



Article

Cultivar-Specific Assessments of Almond Nutritional Status through Foliar Analysis

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Abstract: Mediterranean almond growing is increasing, as confirmed in Italy where new almond orchards in areas not previously interested in this nut crop have been recently established. In these new areas, as in the coastal of Latium region, the knowledge of eco-physiological behavior as a complex interaction among cultivars, pedoclimate conditions and orchard management is still poor. Optimizing fertilization strategies according to cultivar needs is one of the main key factors to guarantee high yields and nut quality, as well as to reduce environmental pollution. With this aim, an almond field collection has been established planting medium- and late-bloom cultivars. During the growing seasons 2019–2020, leaf samples were collected at 90 and 120 days after full bloom (DAFB) analyzed for biometrical and eco-physiological traits and leaf nutrients content through foliar diagnostics. Differences in foliar nutrient content depend on both cultivar and phenological stage. According to Pearson correlation heatmap, leaf nitrogen content showed a medium–high positive correlation with Nitrogen Balance Index (NBI) either at 90 or 120 DAFB, highlighting reciprocal influences among leaf nutrient contents and some eco-physiological traits. The findings of this study will help to develop novel environmentally friendly nutrition and fertigation strategies supported by foliar diagnostics which also consider accurate cultivar requirements.



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1. Introduction

The nut market is currently experiencing a period of steady growth both in terms of production and consumption [1]. Almond is the main globally grown nut crop with a yearly average in-shell nut production of about 4.1 million tons harvested from a cultivated area of about 2 million hectares. California is the largest almond producer with about 2.3 million tons of in-shell nuts per year, whereas in Europe the main producer is Spain, where about 400,000 tons of in-shell nuts per year are produced [2].

The Italian almond harvested area was 53,000 hectares and the in-shell production was about 85,000 tons as of 2020 [3]. During the first half of the 20th century, Italy was the world leader in almond production [4]. Nevertheless, the development of Californian and Spanish almond cultivation led Italy into a deep crisis, determining the loss of production leadership in the 70s. Recently, the interest in Italian almond cultivation is growing up again, as confirmed by the new almond plantings established both in traditional and new growing areas. These new plantations are usually established planting both traditional cultivars as 'Tuono' and 'Genco' or more recently released late-flowering cultivars such as 'Belona', 'Soleta', 'Penta[®]', 'Vialfas', 'Makako', 'Lauranne[®] Avijor' [5].

In California, where soft-shelled almond cultivars are preferred [6], the breeding program started in the 1920s at the Department of Agriculture—Almond Board of California (USDA) and was focused on improving nut quality, self-compatibility and in realizing the suitable pollinators of the main cultivar Nonpareil [7]. In the Mediterranean area, almond breeding started in 1960 in France (INRA-Bordeaux), in 1970 in Spain (CITA-Aragon, IRTA-Catalonia, CEBAS CSIC-Murcia), as well as in Italy (CREA-Rome), mainly with the aim

to obtain late-flowering and new self-compatible cultivars, increase kernel quality and releasing disease-resistant varieties [8].

French almond breeding was very active during the 1970s–80s when the varieties ‘Ferragnes’, ‘Ferraduel’ [9] and in 1989 ‘Lauranne’ [10] were released. More recently, the Spanish almond breeding released in 2006 the varieties ‘Belona’ and ‘Soleta’ [11], Mardia in 2007 [12] and ‘Vialfas’ in 2015 [13]. Additionally, in the same period IRTA released ‘Costanti’, ‘Marinada’ and ‘Vairo’ [14]. Furthermore, the cultivars ‘Antoñeta’ and ‘Marta’ were released in 1990 [15], while the extra-late-flowering cultivars ‘Penta®’ and ‘Tardona’ were released in 2009 [16].

Apart from ‘Ferragnes’ and ‘Ferraduel’, the above-mentioned cultivars are self-compatible thanks to the presence of the dominant allele Sf [17] transmitted in the breeding pedigrees using the Italian self-compatible cultivars ‘Tuono’ and ‘Genco’ as parentals, as well as the Italian cultivar ‘Cristomorto’ was used to transmit the late-flowering trait in the released cultivars [18].

The traditional Italian almond cultivation has been established using local varieties often characterized by early blooming which compromised its spread in new growing areas if prone to late frosts.

Due to the new trend in the Italian almond sector and the climate changes affecting the Mediterranean basin, almond cultivation is at risk of being introduced in areas where the local climate is not suited to almond growing, as well as a lack of knowledge about plant adaptation for phenology, physiology and orchard resilience [5,19].

A key factor that may mitigate the above criticalities is to develop sustainable nutrition models to promote agronomic and physiological performance and minimize the environmental pollution [20]. To achieve these goals, cultivar-site specific foliar diagnostic protocols to monitor the plant nutrition status during the growing season are required.

Some studies carried out on different *Prunus* species [21–26] including almond [27] demonstrated as the best time for monitoring the nutritional status of the plant is between 60 and 120 days after full bloom (DAFB).

The content of leaf nutrients can be compared with their critical values (CVs) determined by Ulrich [28] and expressed as concentration of the nutrients into leaf tissues. These CVs are considered the minimum leaf nutrient content necessary to achieve at least 90% of potential plant yield [20,29].

Hence, the comparison of leaf nutrient content with their CVs and their Sufficiency Ranges (SR) is the most applied method for leaf nutritional diagnosis and both approaches operate through the measurement of total nutrients content in leaf tissues to be compared with reference values [24,30,31].

Considering that the leaf nutrient reference values for almond in the Mediterranean environment are very poor, the present study aims to their determination with a novel approach which includes site-cultivar specific testing of the main almond varieties recently introduced in central Italy. This approach will be useful to develop novel environmentally friendly almond nutrition strategies supported by proper foliar diagnostics.

2. Materials and Methods

2.1. Plant Material and Pedoclimatic Condition

The trial was carried out at the experimental farm of ARSIAL (Regional Agency for Innovation and Development of Agriculture in Latium) located in the municipality of Tarquinia (Latium region-Italy. Latitude 42°16′20″ N; longitude 11°42′26″ E; altitude 32 m a.s.l.), where a collection orchard of almond cultivars was planted in late 2017.

The following eight cultivars were introduced in the trial: ‘Genco’, ‘Guara’, ‘Lauranne®’, ‘Avijor’, ‘Penta®’, ‘Supernova’, ‘Soleta’, ‘Tuono’ and ‘Vialfas’.

Although the cultivars ‘Supernova’ and ‘Guara’ are considered two different commercial varieties and are individually marketed, they have been highlighted in the literature as they may be both synonyms of the cultivar ‘Tuono’ [32–34].

The studied cultivars, each represented by three replicates (three plants per cultivar), were grafted on rootstock GF677 (*Prunus persica* × *Prunus dulcis*), spaced at 6 m × 5 m

and trained as free vase. The experimental almond orchard was managed with a natural green cover crop according to the rules of the almond Integrated Production System and it had yearly application of the following quantities of soil fertilizers: 120 kg ha⁻¹ N, 100 kg ha⁻¹ P₂O₅ and 100 kg ha⁻¹ K₂O. The plants were individually drip irrigated and the amount of water supplied was about 1500–2000 m³ ha⁻¹ per growing season.

The physical and chemical soil characteristics in the experimental almond orchard were reported in Table 1. The clay–loam soil had almost 2% of organic matter mainly located in the topsoil profile and showed sub-alkaline soil reaction (pH = 7.6).

Table 1. Main physical and chemical soil characteristics in the experimental almond orchard located at Tarquinia municipality (Latium region—Italy) at the ARSIAL experimental farm.

Trait	Unit	Value
Texture		
Sand	%	45.4
Lime	%	25.6
Clay	%	30.0
pH	-	7.6
Total limestone	g kg ⁻¹	8.0
Organic matter	%	1.9
Cation exchange capacity	meq	29.1
Calcium (Ca ²⁺)	meq	23.7
Magnesium (Mg ²⁺)	meq	4.1
Potassium (K ⁺)	meq	0.5
Sodium (Na ⁺)	meq	0.8
Basic saturation	%	100.0
Mg/K Ratio	-	7.5

The average climatic parameters recorded during the two research years (2019–2020) are reported in Figure 1. The average annual rainfall at the experimental site was about 740 mm, while the average monthly temperature over the two-year period showed the lowest values in January (+7 °C) and highest values in July and August (+25 °C). May, June and partially July were the critical months in 2019, as well as June, partially July and August in 2020, with very low rainfall and high daily evaporative demands.

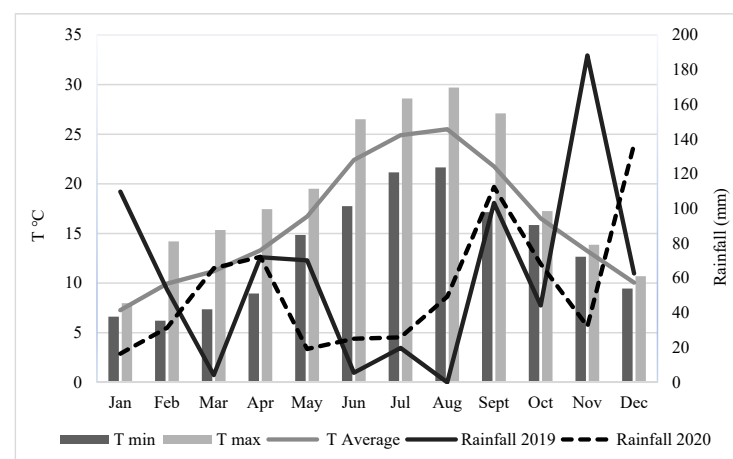


Figure 1. Yearly rainfall per month and mean values of monthly air temperature at the research area over the years 2019–2020. Data source: ISPRA (<http://www.scia.isprambiente.it>, accessed on 30 June 2022).

2.2. Leaf Sampling, Leaf Analysis and DOP Index

Leaf samples of each cultivar under test were collected at 90 and 120 days after full bloom (DAFB), applying the following protocol. A total of 60 mature leaves per cultivar were collected from the middle portion of non-bearing shoots, taking care to sample not damaged leaves oriented in all cardinal points as recommended by Montañés and Sanz [22] and more recently by Milošević and Milošević [35]. The leaf samples were placed into single plastic bags and stored inside a polystyrene box with cooling gel, until they could be processed in the laboratory.

