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# Influence of Living Mulch and Nitrogen Dose on Yield and Fruit Quality Parameters of *Malus domestica* Borkh. cv. 'Sampion'

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**Abstract:** This study was conducted to estimate the yield, and to identify and quantify primary and secondary metabolites in fruit of *Malus domestica* Borkh. cv. 'Sampion' under two agrotechnical factors: the floor management (herbicide fallow and living mulch) and the dose of nitrogen (50, 80, 110, and 140 kg ha$^{-1}$). Compared to herbicide fallow, living mulch did not decrease yield. Research showed a rich composition of phenolic and volatile organic compounds in apples, which varied with the evaluated factors, as well as with the weather conditions during the vegetation season. The precipitation deficit and high summer temperatures did not contribute to proper fruit growth and development and led to a higher content of phenolic compounds in the fruit flesh from trees in herbicide fallow compared to living mulch. Living mulch, which could be a factor regulating the availability of nitrogen to trees, stimulated the synthesis of anthocyanins, which was also potentiated by low average temperatures at harvest time, resulting in a large area of fruit skin red blush.

**Keywords:** apple tree; cover crop; sugar; organic acid; phenolic compounds; volatile organic compounds (VOCs)

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## 1. Introduction

Apples are a rich source of phenolic compounds, which are classified as secondary metabolites and are known for their beneficial effects on human health, especially their onco-preventive and cardiovascular disease roles [1,2]. In the last fifteen years, phenolic compounds and antioxidant activity analyses have been carried out on many cultivars of apple trees grown in various regions of the world [3–6]. In recent studies, more attention has been given to old cultivars [7–9] and local genotypes [10–12] with the aim to increase knowledge regarding apples. Different apple production technologies, such as organic low input [13,14]—similar to integrated [15] and typical integrated production [16,17]—have also been the subject of scientific research as a possibility of influencing the content of the metabolic profile of fruits. Despite ambiguous results, the organic system seems to increase the content of phenolic compounds in apple fruit due to the presence of a higher biotic and abiotic stress in comparison to conventional production [18]. Moreover, selected rootstocks can also have a beneficial effect on the amount of fruit phenolic compounds [19,20]. Similarly, the replacement of the herbicide fallow by living mulch under apple trees can also stimulate the synthesis of secondary metabolites in apple fruit, especially anthocyanins [21,22], which is directly related to the red blush of the apple skin [23]. Fruit quality parameters, such as size and color, as well as their flavor and taste, are important sensory attributes for consumers [24]. In apples, fruit color is mainly determined by anthocyanins [25], which increase when the tree is deprived of excessive amounts of nitrogen and has moderate growth [26]. Nitrogen dose should also improve fruit size and

impact more significantly in secondary rather than on primary metabolism. Tree cultivation technology and cultivar should therefore be selected carefully because they affect the content and biosynthesis of different metabolites, such as phenolic compounds [18,25] and aromatic volatile compounds [27,28].

The use of living mulch reduces the negative impact of agriculture on the environment and specifically on the soil. Its importance as an alternative orchard floor management compared to herbicide fallow increases due to the recent restrictions on the use of glyphosate—the most common active ingredient of herbicides [29]. Living mulch may restrict weed populations in the orchard [30,31] and increase flora biodiversity [32,33] and soil organic matter [34,35]. In addition, plants under tree canopies may accumulate nitrogen in vegetative parts, part of which would be restored to the soil after living mulch sod mowing [36]. However, from the point of view of fruit production, the biggest problem of the use of living mulch is its competition for water and nutrients with the trees [34], which could result in a decrease in the yield [35,37,38] and fruit size [39,40]. The appropriate dose of nitrogen should satisfy the nutritional requirements of trees, ensure high yield, and at the same time avoid negative impacts on the environment and depletion of the soil. Moreover, nitrogen fertilization in the range of 50–100 kg ha$^{-1}$ provides fruit and leaves with sufficient amounts of this element [41]. However, increasing the dose of nitrogen does not always result in a significant increase in tree growth, crop efficiency coefficient, and yield [41,42].

The Czech cultivar 'Sampion' occupies an important place in the ranking of apple production in Central Europe, especially in Poland—a country with the third largest apple production in the world, after China and the U.S.A. It has become the most important cultivar in recent years, preceded only by cultivars 'Idared' and 'Jonagold' [43]. The aim of this study was to evaluate the effect of different doses of nitrogen on *Malus domestica* cv. 'Sampion' tree growth, yield, and fruit quality parameters under two orchard floor management systems: herbicide fallow and living mulch. The evaluated cultivar 'Sampion' was subjected to LC-MS testing, which allowed identification of primary and secondary metabolites, as well as GC to identify the volatile organic compounds of the fruit.

## 2. Materials and Methods

### *2.1. Field Experiment and Plant Material*

The field experiment was established at the Fruit Experimental Station of the Wrocław University of Environmental and Life Sciences in Wrocław (Poland), in Samotw ór (51°06′12″ N, 16°49′52″ E). One-year-old apple trees (*Malus domestica* Borkh.) cv. 'Sampion', grafted on a semi-dwarf M.26 rootstock, were planted in spring 2015, on haplic Luvisol. The chosen spacing was 3.5 m × 1.2 m (2380 trees ha$^{-1}$). The plant material nursery stock was unbranched, with average trunk diameters of 1.15 cm. The apple trees were trained into the slender spindle form of the canopy, and they were cut and protected in accordance with the current commercial orchard recommendations [44]. Manual hand thinning of fruit sets was carried out, only in the over crop years 2016 and 2019. In the year 2015, each tree was manually fertilized with ammonium nitrate (15 g N per tree) under the tree canopy. The was no irrigation in the orchard.

During the last two decades (2001–2020), the average annual temperature was 9.9 °C and the sum of rainfall was 534.7 mm. In 2015, due to unusual weather conditions, heavy hail damaged the leaves and caused wounds on trunks and branches, which caused the slower growth and development of young trees in the first years after the establishment of the orchard.

The experiment was conducted from spring 2016 to autumn 2022. It was established following a two-way randomized block design, with four replications, comprising two orchard floor management systems: herbicide fallow and living mulch, and four doses of nitrogen fertilization: 50, 80, 110, and 140 kg N ha$^{-1}$, resulting in 8 treatments by orthogonal combination of both factors. Each replication consisted of a plot (6 m$^2$) with five trees. In the period 2016–2020, the application of nitrogen was divided into two parts. Ammonium

nitrate was spread over the plots at the end of April and in the middle of June. During the period 2021–2022, fertilization in the orchard was carried out only once, in mid-May.

