also considering the gas-exchange parameters and the photosynthetic water-use efficiency. After the first harvest, biomass allocation was more directed towards the stem growth, which should also cover increased branching and possibly stem thickness, as indicated by the higher number of leaves and the stem dry weight, respectively. There is a consensus that SLA reflects the expected return in dry mass growth of previously acquired resources, leading to the conclusion that leaves with higher SLA are more productive [47]. Hydroponics allowed to consistently deliver nutrients to the plants, which grew in absence of intraspecific competition for resources, ruling out two of the most evoked sources of reduction of SLA [48–50]. Considering that at CT2 leaves are more numerous and succulent, and have a lower An per nitrogen, the variation of leaf structure indicated by the SLA is more likely due to inter-leaf shading [51], with carbon fixation not occurring at maximum rate not because of a limitation of nitrogen-containing photosynthetic compounds [52]. Although there are different mineral elements that affect vegetative growth, nitrogen is crucial for crop yield in virtually all leafy

vegetables [53,54]. Our work indicated that the cut affected nitrogen concentration and NUE in basil. The total nitrogen accumulation in leaves was not different, reflecting the invariable N fertilization in the two phases. Nonetheless after the first cut, the leaf-level nitrogen use efficiency decreased, possibly because nitrogen concentration declines with leaf growth [2]. The higher nitrogen concentration of the leaves of the second cut has two main implications. Firstly, the data denote a luxury consumption [55], leading to elevated tissue concentration of non-protein nitrogen after the first cut. Moreover, nitrate concentration in edible products must be under a safety level according to specific regulations [56]. Although the nitrate amount in leaves of the second cut was not above the legal threshold of other leafy vegetables, a low nitrate content is considered a quality trait considering the multiple sources of this anion in the human diet [57]. For these two reasons, our work underlined the need of more tailored nitrogen fertilization schemes for successive cuts of CC vegetables. However, it remains unaddressed whether the accumulation of nitrates after the first cut is a plastic, juvenile response following tissue loss (e.g., a storage), of little use for basil in the standard short-interval harvest of the crop.

Plant clipping and defoliation have been mainly studied in relation to pasture grasses (which may also be perennial and not always fertilized). These procedures typically result in a biomass reduction, although compensatory positive effects on the growth rate following defoliation have been also reported [58,59]. Defoliation also enhances the above-ground N yield of grasses [60], because of an increase uptake and preferential allocation of nitrogen to the shoots [61]. In the same way as our results, single clipping reduced leaf biomass in ryegrass and increased cytokinin concentration in leaves [62]. Although the morphogenetic activity of cytokinins in leaves is intricate [63], this hormone regulates leaf primordia number by stimulating cell division [64]. Moreover, low levels of the cytokinin isopentenyl adenine associate with stomatal closure [65]. On the other hand, gibberellins were the only detected plant hormones present in smaller quantities at CT2. Their promoting role in leaf growth, cell elongation and photosynthetic activity is well established and for instance, application of gibberellins in leafy vegetables (e.g. lettuce and rocket) increased leaf biomass [66].Metabolomics also indicated at the second harvest a reduced production of phenylpropanoids and an increase of terpenoids. A systems biology approach suggested that in basil the "carbon flow" is competitively directed towards the shikimate or the MEP pathways, used for phenylpropanoid and terpenoid biosynthesis, respectively [67]. The competitive metabolic relation between phenylpropanoids and terpenoids has been evoked to explain why *Ocimum* species and basil cultivars are rich either in phenylpropanoids or terpenoids [44,67]. Differences in terpenoids and phenolic compounds between leaves of different cuts were also described in salad burnet (*Sanguisorba minor* Scop.) and sorrel (*Rumex acetosa* L.) [68,69]. In the future it will be interesting to test if the modulation of agronomic factors (e.g. the nutrient solution or more generally those affecting the photosynthetic rate) [70] will be able to counteract the consequences of the cut, with the aim to have a more constant yield and quality in successive harvests, but also to switch metabolic fluxes for the production of specific classes of secondary metabolites.

5. Conclusions

Basil, as other CC leafy vegetables, offers different possibilities for harvesting. The choice should depend on the destination of the production (e.g., fresh market, oil extraction, food industry) and related technological (e.g., handling, processing) and quality attributes (e.g., phytochemicals). Our study indicated that the differences between two consecutive cuts are wide-ranging and included a modest reduction of some quality attributes and an ample variation of the profile of bioactive compounds. From the agronomic point of view, the CC harvesting strategy may require an adaptation of the NS composition, not only for the diminished return of the second harvest but also to optimize the use of nitrogen input, to limit nitrate accumulation in edible organs and possibly, to specifically alter the leaf metabolic profile.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Volcano plot of the metabolites after filtering and normalization. Each metabolite, represented by a circle, is colored according to the set threshold of the fold change (iris blue: $|\log2FC| > 1$; salmon: $|\log2FC| \leq 1$), Table S1: List of the

identified compounds over-represented in basil leaves of the second cut (CT2). Table S2: List of the identified compounds under-represented in basil leaves of the second cut (CT2).

Author Contributions: Methodology, G.C. (Giandomenico Corrado), L.L. and Y.R.; formal analysis, G.C. (Giandomenico Corrado) and Y.R.; investigation, G.C. (Giandomenico Corrado), L.L., B.M.-M., P.C. and Y.R.; writing—original draft preparation, G.C. (Giandomenico Corrado); writing—review and editing, G.C. (Giandomenico Corrado), P.C., G.C. (Giuseppe Colla) and Y.R. All authors have read and agreed to the published version of the manuscript.

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

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