The determination of leaf macro- and micronutrients has been carried out following the protocol described by Stazi et al. [36]. The samples were dried at +37 °C for 72 h and shredded. Approximately 250 mg of each dry sample was inserted directly into a microwave-closed vessel (tetrafluoromethoxy vessels). Two milliliters of 30% (m/m) H₂O₂, 0.5 mL of 37% HCl and 7.5 mL of HNO₃ solution were added to each vessel. The program of heating was carried out in one step, where the temperature was increased linearly from +25 to +180 °C in 37 min and then maintained at +180 °C for 15 min. After the digestion and cooling, the samples were diluted to a final volume of 25.0 mL with Milli-Q water. Analyses of leaf nutrients per cultivar were performed in triplicate. Each sample replication was composed by sub-samples of ten mature leaves.

For the elements determination the following emission lines were used: phosphorus (P) 213.620 nm, zinc (Zn) 213.857 nm, boron (B) 249.677 nm, manganese (Mn) 257.610 nm, iron (Fe) 259.939 nm, magnesium (Mg) 279.077 nm, calcium (Ca) 317.933 nm, copper (Cu) 324.752 nm, potassium (K) 766.490 nm.

An ICP-OES spectrometry with an axially viewed configuration (8000 DV, PerkinElmer, Shelton, CT, USA) was used. For detections, we chose the frequency showing the lowest interference and high analytical signal and background ratio, for every element. For inorganic carbon (C) and nitrogen (N), an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used.

After almond leaf macro- and microelements determination, their contents were compared with the optimal ones, calculating the deviation from optimum percentage (DOP) index [25,35,37,38] as follows:

$$\text{DOP} = (C \times 100 / C_{\text{ref}}) - 100$$

where C is the leaf concentration determined for a given nutrient and C_{ref} is the optimal leaf concentration for the same nutrient as proposed by Mills et al. [39].

Through the DOP index it is possible to determine the nutritional status of the plant according to Σ DOP index of the investigated leaf nutrients. The Σ DOP index is calculated by adding the absolute values of the DOP index determined for every single nutrient.

If the detected nutrients are in balance with the reference values, the Σ DOP index will have low values [35,40]. For its determination the mean of single nutrient reference values proposed by Mills et al. [39] were used.

A portion of leaf samples collected at 90 and 120 DAFB (20 leaves per cultivar analyzed in duplicate: 10 leaves per sample) were analyzed to determine the Specific Leaf Area (SLA) [41,42] using the software Image J [43]. This leaf trait is obtained by the ratio between the leaf blade area to its relative dry weight, expressed in cm² g^{−1} [44].

Furthermore, the same leaves, previously weighed in the lab to determine their fresh weight, were dried by heating at +103 ± 2 °C to constant weight in order to determine the Leaf Dry Matter Content (LDMC), which is given by the ratio between the dry weight and the relative fresh weight of the leaf blade and expressed in mg g^{−1} [45,46].

2.3. Leaf Content of Total Chlorophyll, Flavonols, Anthocyanins and Nitrogen Balance Index

Leaves analyzed for nutrient contents were previously subjected to in-field measuring of eco-physiological parameters (two measurements per leaf), such as total chlorophyll (Chl), flavonols (Flav) and anthocyanins (Anth), using a hand-held meter (FORCE-A,

Dualex[®], Orsay, France). The instrument also automatically determines the Nitrogen Balance Index (NBI), expressed as ratio between Chl and Flav [47].

The instrument quantifies the Chl content by exciting the leaf tissue with radiation at two different wavelengths (red and near infrared). Furthermore, it quantifies the leaf flavonoids amount as a logarithmic ratio between the infrared fluorescence of Chl excited by a red wavelength and an ultraviolet (UV) wavelength [48].

It is well known that the flavonoids concentrated in the epidermal cells absorb UV light by lowering the fluorescence of Chl in the infrared range [49].

2.4. Statistical Analysis

Statistical analyses were carried out using InfoStat Professional v.1.1 program [50]. For the analysis of variance (two-way ANOVA), the parameters “cultivar”, “year” and their interaction were used, analyzing the sampling dates individually (90 DAFB and 120 DAFB). Differences were accepted as statistically significant when $p < 0.05$. Fischer’s test was carried out to identify significance among the cultivars and the years of samples. Furthermore, correlations among data collected through leaf analyses and measurements at 90 and 120 DAFB, respectively, were determined by Pearson coefficient testing at $p < 0.05$ and represented as Pearson’s correlation heatmap in order to explain possible relationships between analyzed leaf parameters.

3. Results and Discussions

3.1. Specific Leaf Area and Leaf Dry Matter Content

Table 2 shows mean values of SLA and LDMC determined on leaf samples of the almond cultivars tested at 90 and 120 DAFB, respectively.

SLA plays an important role in determining plants’ productivity, since its values may reflect changes in the structure and nutritional content of leaves. It is well known as the SLA is mostly driven by the concentration of starch and sugars due to increased rates of photosynthesis, increasing the C:N ratio and reducing the nutritional value of the leaf [51].

LDMC is closely related to the average density of leaf tissues [46,52] and tends to increase as SLA decreases [53]. LDMC is also strongly correlated with resource availability and with relative growth rate [54] and it has been recommended as a highly reliable correlate of soil fertility, at least in agroecosystems not subject to severe water limitation [55].

The values of SLA at 90 DABF showed differences related to the year, whereas values were not influenced by cultivar, as well as the interaction cultivar $\times$ year was not significant. SLA at 90 DABF ranged between $10.11 \text{ cm}^2 \text{ g}^{-1}$ in cv ‘Tuono’ in 2019 to $7.16 \text{ cm}^2 \text{ g}^{-1}$ in cv ‘Lauranne’[®] Avijor’ in 2020. Furthermore, SLA expressed as mean value of cultivars was higher in the first research year, showing values of 9.20 and $8.05 \text{ cm}^2 \text{ g}^{-1}$ in 2019 and 2020, respectively.

The trait measured at 120 DAFB showed differences related to the effects of cultivar and year, whereas their interaction was not significant (Table 2). At this phenological stage (120 DABF) the highest mean value of SLA was observed in ‘Tuono’ in 2019 ($8.72 \text{ cm}^2 \text{ g}^{-1}$) while the lowest one was noted in ‘Supernova’ in 2020 ($5.81 \text{ cm}^2 \text{ g}^{-1}$).

Even at 120 DABF, the trait was higher in 2019, when expressed as mean value of cultivars. Overall, the mean values of SLA were slightly lower at 120 DABF in comparison to those observed at 90 DABF for all cultivars, except for ‘Lauranne’[®] Avijor’ both in 2019 and 2020.

Leaves with low SLA values tend to be more efficient at high light concentrations and more tolerant to water and nutrient deficiency due mainly to their thicker cuticle and slower leaf turnover [56,57].

Table 2. Specific leaf area (SLA) and leaf dry matter content (LDMC) in almond plants at 90 and 120 DAFB over the years 2019–2020. Data are presented as mean $\pm$ standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher’s test $p < 0.05$). Lowercase letters in italics describe the differences expressed as two-year mean values of the cultivars.

Cultivar	Year	90 DAFB		120 DAFB	
		SLA (cm ² g ^{−1})	LDMC (mg g ^{−1})	SLA (cm ² g ^{−1})	LDMC (mg g ^{−1})
‘Genco’	2019	9.08 $\pm$ 1.01	367.53 $\pm$ 7.29 cd	7.32 $\pm$ 0.95	391.94 $\pm$ 3.90 e
	2020	7.58 $\pm$ 0.58	483.91 $\pm$ 6.55 a	6.53 $\pm$ 0.72	420.81 $\pm$ 6.80 abc
Mean value		8.33 $\pm$ 1.11	425.72 $\pm$ 62.54	6.92 $\pm$ 0.89 <i>bc</i>	406.38 $\pm$ 16.26
‘Guara’	2019	9.05 $\pm$ 2.09	349.77 $\pm$ 3.59 d	7.01 $\pm$ 1.02	381.17 $\pm$ 10.72 fg
	2020	7.62 $\pm$ 2.14	482.14 $\pm$ 13.05 a	6.34 $\pm$ 0.58	416.46 $\pm$ 1.49 bcd
Mean value		8.33 $\pm$ 2.10	415.95 $\pm$ 71.31	6.67 $\pm$ 0.84 <i>c</i>	398.32 $\pm$ 20.15
Lauranne® Avijor	2019	8.73 $\pm$ 0.84	363.96 $\pm$ 4.44 cd	9.79 $\pm$ 1.16	368.57 $\pm$ 12.30 hi
	2020	7.16 $\pm$ 1.39	475.49 $\pm$ 4.19 a	8.06 $\pm$ 1.23	417.52 $\pm$ 16.47 bcd
Mean value		7.94 $\pm$ 1.35	419.73 $\pm$ 59.75	8.93 $\pm$ 1.44 <i>a</i>	393.15 $\pm$ 29.52
‘Penta®’	2019	9.83 $\pm$ 2.24	360.61 $\pm$ 6.10 cd	8.39 $\pm$ 0.96	376.84 $\pm$ 9.46 fg
	2020	9.57 $\pm$ 2.17	432.26 $\pm$ 17.25 b	7.59 $\pm$ 1.23	408.95 $\pm$ 6.57 d
Mean value		9.71 $\pm$ 2.05	396.43 $\pm$ 40.12	7.99 $\pm$ 1.11 <i>ab</i>	392.89 $\pm$ 18.75
‘Soleta’	2019	7.98 $\pm$ 0.66	371.20 $\pm$ 5.85 c	6.65 $\pm$ 1.11	374.51 $\pm$ 0.40 gh
	2020	8.24 $\pm$ 2.94	484.35 $\pm$ 7.49 a	6.99 $\pm$ 0.93	429.91 $\pm$ 2.78 a
Mean value		8.11 $\pm$ 1.98	427.77 $\pm$ 60.80	6.82 $\pm$ 0.96 <i>bc</i>	402.21 $\pm$ 29.67
‘Supernova’	2019	9.56 $\pm$ 2.88	364.16 $\pm$ 15.94 cd	7.98 $\pm$ 0.78	383.92 $\pm$ 0.55 efg
	2020	9.05 $\pm$ 3.89	466.54 $\pm$ 32.87 a	5.81 $\pm$ 1.50	411.27 $\pm$ 1.79 cd
Mean value		9.31 $\pm$ 3.18	415.35 $\pm$ 59.72	6.9 $\pm$ 1.61 <i>bc</i>	397.6 $\pm$ 14.67
‘Tuono’	2019	10.11 $\pm$ 3.07	359.93 $\pm$ 16.89 cd	8.72 $\pm$ 2.26	363.76 $\pm$ 1.54 i
	2020	7.51 $\pm$ 0.96	466.80 $\pm$ 10.14 a	6.35 $\pm$ 0.70	425.47 $\pm$ 6.20 ab
Mean value		8.81 $\pm$ 2.53	413.37 $\pm$ 58.56	7.54 $\pm$ 2.01 <i>bc</i>	394.62 $\pm$ 33.25
‘Vialfas’	2019	9.26 $\pm$ 0.39	365.76 $\pm$ 8.32 cd	6.98 $\pm$ 0.59	386.6 $\pm$ 0.77 ef
	2020	7.69 $\pm$ 1.99	475.55 $\pm$ 8.24 a	7.36 $\pm$ 1.42	415.03 $\pm$ 3.59 cd
Mean value		8.48 $\pm$ 1.57	420.66 $\pm$ 59.18	7.17 $\pm$ 1.03 <i>bc</i>	400.82 $\pm$ 15.39
Average cultivars 2019		9.20 $\pm$ 1.80 a	362.86 $\pm$ 10.54 b	7.86 $\pm$ 1.47 a	378.41 $\pm$ 10.76 b
Average cultivars 2020		8.05 $\pm$ 2.13 b	470.88 $\pm$ 21.02 a	6.88 $\pm$ 1.20 b	418.20 $\pm$ 9.25 a