Herbicide fallow was treated with a mix of glyphosate (1.44–1.96 kg $ha^{-1}$) and MCPA (2-methyl-4-chlorophenoxyacetic acid, 0.60–1.00 kg $ha^{-1}$) three times a year: in spring (April or May), summer (July), and in the case of some years, at the end of the vegetation period. The perennial living mulch was the blue fescue (*Festuca ovina* L.) cvs. 'Noni' and 'Ridu', in ratio 1:1, sown at 50 kg seeds $ha^{-1}$ in May 2016. The grass sod in the rows was mowed manually, twice or thrice per vegetation season, with a string trimmer, which ensured the return of nutrients to the soil after subsequent years of orchard management [35].

Three middle trees on the plot were selected for estimation of the yield (2019–2022). As a measure of tree growth, trunk cross-sectional area (TCSA) and its increment were calculated as an average of the diameter measured in two directions (north–south and east–west), measured 30 cm above the grafting point. The measurements were made in spring 2015 and in autumn 2022. The crop efficiency coefficient (CEC) was computed as a ratio of the total yield of four years (2019−2022) and TCSA in autumn 2022.

Fruits for laboratory analysis were harvested in autumn 2022 at the average harvest time for the 'Sampion' cultivar in the Lower Silesia area (firmness: 6.4 kg per $cm^2$, starch test: 2.8). Eight fruit samples from each treatment (two from each field replication) were selected randomly. Apples were stored at 2 °C for one month before being delivered to the laboratory of the University of Ljubljana (Slovenia), Chair for Fruit, Wine, and Vegetable Growing, and subjected to analysis for the content of primary and secondary metabolites.

### *2.2. Fruit Quality Parameters*

The red blush and size of fruit were measured immediately after harvest. Fruits were sorted manually by red blush area into three groups: >75%, 25–75%, and <25% of blush on the apple skin surface area. Samples were also sorted into three classes based on the fruit diameter: <6.5; 6.5–7.5, and >7.5 cm.

Only in the year 2022, skin color was measured numerically on 6 fruits per treatment. Ground and red-blush color were measured with a colorimeter (CR-10 Chroma, Minolta, Osaka, Japan), and expressed as CIELAB parameters, where L* represents lightness (0: black, 100: white) and a* the color on the red–green axis. The hue angle (h°) was expressed in degrees from 0 to 360° (0°—red, 90°—yellow, 180°—green, and 270°—blue). Fruit weight was measured with a digital scale of 14 fruit per treatment.

### *2.3. Analysis of Individual Sugars and Organic Acids*

Five grams of sample were mixed with 25 mL of double-distilled water and homogenized with a homogenizer (T25 basic Ultra-Turrax, IKA Labortechnik, Janke and Kunkel GmbH, Staufen, Germany). They were left for half an hour at room temperature with constant stirring. Samples were then centrifuged (Eppendorf Centrifuge 5810 R, Hamburg, Germany) at 8000× rpm for 8 min at 4 °C. The supernatants were filtered through a 0.25 µm cellulose mixed ester filter (Macherey-Nagel, Düren, Germany), poured into vials, and analyzed using high-performance liquid chromatography (HPLC; Thermo Scientific, San Jose, CA, USA). For the analysis of organic acids, a Rezex ROA Organic acid column (300 mm × 7.8 mm) (Phenomenex, Torrance, CA, USA), heated to 65 °C, with 4 mM sulfuric acid as the mobile phase at 0.6 mL/min flow rate and a UV detector set to 210 nm, was used. For the determination of sugars, a Rezex RCM-monosaccharide column (300 mm × 7.8 mm) (Phenomenex, Torrance, CA, USA) heated to 85 °C, with double-distilled water as the mobile phase at 0.8 mL/min flow rate and a RI detector, was used. The concentrations were calculated using the calibration curve of corresponding external standards of known concentrations and expressed as mg/g of fresh weight (FW).

### *2.4. Analysis of Individual Phenolic Compounds*

The apples were peeled with a hand peeler, and peel and pulp were shock-frozen in liquid nitrogen separately. The peel was ground to a fine powder in a mortar, while the

pulp was shredded with a knife. The samples (10 g of pulp or 5 g of peel) were extracted with 10 mL of methanol containing 3% (*v*/*v*) formic acid in a cooled ultrasonic bath for 1 h. Later, samples were centrifuged (Eppendorf Centrifuge 5810 R, Hamburg, Germany) at 8000× rpm for 8 min at 4 °C. The supernatant was filtered through a Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany). Phenolic compounds were analyzed on a Dionex UltiMate 3000 series UHPLC+ focused (Thermo Scientific, San Jose, CA, USA) using a Gemini C18 column (150 mm × 4.6 mm, 3 µm, Phenomenex) heated at 25 °C and a diode array detector set at 280 nm (flavanols and phenolic acids), 350 nm (flavonols), and 530 nm (anthocyanins).

Phenolic compounds were identified and quantified by comparing their retention times with external standards and also confirmed with a mass spectrometer (Thermo Fisher Scientific, LCQ Deca XP MAX) with an electrospray interface (ESI) operating in negative (phenolic acids, flavonols, and flavanols) or positive (anthocyanins) ion mode. Full scan scanning was performed from m/z 115–2000. All conditions on HPLC and MS have been reported in detail previously [45].

### *2.5. Determination of the Total Phenolic Content*

Determination of the total phenol content was conducted using the Folin–Ciocalteu phenol reagent procedure [46]. Bi-distilled water (7.9 mL) was pipetted into centrifuge tubes, followed by 100 µL of the extract and 500 µL of the Folin–Ciocalteau reagent. After allowing the samples to stand at room temperature for a few minutes, 1.5 mL of 20% sodium carbonate (*w*/*v*) was added. The extracts were mixed and then heated in an oven at 40 °C for 30 min. Absorbance was measured at 765 nm using a Genesys 10S UV-VIS spectrophotometer (Thermo Scientific, San Jose, CA, USA). The same mixture was used for the blank sample, but 100 µL of methanol was used instead of the extract. Total phenolic content was calculated based on the standard curve of gallic acid and expressed as gallic acid equivalents (GAE) in mg/kg fresh weight (FW).