LDMC showed differences for the effect of year and interaction cultivar $\times$ year, whereas no differences were emerged for cultivar effect (Table 2). The higher mean values were observed for all cultivars in 2020 at both 90 and 120 DABF. After 90 days of full bloom, the highest LDMC mean values were recorded for cultivars ‘Soleta’, ‘Genco’ and ‘Guara’ (484.3, 483.9 and 482.1 mg g^{−1}, respectively), whereas the lowest LDMC were observed for ‘Guara’ in 2019 with mean values of 349.7 mg g^{−1}.

At 120 DABF the LDMC ranged between 429.9 mg g^{−1} recorded in ‘Soleta’ in 2020 to 363.7 mg g^{−1} measured in leaves of cv ‘Tuono’ sampled in 2019.

The mean value of LDMC in 2019, expressed as average of cultivars (Table 2), was slightly lower at 90 DABF (362.8 mg g^{−1}) when compared to 120 DABF (378.4 mg g^{−1}), whereas in 2020 the reverse trend was observed, with slightly higher average values at 90 DABF (470.8 mg g^{−1}) than those recorded at 120 DABF (418.2 mg g^{−1}).

3.2. Specific Almond Cultivar Leaf Nutrient Concentration and Σ DOP Index

In order to achieve almond cultivar-specific diagnostic tools focused on managing the balanced fertilization of almond orchards, highlighting the nutritional requirements of the main cultivars in relation to a given environment or cultivation system [58], leaf diagnosis was applied to the cultivars over the two-year investigation.

Leaf analyses carried out at 90 DAFB showed differences related to the effect of cultivar, year of investigation and their interaction, with the exception for K which showed no significance on statistical analysis, Mg that was not influenced by the effect of cultivar and year of investigation (Table 3) and Cu that was not influenced by cultivar effect (Table 4).

Table 3. Macronutrients content (% DW) in almond leaves collected at 90 DAFB over the years 2019–2020. (N = nitrogen, C = carbon, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium). Data are presented as mean $\pm$ standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$). Lowercase letters in italics describe the differences expressed as two-year mean values of the cultivars.

Cultivar (90 DAFB)	Year	N (% DW)	C (% DW)	P (% DW)	K (% DW)	Ca (% DW)	Mg (% DW)
'Genco'	2019	2.01 $\pm$ 0.02 e-h	44.23 $\pm$ 0.36 cd	0.05 $\pm$ 0.004 i	1.11 $\pm$ 0.10	1.62 $\pm$ 0.16 a	0.24 $\pm$ 0.01 b-e
	2020	3.18 $\pm$ 0.20 a	42.89 $\pm$ 0.12 fg	0.08 $\pm$ 0.003 ed	0.86 $\pm$ 0.03	0.97 $\pm$ 0.26 b-e	0.20 $\pm$ 0.01 fg
Mean value		2.59 $\pm$ 0.64 <i>ab</i>	43.56 $\pm$ 0.76	0.07 $\pm$ 0.02	0.98 $\pm$ 0.15	1.30 $\pm$ 0.40 <i>a</i>	0.22 $\pm$ 0.02
'Guara'	2019	1.74 $\pm$ 0.17 ghi	44.31 $\pm$ 0.12 cd	0.07 $\pm$ 0.009 gh	0.86 $\pm$ 0.09	1.14 $\pm$ 0.05 bcd	0.21 $\pm$ 0.02 d-g
	2020	2.45 $\pm$ 0.38 cd	42.31 $\pm$ 0.30 ghi	0.09 $\pm$ 0.007 bc	0.84 $\pm$ 0.16	0.89 $\pm$ 0.12 def	0.25 $\pm$ 0.02 abc
Mean value		2.09 $\pm$ 0.47 <i>cd</i>	43.31 $\pm$ 1.09	0.08 $\pm$ 0.02	0.85 $\pm$ 0.12	1.01 $\pm$ 0.16 <i>b</i>	0.23 $\pm$ 0.03
'Lauranne® Avijor'	2019	1.87 $\pm$ 0.02 f-i	44.43 $\pm$ 0.02 bc	0.07 $\pm$ 0.003 fgh	0.93 $\pm$ 0.04	1.06 $\pm$ 0.05 bcd	0.19 $\pm$ 0.01 g
	2020	2.85 $\pm$ 0.01 b	42.96 $\pm$ 0.01 fg	0.11 $\pm$ 0.003 a	0.94 $\pm$ 0.08	0.95 $\pm$ 0.05 c-f	0.28 $\pm$ 0.02 ab
Mean value		2.36 $\pm$ 0.52 <i>bc</i>	43.70 $\pm$ 0.80	0.09 $\pm$ 0.02	0.94 $\pm$ 0.06	1.01 $\pm$ 0.08 <i>b</i>	0.23 $\pm$ 0.05
'Penta®'	2019	1.88 $\pm$ 0.02 f-i	44.29 $\pm$ 0.18 cd	0.06 $\pm$ 0.005 hi	1.06 $\pm$ 0.08	1.22 $\pm$ 0.07 b	0.21 $\pm$ 0.01 efg
	2020	2.31 $\pm$ 0.20 cde	42.60 $\pm$ 0.38 gh	0.10 $\pm$ 0.006 ab	0.89 $\pm$ 0.08	0.72 $\pm$ 0.02 ef	0.22 $\pm$ 0.01 d-g
Mean value		2.09 $\pm$ 0.26 <i>cd</i>	43.41 $\pm$ 0.95	0.08 $\pm$ 0.02	0.98 $\pm$ 0.11	0.97 $\pm$ 0.27 <i>b</i>	0.21 $\pm$ 0.01
'Soleta'	2019	1.59 $\pm$ 0.04 i	44.99 $\pm$ 0.04 ab	0.09 $\pm$ 0.01 cd	0.90 $\pm$ 0.07	1.17 $\pm$ 0.28 bc	0.25 $\pm$ 0.06 a-d
	2020	2.17 $\pm$ 0.10 def	41.06 $\pm$ 0.13 j	0.08 $\pm$ 0.002 def	1.11 $\pm$ 0.40	1.04 $\pm$ 0.34 bcd	0.26 $\pm$ 0.01 abc
Mean value		1.88 $\pm$ 0.32 <i>d</i>	43.03 $\pm$ 2.28	0.08 $\pm$ 0.01	1.01 $\pm$ 0.28	1.11 $\pm$ 0.29 <i>ab</i>	0.25 $\pm$ 0.04 <i>fg</i>
'Supernova'	2019	1.72 $\pm$ 0.01 hi	43.71 $\pm$ 0.08 de	0.07 $\pm$ 0.01 efg	1.01 $\pm$ 0.35	1.59 $\pm$ 0.40 a	0.29 $\pm$ 0.06 a
	2020	2.06 $\pm$ 0.27 efg	41.76 $\pm$ 0.44 i	0.09 $\pm$ 0.005 cd	1.02 $\pm$ 0.17	1.07 $\pm$ 0.15 bcd	0.23 $\pm$ 0.01 c-f
Mean value		1.89 $\pm$ 0.26 <i>d</i>	42.73 $\pm$ 1.09	0.08 $\pm$ 0.02	1.01 $\pm$ 0.25	1.33 $\pm$ 0.39 <i>a</i>	0.26 $\pm$ 0.05
'Tuono'	2019	2.42 $\pm$ 0.63 cd	44.27 $\pm$ 0.84 cd	0.07 $\pm$ 0.004 gh	0.90 $\pm$ 0.05	1.18 $\pm$ 0.11 bc	0.21 $\pm$ 0.01 efg
	2020	2.44 $\pm$ 0.09 cd	42.18 $\pm$ 0.13 hi	0.10 $\pm$ 0.005 b	0.84 $\pm$ 0.10	1.08 $\pm$ 0.01 bcd	0.24 $\pm$ 0.02 cde
Mean value		2.43 $\pm$ 0.42 <i>bc</i>	43.23 $\pm$ 1.25	0.08 $\pm$ 0.02	0.87 $\pm$ 0.08	1.13 $\pm$ 0.09 <i>ab</i>	0.23 $\pm$ 0.02
'Vialfas'	2019	2.51 $\pm$ 0.28 bc	45.29 $\pm$ 0.15 a	0.07 $\pm$ 0.001 fgh	0.98 $\pm$ 0.01	1.20 $\pm$ 0.06 bc	0.24 $\pm$ 0.04 b-e
	2020	3.39 $\pm$ 0.22 a	43.43 $\pm$ 0.47 ef	0.11 $\pm$ 0.004 a	0.87 $\pm$ 0.02	0.71 $\pm$ 0.03 f	0.22 $\pm$ 0.01 c-g
Mean values		2.95 $\pm$ 0.52 <i>a</i>	44.36 $\pm$ 1.05	0.09 $\pm$ 0.002	0.93 $\pm$ 0.06	0.96 $\pm$ 0.26 <i>b</i>	0.23 $\pm$ 0.03
Average cultivars 2019		1.97 $\pm$ 0.39 <i>b</i>	44.44 $\pm$ 0.56 <i>a</i>	0.07 $\pm$ 0.01 <i>b</i>	0.97 $\pm$ 0.15	1.27 $\pm$ 0.26 <i>a</i>	0.23 $\pm$ 0.04
Average cultivars 2020		2.60 $\pm$ 0.50 <i>a</i>	42.40 $\pm$ 0.86 <i>b</i>	0.09 $\pm$ 0.01 <i>a</i>	0.92 $\pm$ 0.18	0.93 $\pm$ 0.20 <i>b</i>	0.24 $\pm$ 0.03

Similarly, leaf analyses carried out at 120 DAFB showed as the nutrients N, C, K, Mg and Zn were not influenced by the cultivar, while Ca, Mg, Mn and B were not influenced by year of investigation (Tables 5 and 6). The leaf contents of N detected in the trial were in line with those observed in the literature [59]. At 90 DAFB (Table 3) cultivar 'Vialfas' showed the higher N content (3.38% DW) in 2020, whereas the lowest mean value of N was detected in 'Soleta' in 2019 (1.59% DW). Nitrogen contents were higher in 2020 for all cultivars analyzed, as confirmed by the values expressed as mean of cultivars, which were 1.97% DW in 2019 and 2.60% DW in 2020, respectively. The only cultivar 'Tuono' did not confirm the trend, showing the same values of N both in 2019 and 2020 (2.4% DW) highlighting the high stability for this nutrient in the leaves at 90 DAFB.