### *2.6. Extraction of Aroma Compounds*

The volatiles' profile was obtained by gas chromatography analysis (HS-GC-MS). Frozen apple material, including skin and pulp, was ground to fine powder using liquid nitrogen and an analytical mill (IKA A11 basic, Staufen, Germany). The powder (5 g) was placed in 20 mL vials and 10 µL of internal standard (IS: 3-Nonanone, 1:8000, 0.09 mg/mL in acetonitrile) was added. The vial was closed with a screw cap with a PTFE-silicon septum and transferred to a Shimadzu AOC-20s autosampler, where it was incubated at 50 °C for 10 min with constant shaking at 250 rpm. An amount of 1000 µL of the headspace portion was injected in 1:10 split mode for 0.4 min into the injection port at 250 °C at a 25 mL/min injection rate. A Shimadzu GC-MS QP2020 gas chromatograph, connected to a Single Quadropole MS with an EI detector, was used. A ZB-wax PLUS capillary column (30 m × 0.25 mm, 0.5 µm film thickness) was used for the separation of the volatile compounds. The carrier gas was helium with a 4 mL/min flow rate. The temperature program was set as follows: first, hold at 45 °C for 3 min, then raise to 150 °C at a rate of 4 °C/min, then raise again to 220 °C at 10 °C/min, and finally hold at 220 °C for 5 min. The temperature of the interface and the MS ion source was set at 240 °C, the scan rate at 2.0 scan/s, the ionization energy at 70 eV, and the mass scan range at 50–500 m/z. Volatiles of the samples were identified based on their retention indices (RIs) and commercial libraries of spectra (NIST 11 and FFNSC 4), and they were semi-quantified based on each compound and internal standard peak areas, and the internal standard and sample weight.

### *2.7. Chemicals and Standards*

For the extraction and analysis of metabolites, HPLC- or MS-grade methanol, acetonitrile, and formic acid for the extraction were purchased from Sigma-Aldrich (Steinheim, Germany). The following standards were used for the quantification of phenolic compounds: quercetin-3-glucoside, p-coumaric acid, kaempferol-3-rutinoside, kaempferol-3-glucoside,

delphinidin-3-O-glucoside chloride, peonidin chloride, and pelargonidin chloride (Fluka Chemie, Buch, Switzerland); 3-nonanone, gallic acid, quercetin-3-rutinoside, ferulic acid, luteolin 7-O-β-D-glucoside, 3-caffeoylquinic acid, and 5-caffeoylquinic acid (Sigma-Aldrich); isorhamnetin-3-glucoside, cyanidin 3-O-galactoside chloride, and petunidin chloride (Extrasynthese, Genay, France). Double-distilled water, purified with a Mili-Q Millipore system (Merck Millipore, Billerica, MA, USA) was used to perform the extraction and analyses of all metabolites.

### *2.8. Statistical Evaluation*

All data were evaluated statistically using the one-way analysis of variance (ANOVA) in R software (version 4.2.2). Significant differences between treatment means were calculated with the Tukey test ($p < 0.05$). Means are presented with standard deviations (mean ± SD). In addition, a two-way analysis of variance (ANOVA) was also performed between treatments to identify the effects of the dose of nitrogen (D), floor management (FM), and the interaction of these two factors (D × FM) on the variables. Statistically significant differences were obtained at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

## 3. Results and Discussion

### *3.1. Growth and Yield of Tree, and Fruit Size*

In the presence of living mulch, a decrease in the yield was observed (Table 1), which agrees with previous studies showing the negative impact of living mulch on the yield of young trees [35,40,47], as well as under the crowns of older trees [38]. In the presented experiment, the yield was not affected by the different doses of nitrogen. Similarly, doubling the dose of this nutrient to 100 kg ha$^{-1}$ in an orchard with herbicide fallow did not bring significant changes in the yield [41,42].

**Table 1.** Mean yield and tree growth depending on two floor management systems and dose of nitrogen (mean ± SE, $n = 4$).

| Specification | Dose of Nitrogen (kg·ha$^{-1}$) and Floor Management | | | | | | | | D | FM | D × FM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | | 80 | | 110 | | 140 | | | | |
| | H | LM | H | LM | H | LM | H | LM | | | |
| Mean yield 2019–2022 (kg·tree$^{-1}$) | 9.62 ± 1.81 a | 5.38 ± 1.48 a | 5.67 ± 2.05 a | 4.74 ± 2.42 a | 9.32 ± 3.29 a | 5.94 ± 2.73 a | 6.43 ± 2.26 a | 4.95 ± 1.78 a | NS | ** | NS |
| TCSA increment 2015–2022 (cm$^2$) | 9.8 ± 0.73 a | 7.94 ± 0.92 a | 6.27 ± 3.17 a | 7.61 ± 1.17 a | 8.6 ± 2.11 a | 7.87 ± 1.25 a | 6.16 ± 1.5 a | 7.62 ± 2.6 a | NS | NS | NS |
| Crop efficiency coefficient 2019–2022 (kg·cm$^{-2}$) | 0.98 ± 0.15 a | 0.56 ± 0.18 a | 0.87 ± 0.31 a | 0.89 ± 0.77 a | 0.94 ± 0.23 a | 0.64 ± 0.36 a | 0.90 ± 0.23 a | 0.76 ± 0.46 a | NS | NS | NS |

TCSA—trunk cross-sectional area, H—herbicide fallow, LM—living mulch, D—dose of nitrogen, FM—floor management, NS—not significant ** Statistically significant differences at $p < 0.01$,. Mean marked with different letters in rows represent statistical differences among eight treatments (ANOVA, Tukey test).

Similarly to yield, the growth of apple trees cultivated with living mulch under four different doses of nitrogen were lower compared to herbicide fallow (Table 1), suggesting that the applied nitrogen was eagerly taken up by both apple trees and living mulch, therefore affecting apple tree development. Other research works show that the soil covered with grass mulch had significantly lower nitrogen content compared to mechanical cultivation [38] and legume cover crop [48]. Competitive interaction between apple tree and vegetative cover affects not only tree growth and yield, but also fruit quality and storage properties [38], especially in a young orchard [39,47]. However, in this study, the growth of older apple trees did not differ significantly between treatments, which was also observed in other experiments [37,40] with several-year-old apple trees. Weed species, especially annuals, also appear under the tree canopy every year, a few weeks after the first spring application of herbicides and nitrogen in the orchard [31,49], which also take some of the nitrogen from the soil. Both the periodic presence of weeds in the herbicide fallow and the living mulch, especially in the last years of research (2020–2022) with a deficit of

precipitation in spring (Table S1), did not stimulate tree growth, even when the nitrogen dose was 140 kg ha$^{-1}$.

Compared to standard yielding of cv. 'Sampion', yields were lower in all treatments. This could be related to the quality of planting material, initially with an average trunk diameter of just over 1 cm, and the biotic and abiotic stress factors that affected them in the first year, especially the damage as a result of hail in the summer of 2015 (Table S1), as well as in the second year, the presence of grubs causing damage to the root system. This affected the growth of young trees, and also most likely in the subsequent years. The age of plant material and methods used for improving feathering had an influence on the intensity of the blossoming and on the yielding of apple tree [50,51]. In addition, in the last period of the research (2021–2022) adverse weather conditions were also recorded. The research of Le Bourvellec et al. [15] emphasizes the impact of yearly conditions in the orchard, especially on the quality of fruit, under which the management system has a limited effect. However, in their studies, the yield in the organic system was lower due to lower weight and smaller fruit size, and similarly the fruit weight and size in organic system were significantly lower than those obtained in the conventional one [14]. On the contrary, in our research, an increase in the share of small fruits (<6.5 cm) was observed on trees maintained in herbicide fallow (Table 2). When applying a dose of 140 kg of nitrogen ha$^{-1}$, the share of the smallest size of fruit was significantly lower in living mulch compared to herbicide fallow. Similarly, a significant reduction in fruit weight was also noted at this dose (Table 3). The smaller size of fruits probably resulted from a slightly higher yield in all treatments with herbicide fallow.

**Table 2.** Red blush and size of fruit under two floor management systems and different doses of nitrogen, mean for 2019–2022 (mean ± SE, $n$ = 4).

| Specification | Dose of Nitrogen (kg·ha$^{-1}$) and Floor Management | | | | | | | | D | FM | D × FM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | | 80 | | 110 | | 140 | | | | |
| | H | LM | H | LM | H | LM | H | LM | | | |
| | % of fruit with blush on skin surface area: | | | | | | | | | | |
| >75% | 49.4 ± 5.9 ac | 76.0 ± 7.6 e | 53.5 ± 8.8 acd | 73.1 ± 14.8 de | 43.6 ± 5.5 ab | 61.2 ± 7.4 bce | 39.8 ± 5.7 a | 65.6 ± 8.3 ce | * | *** | NS |
| 25–75% | 38.2 ± 7.9 ad | 21.1 ± 4.2 a | 44.1 ± 9.1 bd | 26.4 ± 14.0 ab | 50.0 ± 6.4 cd | 37.9 ± 7.3 ad | 56.1 ± 5.7 d | 34.0 ± 7.8 abc | ** | *** | NS |
| <25% | 12.3 ± 2.8 b | 2.9 ± 3.7 a | 2.4 ± 3.4 a | 0.5 ± 0.8 a | 6.4 ± 4.9 ab | 0.9 ± 0.8 a | 4.1 ± 0.3 a | 0.3 ± 0.6 a | *** | *** | NS |
| | % of fruit with diameter: | | | | | | | | | | |
| >7.5 cm | 11.5 ± 2.7 a | 13.3 ± 7.9 a | 8.8 ± 5.4 a | 15.8 ± 7.3 a | 8.1 ± 4.3 a | 14.2 ± 6.0 a | 3.8 ± 1.2 a | 18.8 ± 13.1 a | NS | ** | NS |
| 6.5–7.5 cm | 25.4 ± 8.5 ab | 34.3 ± 6.8 ab | 29.0 ± 7.8 ab | 43.0 ± 6.6 b | 24.4 ± 15.4 ab | 44.0 ± 4.5 b | 20.4 ± 10.4 a | 41.3 ± 5.0 b | NS | *** | NS |
| <6.5 cm | 63.1 ± 6.4 ab | 52.4 ± 14.5 ab | 62.2 ± 9.4 ab | 41.2 ± 5.3 a | 67.6 ± 18.9 ab | 41.8 ± 9.5 a | 75.8 ± 11.5 b | 39.9 ± 18.0 a | NS | *** | NS |

H—herbicide fallow, LM—living mulch, D—dose of nitrogen, FM—floor management, NS—not significant, * Statistically significant differences at $p < 0.05$, ** Statistically significant differences at $p < 0.01$, *** Statistically significant differences at $p < 0.001$. Mean marked with different letter in rows represent statistical differences among eight treatments (ANOVA, Tukey test).

In general, in both orchard management systems, the fruit size was smaller than expected. This was probably also related to the weather conditions at the time of fruit setting, when the intensity of cell division of the fruitlets and cell enlargement stage were insufficient due to precipitation deficit and high mean temperatures in the year 2022 (Table S1). On the contrary, as other studies in young orchards have shown, floor management systems, rather than yield, determined a significant reduction in the quantity of large fruits (7.0–8.0 cm of diameter) [39] or fruit weight [40]. When the trees entered the full cropping period, the importance of orchard floor management decreased [22,40].

**Table 3.** Fruit weight and quality parameters depending on two floor management systems and dose of nitrogen, in the year 2022 (mean ± SE, $n = 4$).

| | Dose of Nitrogen (kg·ha$^{-1}$) and Floor Management | | | | | | | | D | FM | D × FM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | | 80 | | 110 | | 140 | | | | |
| | H | LM | H | LM | H | LM | H | LM | | | |
| Fruit weight (g) | 100 ± 13 ab | 119 ± 10 b | 90 ± 9 ab | 124 ± 17 b | 98 ± 18 ab | 115 ± 17 ab | 79 ± 12 a | 118 ± 25 b | NS | *** | NS |
| Color red blush: | | | | | | | | | | | |
| L | 38.1 ± 1.4 a | 34.7 ± 1.8 a | 36.5 ± 1.7 a | 36.2 ± 2.7 a | 37.5 ± 1.3 a | 37.1 ± 1.9 a | 37.8 ± 0.9 a | 37.2 ± 2.2 a | NS | NS | NS |
| a | 34.8 ± 1.5 a | 36.4 ± 1.7 a | 34.5 ± 1.5 a | 36.6 ± 1.6 a | 35.4 ± 0.5 a | 35.2 ± 1.9 a | 34.9 ± 2.2 a | 35.4 ± 1.0 a | NS | NS | NS |
| h | 35.0 ± 1.6 b | 30.5 ± 1.2 a | 33.0 ± 1.7 ab | 31.6 ± 2.4 ab | 33.5 ± 2.1 ab | 33.9 ± 2.4 ab | 33.4 ± 1.6 ab | 33.1 ± 2.1 ab | NS | * | NS |
| Ground side color: | | | | | | | | | | | |
| L | 60.1 ± 0.8 a | 62.7 ± 1.1 a | 61.0 ± 1.3 a | 61.5 ± 2.2 a | 61.6 ± 1.1 a | 60.1 ± 1.0 a | 61.2 ± 1.1 a | 61.6 ± 1.0 a | NS | NS | * |
| a | 3.5 ± 1.4 a | 9.6 ± 1.3 b | 3.8 ± 0.5 a | 5.1 ± 2.2 a | 3.4 ± 1.2 a | 5.0 ± 2.8 a | 3.9 ± 0.7 a | 5.0 ± 2.8 a | NS | *** | * |
| h | 85.7 ± 1.6 b | 78.2 ± 1.5 a | 85.1 ± 0.7 b | 83.5 ± 3.0 ab | 85.7 ± 1.5 b | 83.9 ± 3.3 b | 85.2 ± 1.0 b | 83.7 ± 3.6 b | NS | *** | * |

H—herbicide fallow, LM—living mulch, D—dose of nitrogen, FM—floor management, NS—not significant, * Statistically significant differences at $p < 0.05$, *** Statistically significant differences at $p < 0.001$. Mean marked with different letter in rows represent statistical differences among eight treatments (ANOVA, Tukey test).