Table 4. Micronutrients content ($\mu\text{g g}^{-1}$ DW) in almond leaves collected at 90 DAFB over the years 2019–2020. (Fe = iron, Mn = manganese, B = boron, Cu = copper, Zn = zinc). Data are presented as mean $\pm$ standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher’s test $p < 0.05$). Lowercase letters in italics describe the differences expressed as two-year mean values of the cultivars.

Cultivar (90 DAFB)	Year	Fe ($\mu\text{g g}^{-1}$ DW)	Mn ($\mu\text{g g}^{-1}$ DW)	B ($\mu\text{g g}^{-1}$ DW)	Cu ($\mu\text{g g}^{-1}$ DW)	Zn ($\mu\text{g g}^{-1}$ DW)
‘Genco’	2019	68.13 $\pm$ 7.92 e	18.68 $\pm$ 1.80 fgh	50.43 $\pm$ 14.60 b	7.20 $\pm$ 2.98 ef	33.41 $\pm$ 4.72 cd
	2020	101.00 $\pm$ 5.98 bc	17.79 $\pm$ 1.28 ghi	12.58 $\pm$ 2.25 gh	20.84 $\pm$ 4.05 a	43.61 $\pm$ 0.49 abc
Mean value		84.57 $\pm$ 18.73 <i>ab</i>	18.23 $\pm$ 1.52 <i>cd</i>	31.50 $\pm$ 22.43 <i>abc</i>	14.02 $\pm$ 8.00	38.51 $\pm$ 9.30 <i>ab</i>
‘Guara’	2019	71.55 $\pm$ 20.35 de	22.70 $\pm$ 1.07 cde	34.27 $\pm$ 10.04 cd	3.23 $\pm$ 0.18 gh	28.60 $\pm$ 16.44 cde
	2020	60.34 $\pm$ 10.44 e	17.92 $\pm$ 0.67 gh	13.10 $\pm$ 2.25 gh	15.12 $\pm$ 0.88 cd	6.66 $\pm$ 0.44 e
Mean value		65.95 $\pm$ 16.13 <i>bc</i>	20.31 $\pm$ 2.69 <i>bcd</i>	23.68 $\pm$ 13.17 <i>bc</i>	9.18 $\pm$ 6.38	17.63 $\pm$ 14.92 <i>c</i>
‘Lauranne® Avijor’	2019	50.15 $\pm$ 1.46 e	20.24 $\pm$ 2.54 e–h	43.40 $\pm$ 17.57 bc	1.27 $\pm$ 0.10 h	49.52 $\pm$ 18.73 abc
	2020	99.00 $\pm$ 13.34 bcd	23.53 $\pm$ 1.30 cde	21.86 $\pm$ 4.60 efg	16.75 $\pm$ 5.57 bc	43.47 $\pm$ 10.47 abc
Mean value		74.58 $\pm$ 27.55 <i>abc</i>	21.89 $\pm$ 2.57 <i>abc</i>	32.63 $\pm$ 16.55 <i>ab</i>	9.01 $\pm$ 7.07	46.50 $\pm$ 14.42 <i>ab</i>
‘Penta®’	2019	52.06 $\pm$ 3.34 e	30.73 $\pm$ 2.17 a	50.74 $\pm$ 7.27 b	1.70 $\pm$ 0.62 h	48.57 $\pm$ 6.58 abc
	2020	56.40 $\pm$ 2.77 e	17.41 $\pm$ 1.01 hi	19.31 $\pm$ 20.5 fgh	17.29 $\pm$ 0.66 bc	18.57 $\pm$ 7.70 de
Mean value		54.23 $\pm$ 3.67 <i>c</i>	24.07 $\pm$ 6.29 <i>ab</i>	35.03 $\pm$ 17.52 <i>ab</i>	9.50 $\pm$ 7.53	33.57 $\pm$ 17.35 <i>bc</i>
‘Soleta’	2019	66.75 $\pm$ 19.95 e	20.04 $\pm$ 5.21 e–h	75.96 $\pm$ 1.69 a	3.41 $\pm$ 0.41 gh	56.92 $\pm$ 16.98 a
	2020	130.31 $\pm$ 28.61 a	14.19 $\pm$ 1.09 i	20.11 $\pm$ 5.52 fgh	12.71 $\pm$ 0.69 d	12.06 $\pm$ 1.65 de
Mean value		98.53 $\pm$ 30.93 <i>a</i>	17.12 $\pm$ 5.18 <i>d</i>	48.04 $\pm$ 20.09 <i>a</i>	8.06 $\pm$ 5.00	34.49 $\pm$ 26.45 <i>bc</i>
‘Supernova’	2019	112.85 $\pm$ 32.77 ab	24.93 $\pm$ 1.33 bcd	32.10 $\pm$ 7.87 de	4.21 $\pm$ 1.11 fgh	56.18 $\pm$ 31.20 ab
	2020	69.94 $\pm$ 21.65 e	16.84 $\pm$ 0.39 hi	26.60 $\pm$ 4.99 def	14.46 $\pm$ 2.92 cd	61.12 $\pm$ 19.84 a
Mean value		91.40 $\pm$ 40.82 <i>ab</i>	20.88 $\pm$ 4.42 <i>a–d</i>	29.34 $\pm$ 6.77 <i>bc</i>	9.33 $\pm$ 5.85	58.65 $\pm$ 24.35 <i>a</i>
‘Tuono’	2019	60.83 $\pm$ 16.12 e	25.87 $\pm$ 1.41 bc	16.79 $\pm$ 3.67 fgh	9.02 $\pm$ 0.21 e	43.60 $\pm$ 6.16 abc
	2020	111.27 $\pm$ 19.44 ab	21.64 $\pm$ 1.90 def	10.30 $\pm$ 4.39 h	15.60 $\pm$ 1.64 bcd	34.13 $\pm$ 14.51 bcd
Mean value		86.05 $\pm$ 31.63 <i>ab</i>	23.75 $\pm$ 2.74 <i>ab</i>	13.54 $\pm$ 5.11 <i>c</i>	12.31 $\pm$ 3.68	38.86 $\pm$ 11.49 <i>ab</i>
‘Vialfas’	2019	75.25 $\pm$ 1.66 cde	28.43 $\pm$ 5.92 ab	54.55 $\pm$ 11.34 b	6.09 $\pm$ 1.11 efg	61.37 $\pm$ 31.17 a
	2020	71.49 $\pm$ 6.42 e	21.27 $\pm$ 2.30 efg	13.69 $\pm$ 3.68 gh	18.61 $\pm$ 1.06 ab	17.05 $\pm$ 3.47 de
Mean value		73.36 $\pm$ 4.68 <i>abc</i>	24.85 $\pm$ 5.54 <i>a</i>	34.12 $\pm$ 20.13 <i>ab</i>	12.35 $\pm$ 6.77	39.21 $\pm$ 21.35 <i>ab</i>
Average cultivars 2019		69.70 $\pm$ 26.95 b	23.95 $\pm$ 5.06 a	44.78 $\pm$ 19.19 a	4.52 $\pm$ 2.79 b	47.27 $\pm$ 20.16 a
Average cultivars 2020		87.47 $\pm$ 28.98 a	18.82 $\pm$ 3.06 b	17.19 $\pm$ 6.32 b	16.42 $\pm$ 3.45 a	29.58 $\pm$ 20.28 b

Inorganic carbon (C) was very stable in the leaves collected at 90 DAFB ranging from 44.99% to 41.06% DW recorded in ‘Soleta’ in the years 2019 and 2020, respectively.

The values of P at 90 DAFB recorded in the cultivars tested were slightly low in comparison to the average reference values reported in the literature [39].

Its content ranged between 0.11% in ‘Vialfas’ and ‘Lauranne® Avijor’ in 2020 to 0.05% in ‘Genco’ in 2019. The nutrient was not affected by the cultivar effect and its values, expressed as mean of cultivars, were slightly higher in 2019 (0.09% DW) compared to those observed in 2020 (0.07% DW).

Although the content of K did not show statistical significance and the nutrient was highly stable in the leaves collected at 90 DAFB for all cultivars and for both research-year, its mean value recorded in 2019 (0.97%) was slightly higher than in 2020 (0.92%).

At the same leaf sampling period and in the environment of the trial, low concentrations of Ca were extensively observed in the cultivars tested when compared with reference values for this nutrient [39,58], and highest values were recorded for ‘Genco’ and ‘Supernova’ (1.6% DW) in 2019 for both, whereas the lowest ones occurred for cultivars ‘Penta®’ and ‘Vialfas’ in 2020 (0.7% DW). On average, higher values of Ca were recorded in 2019 compared to those of 2020.

Mg leaf content was affected by the cultivar only, and its values ranged between 0.29% in ‘Supernova’ in 2019 to 0.19% in ‘Lauranne® Avijor’ in the same year of investigation. In addition, the average Mg content expressed as mean of cultivars did not show any noteworthy differences between the two study years (mean values of 0.23–0.24% DW).

Table 5. Macronutrients content (% DW) in almond leaves collected at 120 DAFB over the years 2019–2020. (N = nitrogen, C = carbon, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium). Data are presented as mean $\pm$ standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher’s test $p < 0.05$). Lowercase letters in italics describe the differences expressed as two-year mean values of the cultivars.

Cultivar (120 DABF)	Year	N (% DW)	C (% DW)	P (% DW)	K (% DW)	Ca (% DW)	Mg (% DW)
‘Genco’	2019	1.80 $\pm$ 0.01 i	45.05 $\pm$ 0.04 bcd	0.07 $\pm$ 0.01 f	1.68 $\pm$ 0.17 b	1.52 $\pm$ 0.19 bcd	0.22 $\pm$ 0.02 efg
	2020	3.03 $\pm$ 0.33 abc	44.48 $\pm$ 0.23 def	0.11 $\pm$ 0.02 bc	0.31 $\pm$ 0.03 g	1.67 $\pm$ 0.13 abc	0.26 $\pm$ 0.03 abc
Mean value		2.42 $\pm$ 0.69	44.76 $\pm$ 0.34	0.08 $\pm$ 0.02 <i>b</i>	1.00 $\pm$ 0.74	1.60 $\pm$ 0.17 <i>ab</i>	0.24 $\pm$ 0.03
‘Guara’	2019	2.28 $\pm$ 0.12 gh	45.39 $\pm$ 0.36 bc	0.09 $\pm$ 0.01 e	0.77 $\pm$ 0.75 ef	1.73 $\pm$ 0.11 abc	0.23 $\pm$ 0.03 b–g
	2020	2.71 $\pm$ 0.52 cde	43.77 $\pm$ 0.53 fgh	0.09 $\pm$ 0.01 de	0.34 $\pm$ 0.04 g	1.56 $\pm$ 0.33 bcd	0.27 $\pm$ 0.02 ab
Mean value		2.49 $\pm$ 0.42	44.58 $\pm$ 0.96	0.09 $\pm$ 0.01 <i>b</i>	0.55 $\pm$ 0.54	1.65 $\pm$ 0.24 <i>a</i>	0.25 $\pm$ 0.03
‘Lauranne® Avijor’	2019	2.33 $\pm$ 0.04 fgh	46.58 $\pm$ 0.28 a	0.12 $\pm$ 0.02 a	2.12 $\pm$ 0.31 a	1.51 $\pm$ 0.25 bcd	0.25 $\pm$ 0.02 a–e
	2020	2.54 $\pm$ 0.19 d–g	43.40 $\pm$ 0.28 gh	0.10 $\pm$ 0.01 bc	0.41 $\pm$ 0.01 g	1.25 $\pm$ 0.38 efg	0.23 $\pm$ 0.03 c–g
Mean value		2.44 $\pm$ 0.17	44.99 $\pm$ 1.72	0.11 $\pm$ 0.01 <i>a</i>	1.26 $\pm$ 0.94	1.38 $\pm$ 0.33 <i>bc</i>	0.24 $\pm$ 0.03
‘Penta®’	2019	2.21 $\pm$ 0.07 gh	45.62 $\pm$ 0.05 b	0.09 $\pm$ 0.01 de	1.20 $\pm$ 0.12 d	1.34 $\pm$ 0.04 d–g	0.28 $\pm$ 0.02 a
	2020	2.97 $\pm$ 0.09 bc	43.02 $\pm$ 1.35 hi	0.09 $\pm$ 0.01 de	0.48 $\pm$ 0.08 fg	1.86 $\pm$ 0.11 a	0.22 $\pm$ 0.04 d–g
Mean value		2.58 $\pm$ 0.42	44.32 $\pm$ 1.64	0.09 $\pm$ 0.01 <i>b</i>	0.84 $\pm$ 0.41	1.60 $\pm$ 0.29 <i>ab</i>	0.25 $\pm$ 0.04
‘Soleta’	2019	1.72 $\pm$ 0.07 i	45.80 $\pm$ 0.27 ab	0.10 $\pm$ 0.01 cde	1.28 $\pm$ 0.18 cd	1.40 $\pm$ 0.07 def	0.24 $\pm$ 0.04 b–f
	2020	2.66 $\pm$ 0.71 c–f	42.28 $\pm$ 1.38 i	0.09 $\pm$ 0.01 cde	0.29 $\pm$ 0.02 g	1.23 $\pm$ 0.08 efg	0.25 $\pm$ 0.04 a–d
Mean value		2.19 $\pm$ 0.68	44.04 $\pm$ 2.09	0.09 $\pm$ 0.01 <i>b</i>	0.78 $\pm$ 0.45	1.32 $\pm$ 0.12 <i>c</i>	0.24 $\pm$ 0.04
‘Supernova’	2019	1.82 $\pm$ 0.01 i	44.75 $\pm$ 0.11 cde	0.07 $\pm$ 0.01 f	1.23 $\pm$ 0.02 cd	1.47 $\pm$ 0.04 cde	0.22 $\pm$ 0.01 efg
	2020	2.90 $\pm$ 0.34 bcd	43.55 $\pm$ 0.47 gh	0.11 $\pm$ 0.02 ab	0.43 $\pm$ 0.03 g	1.10 $\pm$ 0.11 g	0.23 $\pm$ 0.01 c–g
Mean value		2.36 $\pm$ 0.62	44.15 $\pm$ 0.71	0.09 $\pm$ 0.02 <i>b</i>	0.83 $\pm$ 0.33	1.28 $\pm$ 0.21 <i>c</i>	0.22 $\pm$ 0.01
‘Tuono’	2019	2.03 $\pm$ 0.04 hi	45.17 $\pm$ 0.59 bcd	0.07 $\pm$ 0.01 f	1.05 $\pm$ 0.07 de	1.41 $\pm$ 0.05 def	0.23 $\pm$ 0.02 c–g
	2020	3.08 $\pm$ 0.15 ab	43.32 $\pm$ 0.84 gh	0.10 $\pm$ 0.01 bcd	0.42 $\pm$ 0.04 g	1.21 $\pm$ 0.021 fg	0.20 $\pm$ 0.03 g
Mean value		2.55 $\pm$ 0.57	44.25 $\pm$ 1.21	0.09 $\pm$ 0.01 <i>b</i>	0.73 $\pm$ 0.34	1.31 $\pm$ 0.17 <i>c</i>	0.21 $\pm$ 0.03
‘Vialfas’	2019	2.51 $\pm$ 0.05 efg	45.76 $\pm$ 0.04 ab	0.09 $\pm$ 0.01 e	1.56 $\pm$ 0.39 bc	1.53 $\pm$ 0.14 bcd	0.21 $\pm$ 0.02 fg
	2020	3.39 $\pm$ 0.10 a	44.17 $\pm$ 0.24 efg	0.09 $\pm$ 0.01 cde	0.35 $\pm$ 0.02 g	1.74 $\pm$ 0.22 ab	0.24 $\pm$ 0.01 b–f
Mean value		2.95 $\pm$ 0.48	44.96 $\pm$ 0.87	0.09 $\pm$ 0.01 <i>b</i>	0.95 $\pm$ 0.54	1.63 $\pm$ 0.21 <i>a</i>	0.23 $\pm$ 0.02
Average cultivars 2019		2.08 $\pm$ 0.28 b	45.51 $\pm$ 0.59 a	0.09 $\pm$ 0.02 b	1.36 $\pm$ 0.49 a	1.49 $\pm$ 0.16	0.23 $\pm$ 0.03
Average cultivars 2020		2.91 $\pm$ 0.41 a	43.50 $\pm$ 0.96 b	0.10 $\pm$ 0.01 a	0.38 $\pm$ 0.07 b	1.45 $\pm$ 0.34	0.24 $\pm$ 0.03

Almond leaf micronutrients detected at 90 DAFB showed differences related to the effect of cultivar, year of investigation and their interaction, as shown in Table 4. Particularly, the leaf content of Fe showed wide variability ranged between 130.31 $\mu\text{g g}^{-1}$ DW recorded in 2020 for cv ‘Soleta’ to 50.15 $\mu\text{g g}^{-1}$ DW in ‘Lauranne® Avijor’ in 2019. Furthermore, its mean value was higher in 2020 than in 2019 (87.5 $\mu\text{g g}^{-1}$ DW vs. 69.7 $\mu\text{g g}^{-1}$ DW), meanwhile the cultivars ‘Guara’, ‘Penta®’ and ‘Vialfas’ showed high stability of leaf content of Fe over the two research-year.

Mn in mid-June showed highest mean values in ‘Penta®’ (30.73 $\mu\text{g g}^{-1}$ DW in 2019) and the lowest ones in ‘Soleta’ (14.19 $\mu\text{g g}^{-1}$ DW in 2020). High mean values of this nutrient were also detected for ‘Vialfas’ (28.43 $\mu\text{g g}^{-1}$ DW) and ‘Tuono’ (25.87 $\mu\text{g g}^{-1}$ DW) in 2019 for both cultivars, whereas the Mn leaf content expressed as mean of cultivars was significantly higher in 2019 than in 2020.

Referring to the leaf content of B, its values were consistently higher in 2019 for all cultivars except for ‘Supernova’, where values measured in 2019 were similar to those of 2020 (32.1 vs. 26.6), as well as partially for ‘Tuono’ (16.79 vs. 10.30). Moreover, although the nutrient was characterized by wide variability in content, its values were in line with the reference values proposed in the literature [60]. The highest values were observed in ‘Soleta’ in 2019 (75.96 $\mu\text{g g}^{-1}$ DW), while the lowest ones were recorded in ‘Tuono’ in 2020 (10.30 $\mu\text{g g}^{-1}$ DW).

Table 6. Micronutrients content ($\mu\text{g g}^{-1}$ DW) in almond leaves collected at 120 DAFB over the years 2019–2020. (Fe = iron, Mn = manganese, B = boron, Cu = copper, Zn = zinc). Data are presented as mean $\pm$ standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher's test $p < 0.05$). Lowercase letters in italics describe the differences expressed as two-year mean values of the cultivars.