### 3.2. Red Blush Area and Fruit Color

As previous studies have shown, living mulch has a beneficial effect on fruit color [38,40,52]. Our results show that under living mulch, when only 50 kg N ha$^{-1}$ was applied, the highest share of fully colored apples (>75% of the fruit skin area) was obtained (Table 2). High blushing of apples was determined by a lower availability of nitrogen for trees [38]. The synthesis of anthocyanins is responsible for the red color of fruit skin [23], and it is inhibited when high nitrogen fertilization stimulates strong shoot growth of the tree [25]. In the present experiment, although no significant development of tree vigor (TCSA) was obtained with an increase in the nitrogen dose, the importance of fertilization, as a decisive factor in reducing the red color area on the skin has been demonstrated. Increasing the nitrogen dose to 110–140 kg ha$^{-1}$ had an adverse effect on the share of apples with >75% of red blush on the skin surface. The presence of the living mulch mitigated this phenomenon. The effect of the floor management on the parameters of the red blush color of apple was negligible (Table 3), as has been demonstrated previously with other apple cultivars and management systems [14,21,22]. Minor statistical differences were found only in ground color parameters.

### 3.3. Sugars and Acids Content

The contents of sucrose, fructose, glucose, and sorbitol were measured (Table 4). The analyzes showed that sucrose and glucose were present in similar amounts. The dominant sugar was fructose. Apples from trees grown in living mulch, which were fertilized with a dose of 50 kg ha$^{-1}$, showed the highest content of this sugar. Only when the nitrogen dose was increased to 140 kg ha$^{-1}$ was the content of this sugar significantly lower. Apples from trees cultivated in the presence of living mulch had significantly less glucose compared to those obtained in herbicide fallow, but the concentration of sucrose in them increased. Despite these differences, total sugar content was not significantly different between the treatments. In some studies [21], the content of total sugars, including fructose, was significantly lower when the herbicide fallow in the tree rows was replaced by living mulch, while in other studies [22], the results were not coincident. The replacement of integrated apple tree management with an organic system, similarly to the change in floor management of our research, did not affect the total sugars in fruit [53]. A similar three-year study showed a significant effect of the cultivar, and above all the weather conditions during the growing season of trees, on the content of sucrose, glucose, fructose, and sorbitol

residue in the fruit. Tree management often had no significant effect on their content in the flesh [15].

**Table 4.** Effect of two floor management systems and dose of nitrogen on the content of individual and total (bold) sugars and organic acids in fruit, in the year 2022 (mean ± SE, mg $g^{-1}$ FW, $n = 8$).

| Specification | Dose of Nitrogen (kg·ha$^{-1}$) and Floor Management | | | | | | | | D | FM | D × FM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | | 80 | | 110 | | 140 | | | | |
| | H | LM | H | LM | H | LM | H | LM | | | |
| Sucrose | 24.97 ± 6.99 ab | 34.66 ± 7.26 b | 26.78 ± 3.68 ab | 28.70 ± 4.02 ab | 23.25 ± 5.25 a | 25.28 ± 9.18 ab | 22.84 ± 7.60 a | 29.95 ± 6.07 ab | NS | ** | NS |
| Glucose | 29.31 ± 3.60 bc | 20.83 ± 3.85 a | 28.61 ± 2.20 bc | 20.65 ± 3.92 a | 29.87 ± 3.67 bc | 24.79 ± 5.43 ab | 31.06 ± 2.61 c | 22.4 ± 3.29 a | NS | *** | NS |
| Fructose | 58.03 ± 2.46 ab | 59.6 ± 6.13 b | 57.79 ± 4.33 ab | 54.76 ± 1.55 ab | 57.04 ± 3.64 ab | 54.07 ± 2.66 ab | 55.79 ± 3.09 ab | 52.56 ± 3.05 a | ** | * | NS |
| Sorbitol | 3.44 ± 0.86 a | 4.92 ± 1.02 b | 3.92 ± 0.61 ab | 3.36 ± 0.67 a | 3.42 ± 0.89 a | 3.76 ± 1.12 ab | 4.02 ± 0.62 ab | 3.85 ± 0.91 ab | NS | NS | ** |
| **Total sugars** | **115.75 ± 8.76 a** | **120.01 ± 13.39 a** | **117.10 ± 7.88 a** | **107.47 ± 4.38 a** | **113.57 ± 12.33 a** | **107.91 ± 8.91 a** | **113.72 ± 9.66 a** | **108.76 ± 8.39 a** | **NS** | **NS** | **NS** |
| Citric | 1.88 ± 0.68 a | 1.53 ± 0.25 a | 1.92 ± 0.52 a | 1.48 ± 0.34 a | 1.77 ± 0.36 a | 1.81 ± 0.88 a | 2.10 ± 0.39 a | 1.34 ± 0.26 a | NS | ** | NS |
| Malic | 7.34 ± 1.23 ab | 7.57 ± 0.36 ab | 8.07 ± 1.11 b | 6.98 ± 0.40 ab | 6.79 ± 1.14 a | 7.14 ± 0.68 ab | 6.94 ± 0.26 ab | 6.92 ± 0.26 ab | NS | NS | * |
| Shikimic | 0.03 ± 0.01 a | 0.02 ± 0.00 a | 0.03 ± 0.00 a | 0.02 ± 0.00 a | 0.03 ± 0.01 a | 0.03 ± 0.01 a | 0.03 ± 0.00 a | 0.02 ± 0.00 a | NS | ** | NS |
| **Total organic acids** | **9.26 ± 1.87 a** | **9.12 ± 0.43 a** | **10.02 ± 1.58 a** | **8.48 ± 0.60 a** | **8.58 ± 1.47 a** | **8.97 ± 1.43 a** | **9.07 ± 0.49 a** | **8.28 ± 0.35 a** | **NS** | **NS** | **NS** |

H—herbicide fallow, LM—living mulch, D—dose of nitrogen, FM—floor management, NS—not significant, * Statistically significant differences at $p$ value < 0.05, ** Statistically significant differences at $p < 0.01$, *** Statistically significant differences at $p < 0.001$. Mean marked with different letters in rows represent statistical differences among eight treatments (ANOVA, Tukey test).

Among organic acids, malic, citric, and shikimic acids were determined (Table 4); the most abundant acid was malic acid. Their content most often did not differ significantly between the treatments, as was demonstrated in other cultivars [21]. In our experiment, the floor management influenced the amount of citric acid despite N dose. Other research shows that the content of these two acids in the flesh is not influenced by tree management systems [15].

### *3.4. Content of Phenolic Compounds in Apple Peel and Flesh*

Forty-two different individual phenolic compounds or their derivatives have been identified in cv. 'Sampion': 6 phenolic acids, 13 flavanols, 2 dihydrochalcones, 8 flavonols, and 3 anthocyanins (Table S2). The peel was a richer source of phenolic compounds than the pulp (Table S3). Phenolic compounds are secondary metabolites that are known to have beneficial effects on human health. Among them, quercetin is one of the most important ones in apples, as it is the most efficiently absorbed compound [2]. In addition, chlorogenic acid and procyanidins, as well as phloretin and phloridzin, which are all abundant, especially in the apple flesh, have a large impact on anticancer treatments [2].

#### 3.4.1. Phenolic Acids

Phenolic acids were identified in the peel and flesh of apple fruits (Tables 5 and 6). Three times more phenolic acids and their derivatives were identified in the flesh than in the skin (Tables S4 and S5). Chlorogenic acid, the most abundant phenolic acid in apple [2], was also the dominant one in cv. 'Sampion'. Compared to other cultivars, e.g., 'Idared' and 'Jonagold', the fruits of cv. 'Sampion' show lower amounts of phenolic acids [54], including chlorogenic acid [3,4]. In the present study, the method of floor management and nitrogen fertilization did not have a significant effect on the content of phenolic acids in the fruit skin (Table 5). However, the increase in the nitrogen dose and interaction with living mulch limited their synthesis in the flesh (Table 6). At the dose of 140 kg $ha^{-1}$ of nitrogen, the content of chlorogenic acid in apple pulp from trees grown in living mulch was almost two times lower compared to herbicide fallow (Table S5). Similarly, fruits from organic production were most often a rich source of chlorogenic acid [13]; however, sometimes the

synthesis of this compound was significantly higher in the conventional system, which can be compared to the herbicide fallow in our experiment. However, in other studies on living mulch [21], the synthesis of chlorogenic acid was most often not dependent on the presence of living mulch in the rows of apple trees.

**Table 5.** Effect of two floor management systems and dose of nitrogen on the content of different phenolic compounds' groups, and the total content of all analyzed phenolics (bold) in the peel of the fruit, in the year 2022 (mean ± SE, mg 100 $g^{-1}$ FW, $n$ = 8).

| | Dose of Nitrogen (kg·ha$^{-1}$) and Floor Management | | | | | | | | D | FM | D × FM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | | 80 | | 110 | | 140 | | | | |
| | H | LM | H | LM | H | LM | H | LM | | | |
| Total phenolic acids | 16.37 ± 3.59 a | 16.58 ± 2.27 a | 17.75 ± 1.55 a | 16.96 ± 3.59 a | 17.78 ± 1.7 a | 18.19 ± 5.69 a | 20.43 ± 2.8 a | 15.25 ± 4.51 a | NS | NS | NS |
| Total flavanols | 113.00 ± 21.05 ab | 120.15 ± 20.72 ab | 115.57 ± 12.07 ab | 126.04 ± 26.71 ab | 123.56 ± 12.17 ab | 139.72 ± 30.71 b | 135.03 ± 12.50 ab | 106.00 ± 21.34 a | NS | NS | * |
| Total dihydrochalcones | 11.62 ± 2.73 ab | 9.57 ± 1.46 a | 11.72 ± 1.58 ab | 9.52 ± 1.87 a | 11.36 ± 1.62 ab | 8.98 ± 1.46 a | 12.72 ± 1.76 b | 9.47 ± 2.50 a | NS | *** | NS |
| Total flavonols | 50.71 ± 8.46 a | 46.67 ± 5.63 a | 52.60 ± 8.43 a | 52.49 ± 16.74 a | 56.66 ± 3.55 a | 52.97 ± 13.34 a | 62.59 ± 10.22 a | 53.97 ± 8.91 a | NS | NS | NS |
| Total anthocyanins | 3.27 ± 1.25 a | 6.26 ± 1.35 b | 3.97 ± 0.59 a | 4.99 ± 1.92 ab | 3.80 ± 1.03 a | 4.49 ± 1.57 ab | 3.76 ± 0.86 a | 4.22 ± 0.91 a | NS | *** | * |
| **Total APC** | **194.97 ± 31.81 a** | **199.24 ± 22.93 a** | **201.62 ± 18.37 a** | **210.01 ± 45.56 a** | **213.17 ± 14.06 a** | **224.35 ± 44.58 a** | **234.53 ± 26.42 a** | **188.91 ± 30.06 a** | **NS** | **NS** | * |

APC—analyzed phenolic compounds, H—herbicide fallow, LM—living mulch, D—dose of nitrogen, FM—floor management, NS—not significant, * Statistically significant differences at $p < 0.05$, *** Statistically significant differences at $p < 0.001$. Mean marked with different letters in rows represent statistical differences among eight treatments (ANOVA, Tukey test).

**Table 6.** Effect of two floor management systems and dose of nitrogen on the content on the content of different phenolic compounds ' groups, and the total content of all analyzed phenolics (bold)in the flesh of the fruit in the year 2022 (mean ± SE, mg 100 $g^{-1}$ FW, $n$ = 8).