Cultivar (120 BAFB)	Year	Fe ($\mu\text{g g}^{-1}$ DW)	Mn ($\mu\text{g g}^{-1}$ DW)	B ($\mu\text{g g}^{-1}$ DW)	Cu ($\mu\text{g g}^{-1}$ DW)	Zn ($\mu\text{g g}^{-1}$ DW)
'Genco'	2019	68.45 $\pm$ 8.79 def	13.72 $\pm$ 0.47 ef	39.84 $\pm$ 14.53 a	19.21 $\pm$ 4.55 d–g	45.26 $\pm$ 26.75 a
	2020	66.80 $\pm$ 16.93 def	19.70 $\pm$ 2.98 abc	15.46 $\pm$ 0.83 e	37.68 $\pm$ 4.28 bc	9.50 $\pm$ 1.99 c
Mean value		67.62 $\pm$ 12.52 <i>b</i>	16.71 $\pm$ 3.76 <i>c</i>	27.65 $\pm$ 16.14 <i>ab</i>	28.44 $\pm$ 8.68 <i>ab</i>	27.38 $\pm$ 16.11
'Guara'	2019	79.60 $\pm$ 5.36 bcd	19.99 $\pm$ 0.51 bcd	18.51 $\pm$ 2.71 de	13.54 $\pm$ 3.52 efg	12.98 $\pm$ 0.25 bc
	2020	50.55 $\pm$ 13.04 f	17.82 $\pm$ 4.09 bcd	13.16 $\pm$ 3.04 ef	51.45 $\pm$ 19.32 a	9.66 $\pm$ 3.57 c
Mean value		65.07 $\pm$ 18.06 <i>b</i>	17.90 $\pm$ 2.70 <i>bc</i>	15.83 $\pm$ 3.91 <i>c</i>	32.49 $\pm$ 20.11 <i>a</i>	11.32 $\pm$ 2.94
'Lauranne® Avijor'	2019	93.51 $\pm$ 6.89 b	17.22 $\pm$ 3.26 cd	16.59 $\pm$ 2.33 de	21.12 $\pm$ 1.87 def	10.72 $\pm$ 1.04 bc
	2020	67.40 $\pm$ 6.21 def	22.37 $\pm$ 0.74 a	28.88 $\pm$ 11.10 b	26.34 $\pm$ 1.91 d	7.46 $\pm$ 0.91 c
Mean value		80.45 $\pm$ 15.22 <i>ab</i>	19.79 $\pm$ 3.51 <i>ab</i>	22.73 $\pm$ 9.92 <i>abc</i>	23.73 $\pm$ 3.29 <i>abc</i>	9.09 $\pm$ 1.97
'Penta®'	2019	131.32 $\pm$ 1.48 a	17.87 $\pm$ 0.27 bcd	27.29 $\pm$ 5.76 bc	13.65 $\pm$ 3.24 efg	19.01 $\pm$ 9.95 bc
	2020	69.62 $\pm$ 10.32 de	19.08 $\pm$ 2.00 bc	17.92 $\pm$ 6.62 de	28.33 $\pm$ 6.63 cd	7.41 $\pm$ 1.22 c
Mean value		100.47 $\pm$ 33.68 <i>a</i>	18.47 $\pm$ 1.47 <i>bc</i>	22.60 $\pm$ 7.62 <i>abc</i>	20.99 $\pm$ 9.22 <i>abc</i>	13.21 $\pm$ 9.03
'Soleta'	2019	90.51 $\pm$ 19.34 bc	13.07 $\pm$ 1.51 ef	21.02 $\pm$ 1.92 b–e	15.09 $\pm$ 4.12 efg	12.30 $\pm$ 0.66 bc
	2020	52.65 $\pm$ 9.94 ef	11.09 $\pm$ 1.93 f	15.35 $\pm$ 0.59 e	22.89 $\pm$ 3.17 de	10.10 $\pm$ 2.29 c
Mean values		71.58 $\pm$ 24.74 <i>b</i>	12.08 $\pm$ 1.92 <i>d</i>	18.17 $\pm$ 3.32 <i>bc</i>	18.99 $\pm$ 5.39 <i>bc</i>	11.20 $\pm$ 2.28
'Supernova'	2019	64.96 $\pm$ 6.65 def	15.32 $\pm$ 0.46 de	19.85 $\pm$ 2.48 cde	12.14 $\pm$ 0.61 fg	13.63 $\pm$ 3.37 bc
	2020	72.67 $\pm$ 16.46 cd	17.14 $\pm$ 1.35 cd	13.93 $\pm$ 4.02 ef	19.32 $\pm$ 2.54 d–g	28.85 $\pm$ 13.36 ab
Mean value		68.81 $\pm$ 12.33 <i>b</i>	16.23 $\pm$ 1.35 <i>c</i>	16.89 $\pm$ 4.42 <i>c</i>	15.73 $\pm$ 4.21 <i>c</i>	21.24 $\pm$ 12.15
'Tuono'	2019	129.50 $\pm$ 32.21 a	18.15 $\pm$ 1.29 bc	23.60 $\pm$ 7.14 bcd	11.25 $\pm$ 0.57 g	37.29 $\pm$ 10.86 a
	2020	61.94 $\pm$ 2.03 def	20.48 $\pm$ 1.75 ab	7.03 $\pm$ 0.50 f	16.02 $\pm$ 3.72 efg	11.55 $\pm$ 1.11 bc
Mean value		95.72 $\pm$ 41.84 <i>a</i>	19.32 $\pm$ 1.89 <i>b</i>	15.32 $\pm$ 9.02 <i>c</i>	13.63 $\pm$ 3.54 <i>c</i>	24.42 $\pm$ 15.51
'Vialfas'	2019	88.22 $\pm$ 2.56 bc	22.40 $\pm$ 2.35 a	18.16 $\pm$ 0.22 de	14.48 $\pm$ 5.06 efg	12.64 $\pm$ 1.21 bc
	2020	67.97 $\pm$ 1.53 def	22.01 $\pm$ 1.68 a	42.10 $\pm$ 2.33 a	44.48 $\pm$ 13.62 ab	8.09 $\pm$ 1.45 c
Mean value		78.10 $\pm$ 11.32 <i>ab</i>	22.20 $\pm$ 1.91 <i>a</i>	30.13 $\pm$ 12.89 <i>a</i>	29.48 $\pm$ 15.64 <i>ab</i>	10.36 $\pm$ 2.73
Average cultivars 2019		93.26 $\pm$ 26.91 a	16.47 $\pm$ 3.15	23.11 $\pm$ 9.08	15.06 $\pm$ 4.39 b	20.48 $\pm$ 12.08 a
Average cultivars 2020		63.70 $\pm$ 12.38 b	18.31 $\pm$ 3.95	19.23 $\pm$ 11.42	30.81 $\pm$ 13.33 a	11.58 $\pm$ 7.13 b

Cu in almond leaves at mid-June was the only micronutrient that did not show statistical significance related to the cultivar effect. Its value expressed as mean of cultivars in 2019 was much lower than in 2020 ($4.52 \mu\text{g g}^{-1}$ DW vs. $16.42 \mu\text{g g}^{-1}$ DW, respectively). The highest Cu leaf content was observed in 'Genco' in 2020 ($20.84 \mu\text{g g}^{-1}$ DW), while the lowest one was expressed in leaves of 'Lauranne® Avijor' in 2019 ($1.27 \mu\text{g g}^{-1}$ DW).

Contrariwise to Cu, the micronutrient Zn showed contents as mean of cultivars in 2019 significantly higher than those detected in 2020, with values of $47.27 \mu\text{g g}^{-1}$ DW and $29.58 \mu\text{g g}^{-1}$ DW, respectively (Table 4). 'Guara' was the cultivar characterized by lower content of Cu in the leaves with values expressed as mean of year investigation of $6.66 \mu\text{g g}^{-1}$ DW, whereas cultivar 'Supernova' showed the higher mean values ($58.65 \mu\text{g g}^{-1}$ DW).

Leaf macronutrients detected in mid-July (120 DAFB) were reported in Table 5. Content of N was significantly influenced by the year and interaction cultivar $\times$ year, while the nutrient was not influenced by the effect of cultivar. Its mean content was higher in 2020 for all cultivars tested and its values ranged between 3.39% DW, recorded in 'Vialfas' in 2020, to 1.72% DW, detected in 'Soleta' in 2019. Furthermore, 'Lauranne® Avijor' was characterized by a higher N stability in content within the two-year research period in comparison to the other cultivars studied.

Similarly to N, C detected at 120 DAFB was also influenced by the year and interaction cultivar $\times$ year. Nevertheless, as observed at 90 DAFB, the leaf trait was very stable as confirmed by its values that ranked within 43–45% DW.

P ranged between values of 0.12% DW observed in 'Lauranne® Avijor' to 0.07% DW detected in 'Genco', 'Supernova' and 'Tuono' in the first research-year for all cultivars. This

macronutrient was influenced by both effects and their interaction, as shown by statistical analysis. Furthermore, leaf sampling at 120 DAFB showed, in general, P values slightly lower in comparison to the reference values reported in the literature [61].

The content of K at mid-July showed differences for year and interaction of cultivar $\times$ year, with the highest average values being observed in 'Lauranne® Avijor' in 2019 (2.12% DW) and the lowest ones in 'Soleta' in 2020 (0.29% DW). The nutrient was also much higher during the first research year, showing values expressed as mean of cultivars of 1.36% DW compared with 0.38% DW in 2020.

Cultivars 'Vialfas' and 'Guara' showed the higher Ca leaf content at 120 DAFB expressed as mean of two years, with similar values between them (1.65% and 1.63% DW respectively), meanwhile the highest values of Ca were detected for 'Penta®' in 2020 (1.86% DW). Contrariwise, lowest content was observed in 'Supernova' in 2020 with mean values of 1.10% DW. For this nutrient no differences were observed in relation to the research-year.

Mg leaf content was affected by interaction between cultivar and year, and its values ranged between 0.28% DW in 'Penta®' in 2019 to 0.20% DW in 'Tuono' in 2020. In addition, the Mg content expressed as mean of cultivars was the same over the two research years, as shown in Table 5.

Table 6 describes the leaf micronutrients content at 120 DAFB. Fe ranged between values of 131.32 $\mu\text{g g}^{-1}$ DW recorded in 'Penta®' in 2019 to 50.55 $\mu\text{g g}^{-1}$ DW in 'Guara' in 2020. On average, 'Penta®' showed the highest mean value for the nutrient when expressed as mean of two research years (100.47 $\mu\text{g g}^{-1}$ DW) contrariwise to 'Guara', 'Genco' and 'Supernova' that were characterized by the lowest mean values (65.07, 67.62 and 68.81 $\mu\text{g g}^{-1}$ DW, respectively). In addition, its values expressed as mean of cultivars were significantly higher in 2019 (93.26 $\mu\text{g g}^{-1}$ DW) than in 2020 (63.70 $\mu\text{g g}^{-1}$ DW).

Mn was influenced by the effect of cultivar and interaction cultivar $\times$ year. The highest mean values at 120 DABF were observed for 'Vialfas' in both research years (22.40 $\mu\text{g g}^{-1}$ DW in 2019 and 22.01 $\mu\text{g g}^{-1}$ DW in 2020) and for 'Lauranne® Avijor' in 2020 (22.37 $\mu\text{g g}^{-1}$ DW), while the lowest ones were recorded for 'Soleta' in 2020 (11.09 $\mu\text{g g}^{-1}$ DW).

Similarly to Mn, B also showed differences for cultivar and interaction of cultivar $\times$ year. The micronutrient at 120 DAFB was characterized by the high variability of leaf content, ranging from values of 42.10 $\mu\text{g g}^{-1}$ DW in 'Vialfas' to 7.03 $\mu\text{g g}^{-1}$ DW in 'Tuono' detected for both cultivars at the second research year. Furthermore, 'Vialfas' and 'Genco' showed the highest content of B in the leaves when expressed as mean of two-year investigation (30.13 and 27.65 $\mu\text{g g}^{-1}$ DW, respectively) in contrast to 'Tuono' and 'Guara' that were characterized by lowest values (about 15–16 $\mu\text{g g}^{-1}$ DW).

The content of Cu was influenced by the effect of cultivar, year and their interaction (Table 6). Its contents, expressed as mean of cultivars, were doubled in value in 2020 in comparison to those detected in 2019 (30.81 vs. 15.06). The highest Cu foliar content was observed in 'Guara' in 2020 (51.45 $\mu\text{g g}^{-1}$ DW), while the lowest one was recorded in 'Tuono' in 2019 (11.25 $\mu\text{g g}^{-1}$ DW).

The highest foliar Zn content were observed in 'Genco' in 2019 with mean values of 45.26 $\mu\text{g g}^{-1}$ DW. 'Penta®' showed the lowest Zn content in the leaves particularly at mid-July 2020, with values of 7.41 $\mu\text{g g}^{-1}$ DW. Since it is noticed as Zn transport in plants is affected by its supply level [62], notable variability in Zn content was highlighted by its values expressed as mean of cultivars, that were significantly higher in 2019 (20.48 $\mu\text{g g}^{-1}$ DW) than in 2020 (11.58 $\mu\text{g g}^{-1}$ DW), as shown in Table 6.

Table 7 shows values of the ΣDOP calculated from the average reference values (Cref) proposed by Mills et al. [39] for each cultivar, year of investigation and DAFB. Differences related to the year were observed for ΣDOP at 120 DAFB, while the interaction cultivar $\times$ year was statistically significant for both ΣDOP at 90 and 120 DAFB.

Table 7. Values of Σ DOP calculated by adding the absolute values of the DOP index determined for every nutrient. Single nutrient DOP indexes were obtained comparing mean values to the average C_{ref} proposed by Mills et al. [39]). Data are presented as mean $\pm$ standard deviation. Different lowercase letters on the columns indicate differences between means (Fisher's test $p < 0.05$).

Cultivar	Year	Σ DOP (90 DAFB)	Σ DOP (120 DAFB)
'Genco'	2019	392 $\pm$ 27 d	525 $\pm$ 88 fgh
	2020	508 $\pm$ 19 ab	718 $\pm$ 47 bc
Mean values		540 $\pm$ 65	622 $\pm$ 122
'Guara'	2019	479 $\pm$ 51 bc	468 $\pm$ 40 gh
	2020	519 $\pm$ 25 ab	845 $\pm$ 155 a
Mean values		499 $\pm$ 43	656 $\pm$ 227
'Lauranne® Avijor'	2019	510 $\pm$ 11 ab	531 $\pm$ 42 fgh
	2020	395 $\pm$ 66 d	587 $\pm$ 39 def
Mean values		452 $\pm$ 75	559 $\pm$ 48
'Penta®'	2019	430 $\pm$ 17 cd	355 $\pm$ 18 i
	2020	501 $\pm$ 20 ab	627 $\pm$ 56 de
Mean values		466 $\pm$ 42	491 $\pm$ 150
'Soleta'	2019	505 $\pm$ 90 ab	456 $\pm$ 11 gh
	2020	430 $\pm$ 19 cd	635 $\pm$ 36 cd
Mean values		468 $\pm$ 73	565 $\pm$ 99
'Supernova'	2019	434 $\pm$ 50 cd	446 $\pm$ 2 h
	2020	425 $\pm$ 35 cd	540 $\pm$ 78 efg
Mean values		430 $\pm$ 41	493 $\pm$ 72
'Tuono'	2019	436 $\pm$ 35 cd	384 $\pm$ 42 i
	2020	425 $\pm$ 48 cd	578 $\pm$ 19 def
Mean values		430 $\pm$ 40	463 $\pm$ 127
'Vialfas'	2019	429 $\pm$ 36 cd	461 $\pm$ 38 gh
	2020	551 $\pm$ 20 a	732 $\pm$ 105 b
Mean values		490 $\pm$ 70	596 $\pm$ 162
Year 2019 (Mean values)		452 $\pm$ 56	449 $\pm$ 74 b
Year 2020 (Mean values)		469 $\pm$ 62	657 $\pm$ 118 a

At 90 DAFB the Σ DOP index ranged from 551 in 'Vialfas' in the 2020 to 392 in 'Genco' in 2019. Considering that lower values of Σ DOP indicate high balance in macro- and micronutrients in the leaves as a result of an adequate nutritional status of the plant, the cultivar that performed similarly to 'Genco' was 'Lauranne® Avijor' in 2020, whereas in 2019 it showed one of the worst performances (mean values of 510). Even 'Tuono' showed low values of Σ DOP at 90 DAFB both in 2019 and 2020. Furthermore, at 90 DAFB this trait was quite stable during the two research years, as shown by the values expressed as the average of cultivars.

The Σ DOP calculated at 120 DAFB showed high variability in values, and it was significantly higher in 2020 (mean values of 657) versus 2019 (mean value of 449), when expressed as mean of cultivars. Additionally, all cultivars showed an increase in value of Σ DOP between the first and second year of the trial. Cultivar 'Guara' showed the highest Σ DOP values mid-July in 2020 (845) in comparison to other cultivars, while in 2019 had mean values approximately half of those calculated for the second research year. Conversely, the lowest Σ DOP were obtained for 'Penta®' and 'Tuono' in 2019 with values of 355 and 384, respectively.

According to the findings obtained through the leaf analyses, Supplemental Table S1 summarizes the optimal ranges of leaf macro- and micronutrients for the cultivars studied at 90 and 120 DAFB, respectively.

The average N content varied between 2 and 3% DW at both 90 and 120 DAFB. 'Vialfas' was characterized by the highest average N contents (2.9–3.0 % DW) while 'Soleta' and 'Supernova', with average values of 1.9% DW at 90 DAFB and about 2.4% DW at 120 DAFB, showed the lowest leaf N contents.

P and Mg were stable in the two leaf sampling times with no noteworthy differences between cultivars.

K was also very stable between cultivars, showing however a slightly wider variability of content at 120 DAFB, going from average values of 0.55% DW in 'Guara' to 1.25% DW in 'Lauranne® Avijor'.

Ca showed a slight increase in content at 120 DAFB (1.3–1.6% DW) compared to the mean contents found at 90 DAFB (1.0–1.3% DW), and with 'Genco', 'Guara', 'Penta®' and 'Vialfas' characterized by mean contents above 1.50% DW.

Among the micronutrients, while Mn was rather stable in average content in relation to both cultivar and leaf sampling time, Fe showed the widest variability in content, ranging from average values of $55 \mu\text{g g}^{-1}$ DW in 'Penta®' at 90 DAFB to values of $100.5 \mu\text{g g}^{-1}$ DW at 120 DAFB for the same cultivar, which also showed the greatest variability in content between the two sampling dates. Similarly, B also showed some variability between cultivars, with the highest contents observed in 'Soleta' at 90 DAFB ($48 \mu\text{g g}^{-1}$ DW) and showing a decreasing trend in average content between the first and second sampling dates. Conversely, Cu, although characterized by rather similar average contents among the cultivars studied, showed a general increase in content from 90 DAFB to 120 DAFB. Finally, Zn showed a decreasing trend between the two sampling dates, although characterized by a wide variability in content, and going from average values of $58 \mu\text{g g}^{-1}$ DW in 'Supernova' at 90 DAFB to values of $9.10 \mu\text{g g}^{-1}$ DW in 'Lauranne® Avijor' at 120 DAFB.

3.3. Content of Total Chlorophyll, Flavonols, Anthocyanins and Nitrogen Balance Index in the Almond Leaves

The leaf content of total Chl helps to quantify the photosynthesis rate of the plant [63], and it can be used to assess the adaptability of a certain cultivar in a new growing environment [64].

Eco-physiological parameters of almond leaves measured at 90 DABF are reported in Table 8. Total Chl content at 90 DAFB was influenced by cultivar, year and their interaction. The Chl values ranged between $32.87 \mu\text{g cm}^{-2}$ recorded in 'Lauranne® Avijor' in 2020 to $18.87 \mu\text{g cm}^{-2}$ in 'Soleta' in 2019. High values of total Chl at 90 DAFB, generally higher than $30 \mu\text{g cm}^{-2}$, were observed in 2020 also for 'Soleta' 'Supernova', 'Tuono' and 'Vialfas'. Furthermore, the mean values of total Chl, expressed as mean of cultivars, was significantly higher in 2020 ($30.48 \mu\text{g cm}^{-2}$) in comparison to those recorded in 2019 ($23.97 \mu\text{g cm}^{-2}$).

Conversely, the leaf contents of Flav and Anth at 90 DAFB were more stable than those of Chl, as shown by their values expressed as mean of cultivars (Table 8). Indeed, Flav had mean values of 2.35 and $2.31 \mu\text{g cm}^{-2}$ in 2019 and 2020 respectively, as well as the Anth content, that showed values of 0.10 (2019) and $0.08 \mu\text{g cm}^{-2}$ (2020).

The NBI in the almond leaves at 90 DAFB ranged between mean values of 14.62 in 'Lauranne® Avijor' in 2020 to 8.00 in 'Soleta' in 2019. Even cultivars 'Soleta', 'Supernova', 'Tuono' and 'Vialfas' showed high values of NBI (over 13) in 2020, whereas 'Guara' and 'Tuono' showed low NBI mean values (below 10) in 2019. This was also confirmed by the NBI expressed as mean of cultivars, significantly higher in 2020 than 2019, as shown in Table 8.

Table 8. Leaf total (Chl), flavonol (Flav) anthocyanin (Anth) and nitrogen balance index (NBI) in almond leaves collected at 90 DAFB over the years 2019–2020. Data are presented as mean $\pm$ standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher’s test $p < 0.05$). Lowercase letters in italics describe the differences expressed as two-year mean values of the cultivars.