| Specification | Dose of Nitrogen (kg·ha$^{-1}$) and Floor Management | | | | | | | | D | FM | D × FM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | | 80 | | 110 | | 140 | | | | |
| | H | LM | H | LM | H | LM | H | LM | | | |
| Total phenolic acids | 9.39 ± 1.99 bd | 8.35 ± 2.00 abc | 11.20 ± 2.19 d | 7.84 ± 0.72 ab | 10.67 ± 1.18 cd | 7.87 ± 1.58 ab | 11.80 ± 1.15 d | 6.39 ± 1.87 a | NS | *** | ** |
| Total flavanols | 15.29 ± 2.84 cd | 12.77 ± 2.07 bc | 13.87 ± 1.94 bd | 12.00 ± 1.48 ab | 15.01 ± 1.21 bd | 11.86 ± 2.68 ab | 16.86 ± 1.90 d | 9.20 ± 1.39 a | NS | *** | *** |
| Total dihydrochalcones | 0.81 ± 0.16 bd | 0.56 ± 0.11 a | 0.83 ± 0.13 cd | 0.58 ± 0.10 ab | 0.82 ± 0.16 bd | 0.62 ± 0.22 abc | 0.97 ± 0.19 d | 0.50 ± 0.10 a | NS | *** | NS |
| Total flavonols | 0.04 ± 0.01 b | 0.02 ± 0.01 ab | 0.03 ± 0.01 b | 0.02 ± 0.01 ab | 0.03 ± 0.01 b | 0.02 ± 0.01 ab | 0.03 ± 0.01 b | 0.02 ± 0.00 a | NS | *** | NS |
| **Total APC** | **25.53 ± 4.46 bd** | **21.70 ± 3.11 bc** | **25.94 ± 3.55 cd** | **20.45 ± 2.07 ab** | **26.53 ± 2.14 cd** | **20.37 ± 4.34 ab** | **29.67 ± 2.90 d** | **16.11 ± 3.04 a** | **NS** | *** | *** |

APC—analyzed phenolic compounds. H—herbicide fallow. LM—living mulch. D—dose of nitrogen. FM—floor management. NS—not significant. ** Statistically significant differences at $p < 0.01$. *** Statistically significant differences at $p < 0.001$. Mean marked with different letters in rows represent statistical differences among eight treatments (ANOVA, Tukey test).

### 3.4.2. Flavanols

Apple is a rich source of monomeric flavanols (catechin and epicatechin) and polymeric flavanols—procyanidins [2], and cv. 'Sampion' contains more of them than the world's most important apple cultivars [3,4]. As in the case of phenolic acids, the content of total flavanols in the fruit skin was not affected by the floor management and the dose of nitrogen separately (Table 5), but the interaction of both factors did. In both floor managements, an increase in the nitrogen dose from 50 to 110 kg $ha^{-1}$ was connected with a tendency to increase the content of total flavanols, especially in some procyanidin dimers, procyanidin trimers, and epicatechin, which are also the most abundant individual flavanols in apple skin (Table S4). A significant increase in the total flavanols of the skin of apples was also

observed in cv. 'Ligol' grown in various living mulches [21], but analysis of the pulp did not follow the same pattern. In the presented research with cv. 'Sampion', the content of flavanols was slightly lower when trees were cultivated with living mulch, especially in combination with the highest dose of nitrogen (Table 6). In other studies, not only changes in fertilization and floor management, but also replacement of conventional apple production with an organic system had no significant effect on total flavanols in both parts of the fruit [14]. In a similar three-year study, the influence of weather conditions during the vegetation period, and not the management system, had more influence on the synthesis of this group of phenolic compounds [13,15]. The higher concentration of procyanidin dimers and epicatechins, which were abundant in the pulp of cv. 'Sampion' (Table S5), was probably related to very high summer temperatures and periodic rainfall shortages. Under such conditions, the smaller but more numerous fruits on slightly better yielding trees in the herbicide fallow (Tables 1 and 3) may have a greater synthesis of flavanols in the pulp.

#### 3.4.3. Dihydrochalcones

Earlier research in cv. 'Sampion' showed that the content of dihydrochalcones in fruits is lower [4] or at a mean level [3,54] compared to other currently cultivated cultivars. In both peel and flesh, total dihydrochalcone content was influenced only by the floor management (Tables 5 and 6). Analyses of the flesh in most treatments with herbicide fallow showed a significantly higher concentration of this phenolic group than in living mulch. This was mainly due to phloretin-2-O-xyloside (Table S5), whose synthesis in both peel and pulp (Table S4) was most often significantly higher in fruit when herbicide fallow was applied. Earlier studies on the impact of living mulch on other cultivars, however, are not coincident with such observations [21]. In the study of Le Bourvellec et al. [15], its content was conditioned by the weather conditions during the growing season. The significantly higher content of total dihydrochalcones, mainly in fruit pulp from trees in herbicide fallow, similarly to flavanols, was probably related to their yield level (Table 1), fruit size, and weight (Tables 2 and 3), and unfavorable weather conditions for cultivation in 2022 (Table S1).

#### 3.4.4. Flavonols

Among flavonols, quercetin glycosides are the most efficiently absorbed by the human body [55]. These compounds are found mainly in the skin of the fruit, and their share in the pulp is marginal [2], which was confirmed by our results in cv. 'Sampion' (Tables 5 and 6). In our study, the content of flavonols in fruits was higher than in the cultivars 'Jonagold' and 'Idared' [54]. Both floor management and different nitrogen doses did not affect the level of synthesis of these phenolic compounds in the fruit skin. However, other research has shown that the replacement of herbicide fallow with living mulch often resulted in a significant increase in the synthesis of flavonols in other cultivars [21]. In addition, replacing conventional or low input production with an organic one contributed to a significant increase in the total flavonol content [14,15]. The synthesis of these compounds was also influenced by weather conditions [13,15,17].

#### 3.4.5. Anthocyanins

Anthocyanins content in the fruit is quite low compared to other phenolic compounds [56]. In cv. 'Sampion', their concentration was higher in living mulch (Table 5). At the basic N dose of 50 kg $ha^{-1}$, the synthesis of total anthocyanins, as well as all individual cyanidins (Table S4), was significantly higher than in herbicide fallow. With more abundant fertilization, the concentration of anthocyanins decreased less with the presence of living mulch. Nitrogen uptake by living mulch probably significantly limited the availability of this element for the trees, as mentioned previously [38,48]. As described by Treutter [25], excessive tree growth inhibits the red pigmentation of the fruit. In our research, it did not limit the synthesis of anthocyanins in the peel of cv. 'Sampion', as was confirmed in earlier reports by Slatnar et al. [21,22]. The introduction of organic apple production instead

of other higher input tree management systems also had a similar impact, although the anthocyanin content was also dependent on weather conditions [15,17]. Cool temperature and full sunlight conditions during fruit ripening stimulate the accumulation of these compounds in the skin, and in the case of red-fleshed apple also in pulp [23]. Therefore, cold days just before fruit harvest in September, when the average monthly air temperature was only 13.9 °C (Table S1), favored the synthesis of these compounds in the case of cv. 'Sampion'.

### *3.5. Apple Volatile Organic Compounds (VOCs) Content*

The total concentration of VOCs was not influenced by either the N dose or the floor management (Table 7). In total, 22 volatile compounds were identified and quantified in cv. 'Sampion' fruits (Table S6). They belong to different chemical groups: esters (13), aldehydes (3), alcohols (2), organic acids (1), and alkanes (1), among others, which are produced by different biochemical pathways [57]. The total amount of VOCs in cv. 'Sampion' was comparable to cv. 'Jonagold'. Among the identified VOCs, esters and aldehydes were dominant in terms of abundance. Similarly, esters were also identified as the most abundant group in 85 different apple cultivars [28]. Some individual esters were also significantly increased by the presence of living mulch, namely hexyl acetate and amyl acetate (Table S6). On the contrary, in the study of Medina et al. [27], cultivars 'Festa', 'Branco', and 'Domigos' contained little or no presence of these two compounds.

**Table 7.** Effect of two floor management systems and dose of nitrogen on the content of individual and total (bold) volatile organic compounds of the fruit, in the year 2022 (mean ± SE. μg $kg^{-1}$ FW. *n* = 4).

| | Dose of Nitrogen (kg·ha−1) and Floor Management | | | | | | | | D | FM | D × FM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50 | | 80 | | 110 | | 140 | | | | |
| | H | LM | H | LM | H | LM | H | LM | | | |
| Total esters | 1221.97 ± 256.46 a | 1157.77 ± 442.65 a | 866.04 ± 183.93 a | 1451.64 ± 353.41 a | 931.75 ± 153.41 a | 1117.93 ± 259.05 a | 1130.46 ± 398.82 a | 1120.19 ± 107.44 a | NS | NS | NS |
| Total aldehydes | 1238.82 ± 150.20 a | 908.50 ± 141.67 a | 1398.7 ± 163.37 a | 966.42 ± 514.37 a | 1307.29 ± 106.38 a | 1178.08 ± 292.65 a | 1235.61 ± 194.21 a | 1182.22 ± 390.21 a | NS | * | NS |
| Total alcohols | 118.76 ± 25.75 a | 97.38 ± 18.39 a | 121.52 ± 34.49 a | 112.4 ± 13.78 a | 124.98 ± 17.83 a | 108.9 ± 16.35 a | 134.91 ± 14.90 a | 116.85 ± 11.67 a | NS | * | NS |
| Total organic acids | 8.43 ± 3.49 b | 11.36 ± 2.09 b | 10.50 ± 2.83 b | 0.00 ± 0.00 a | 8.03 ± 3.43 ab | 0.00 ± 0.00 a | 0.00 ± 0.00 a | 9.31 ± 7.35 b | * | NS | *** |
| Total alkanes | 0.00 ± 0.00 a | 0.00 ± 0.00 a | 17.91 ± 5.07 b | 0.00 ± 0.00 a | 13.67 ± 4.12 b | 18.77 ± 7.51 b | 12.60 ± 0.28 b | 29.54 ± 4.59 c | *** | NS | *** |
| **Total VOC** | **2587.99 ± 255.14 a** | **2169.33 ± 505.58 a** | **2404.94 ± 321.75 a** | **2530.45 ± 355.45 a** | **2374.87 ± 190.22 a** | **2418.99 ± 262.59 a** | **2507.29 ± 369.42 a** | **2453.45 ± 471.21 a** | **NS** | **NS** | **NS** |

VOC—volatile organic compounds. H—herbicide fallow. LM—living mulch. D—dose of nitrogen. FM—floor management. NS—not significant. * Statistically significant differences at $p < 0.05$. *** Statistically significant differences at $p < 0.001$. Mean marked with different letters in rows represent statistical differences among eight treatments (ANOVA, Tukey test).

Our results show that total alcohols and aldehydes were higher in herbicide fallow compared to living much, mainly due to the increase in butanal and hexanal (Table S6). The fruit of cv. 'Sampion' could be considered a rich source of aldehydes compared to other cultivars [27,28]. The concentration of 2-hexenal was even 4-times higher, and in the case of hexanal, 10-times higher. In addition, cv. 'Sampion' contained butanal and 2-butanol, which were not yet detected in any other cultivar [27], but the content of alcohols was similar to other cultivars [28], or lower [27]. The most abundant alcohol was butanol. Other compounds, such as organic acids and alkanes, were only in minor amounts, and were not affected by the floor management system alone, but they were significantly affected by nitrogen dose, especially in living mulch (Table 7 and Table S6). In wild apples [58], the same groups of VOCs were recorded, namely esters, aldehydes, and alcohols, and additionally terpenes and hydrocarbons, which were not found in currently cultivated cultivars, and only in traces in our study. However, it is worth underlining that our research

showed a unique VOCs profile of cv. 'Sampion', which constitutes a fingerprint that defines an apple variety [27].

## 4. Conclusions

Growth and yield of cv. 'Sampion' were influenced by biotic and abiotic stress, and the impact was stronger than the replacement of herbicide fallow by living mulch and the use of higher nitrogen doses. The use of living mulch as an alternative method of floor management in the tree rows compared to herbicide fallow did not show significant differences in yield, and often even reduced the content of phenolic compounds in the fruit flesh. However, the presence of living mulch significantly increased the synthesis of anthocyanins in the peel of the fruit, and its presence mitigated the restrictive effect of using higher doses of nitrogen on their synthesis. High concentrations of this group of phenolic compounds resulted in better-colored apples, which is a desirable trait for consumers. Considering that the content of volatile organic compounds also showed an increase in living mulch, although to a small extent, it could be suggested that under proper nitrogen doses and weather conditions, the use of living mulch could improve the attractiveness, comprising appearance and aroma, of fruits of cv. 'Sampion' for the consumer.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13050921/s1. Table S1: Total precipitation and mean temperatures at the Wrocław-Strachowice Station (51°12′ N. 16°87′ E) in the years 2015–2022; Table S2: Retention times, molecular weights, negative ion MS2, and MS3 fragmentation of phenolic compounds and positive ion frag-mentation of anthocyanins; Table S3: Effect of two floor management systems and dose of nitrogen on the content of total phenolic compounds in the peel and flesh of fruit in the year 2022 (mean ± SE. mg GAE $kg^{-1}$ FW $n$ = 8); Table S4: Effect of two floor management systems and dose of nitrogen on the content of individual phenolic compounds in the peel of fruit in the year 2022 (mean ± SE. mg 100 $g^{-1}$ FW. $n$ = 8); Table S5: Effect of two floor management systems and dose of nitrogen on the content of individual phenolic compounds in flesh of fruit in the year 2022 (mean ± SE. mg 100 $g^{-1}$ FW. $n$ = 8); Table S6: Effect of two floor management systems and dose of nitrogen on the content of individual volatile organic compounds of fruit in the year 2022 (mean ± SE. µg $kg^{-1}$ FW. $n$ = 4).

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