Cultivar. (90 DAFB)	Year	Chl ($\mu\text{g cm}^{-2}$)	Flav ($\mu\text{g cm}^{-2}$)	Anth ($\mu\text{g cm}^{-2}$)	NBI
‘Genco’	2019	25.07 $\pm$ 4.47 gh	2.37 $\pm$ 0.06 abc	0.10 $\pm$ 0.03 cde	10.61 $\pm$ 1.96 hi
	2020	28.08 $\pm$ 3.02 f	2.35 $\pm$ 0.10 bcd	0.09 $\pm$ 0.02 def	11.97 $\pm$ 1.29 g
Mean value		26.57 $\pm$ 4.09 cde	2.36 $\pm$ 0.08 a	0.09 $\pm$ 0.03 c	11.29 $\pm$ 1.79 cd
‘Guara’	2019	22.43 $\pm$ 3.29 j	2.36 $\pm$ 0.07 a–d	0.12 $\pm$ 0.03 b	9.51 $\pm$ 1.43 k
	2020	29.46 $\pm$ 4.27 de	2.30 $\pm$ 0.08 fg	0.09 $\pm$ 0.02 def	12.87 $\pm$ 1.76 de
Mean value		25.95 $\pm$ 5.19 de	2.33 $\pm$ 0.08 abc	0.10 $\pm$ 0.03 b	11.19 $\pm$ 2.32 cd
‘Lauranne® Avijor’	2019	23.66 $\pm$ 2.46 ij	2.34 $\pm$ 0.06 cde	0.10 $\pm$ 0.02 cd	10.13 $\pm$ 1.17 ij
	2020	32.87 $\pm$ 3.39 a	2.28 $\pm$ 0.11 gh	0.07 $\pm$ 0.02 i	14.62 $\pm$ 1.56 a
Mean value		28.26 $\pm$ 5.49 bc	2.31 $\pm$ 0.10 c	0.09 $\pm$ 0.02 cd	12.37 $\pm$ 2.64 ab
‘Penta®’	2019	24.32 $\pm$ 3.44 hi	2.29 $\pm$ 0.07 fg	0.10 $\pm$ 0.03 cde	10.59 $\pm$ 1.53 hi
	2020	28.23 $\pm$ 2.52 ef	2.32 $\pm$ 0.06 ef	0.08 $\pm$ 0.02 gh	12.21 $\pm$ 1.18 fg
Mean value		26.27 $\pm$ 3.58 de	2.31 $\pm$ 0.07 c	0.09 $\pm$ 0.03 cd	11.40 $\pm$ 1.58 cd
‘Soleta’	2019	18.87 $\pm$ 2.84 k	2.36 $\pm$ 0.06 a–d	0.15 $\pm$ 0.03 a	8.00 $\pm$ 1.26 l
	2020	30.62 $\pm$ 4.29 cd	2.33 $\pm$ 0.06 de	0.08 $\pm$ 0.03 fg	13.12 $\pm$ 2.00 cde
Mean value		24.74 $\pm$ 6.92 e	2.35 $\pm$ 0.06 ab	0.12 $\pm$ 0.05 a	10.56 $\pm$ 3.06 d
‘Supernova’	2019	25.70 $\pm$ 3.62 g	2.38 $\pm$ 0.08 ab	0.09 $\pm$ 0.03 ef	10.86 $\pm$ 1.74 h
	2020	32.08 $\pm$ 4.69 ab	2.29 $\pm$ 0.09 fgh	0.08 $\pm$ 0.03 gh	14.14 $\pm$ 2.03 ab
Mean value		28.89 $\pm$ 5.26 ab	2.33 $\pm$ 0.10 abc	0.09 $\pm$ 0.03 cd	12.50 $\pm$ 2.50 a
‘Tuono’	2019	22.93 $\pm$ 3.89 j	2.38 $\pm$ 0.06 a	0.10 $\pm$ 0.03 cde	9.62 $\pm$ 1.61 jk
	2020	30.93 $\pm$ 4.25 bc	2.26 $\pm$ 0.11 h	0.08 $\pm$ 0.02 gh	13.68 $\pm$ 1.66 bc
Mean value		26.94 $\pm$ 5.71 cd	2.32 $\pm$ 0.11 bc	0.09 $\pm$ 0.02 cd	11.65 $\pm$ 2.60 bc
‘Vialfas’	2019	28.75 $\pm$ 4.45 ef	2.30 $\pm$ 0.10 fg	0.07 $\pm$ 0.02 hi	12.59 $\pm$ 2.04 ef
	2020	31.58 $\pm$ 3.88 abc	2.36 $\pm$ 0.09 a–d	0.09 $\pm$ 0.03 ef	13.30 $\pm$ 1.99 cd
Mean value		30.16 $\pm$ 4.39 a	2.33 $\pm$ 0.10 abc	0.08 $\pm$ 0.03 d	12.94 $\pm$ 2.04 a
Average cultivars 2019		23.97 $\pm$ 4.47 b	2.35 $\pm$ 0.11 a	0.10 $\pm$ 0.03 a	10.24 $\pm$ 2.02 b
Average cultivars 2020		30.48 $\pm$ 4.16 a	2.31 $\pm$ 0.10 b	0.08 $\pm$ 0.02 b	13.24 $\pm$ 1.90 a

The same traits measured at 120 DABF are reported in Table 9. Even in mid-July, total Chl content was influenced by cultivar, year and their interaction. The Chl mean values ranged between 39.31 $\mu\text{g cm}^{-2}$ recorded in ‘Vialfas’ in 2020 to 19.83 $\mu\text{g cm}^{-2}$ in ‘Soleta’ in 2019. Values of total leaf Chl, higher than 30 $\mu\text{g cm}^{-2}$, were observed also for ‘Lauranne® Avijor’ for both research-year and for ‘Guara’, ‘Penta®’, ‘Soleta’ and ‘Tuono’ in 2020. Furthermore, total Chl expressed as mean of cultivars was significantly higher in 2020 (32.58 $\mu\text{g cm}^{-2}$) in comparison to those recorded in 2019 (25.21 $\mu\text{g cm}^{-2}$).

Leaf contents of Flav were slightly lower than those observed in mid-June (90 DAFB) as shown by their values expressed as mean of cultivars (2.21 and 2.27 $\mu\text{g cm}^{-2}$ in the growing seasons 2019 and 2020, respectively). Furthermore, the values recorded for each cultivar over the two-year investigation were very similar between them (Table 9).

The leaf Anth contents ranged between 0.12 $\mu\text{g cm}^{-2}$ measured in ‘Supernova’ in 2019 to 0.04 $\mu\text{g cm}^{-2}$ in ‘Penta®’ in 2020, meanwhile Anth expressed as mean of cultivars had mean values of 0.08 and 0.07 $\mu\text{g cm}^{-2}$ in 2019 and 2020, respectively.

The NBI at 120 DAFB ranged between values of 17.16 in ‘Vialfas’ in 2020 to 9.18 in ‘Soleta’ in 2019. This leaf trait, expressed as mean of cultivars, was slightly higher in mid-July when compared to 90 DAFB. During 2019, the NBI at 120 DAFB had mean values of 11.49, higher than over one point in comparison to 90 DAFB (10.24), as well as in 2020 with values of 14.58 in mid-July versus values of 13.24 recorded in mid-June.

Table 9. Leaf total chlorophyll (Chl), flavonol (Flav) anthocyanin (Anth) and nitrogen balance index (NBI) in almond leaves collected at 120 DAFB over the years 2019–2020. Data are presented as mean $\pm$ standard deviation. Different lowercase letters on the columns indicate significant differences between means (Fisher’s test $p < 0.05$). Lowercase letters in italics describe the differences expressed as two-year mean values of the cultivars.

Cultivar (120 DAFB)	Year	Chl ($\mu\text{g cm}^{-2}$)	Flav ($\mu\text{g cm}^{-2}$)	Anth ($\mu\text{g cm}^{-2}$)	NBI
‘Genco’	2019	24.63 $\pm$ 2.48 k	2.30 $\pm$ 0.11 bc	0.08 $\pm$ 0.02 ef	10.74 $\pm$ 1.16 f
	2020	28.27 $\pm$ 6.11 hi	2.30 $\pm$ 0.16 abc	0.09 $\pm$ 0.03 cd	12.94 $\pm$ 2.60 d
Mean value		26.45 $\pm$ 4.99 c	2.30 $\pm$ 0.14 a	0.08 $\pm$ 0.03 c	11.84 $\pm$ 2.29 cd
‘Guara’	2019	22.20 $\pm$ 3.46 l	2.22 $\pm$ 0.15 d	0.11 $\pm$ 0.02 b	9.92 $\pm$ 1.37 fg
	2020	32.51 $\pm$ 7.19 cd	2.31 $\pm$ 0.17 ab	0.08 $\pm$ 0.04 def	14.43 $\pm$ 3.48 c
Mean value		27.35 $\pm$ 7.64 c	2.27 $\pm$ 0.16 ab	0.09 $\pm$ 0.04 bc	12.17 $\pm$ 3.47 c
‘Lauranne® Avijor’	2019	30.62 $\pm$ 5.38 efg	2.03 $\pm$ 0.18 g	0.06 $\pm$ 0.02 g	15.05 $\pm$ 2.84 c
	2020	31.96 $\pm$ 6.81 cde	2.23 $\pm$ 0.18 d	0.07 $\pm$ 0.03 f	14.45 $\pm$ 3.15 c
Mean value		31.29 $\pm$ 6.15 b	2.13 $\pm$ 0.21 e	0.06 $\pm$ 0.03 de	14.75 $\pm$ 3.00 ab
‘Penta®’	2019	26.19 $\pm$ 4.47 jk	2.21 $\pm$ 0.09 de	0.07 $\pm$ 0.03 f	11.86 $\pm$ 2.11 e
	2020	34.55 $\pm$ 4.52 b	2.15 $\pm$ 0.19 f	0.04 $\pm$ 0.03 h	16.14 $\pm$ 2.26 b
Mean value		30.37 $\pm$ 6.13 b	2.18 $\pm$ 0.15 de	0.06 $\pm$ 0.03 de	14.00 $\pm$ 3.06 b
‘Soleta’	2019	19.83 $\pm$ 4.81 m	2.16 $\pm$ 0.10 f	0.13 $\pm$ 0.04 a	9.18 $\pm$ 2.23 g
	2020	31.21 $\pm$ 7.36 def	2.31 $\pm$ 0.14 ab	0.09 $\pm$ 0.03 cde	13.45 $\pm$ 2.89 d
Mean value		25.52 $\pm$ 8.43 c	2.24 $\pm$ 0.15 bc	0.11 $\pm$ 0.04 a	11.32 $\pm$ 3.35 cd
‘Supernova’	2019	21.57 $\pm$ 2.82 lm	2.30 $\pm$ 0.05 bc	0.12 $\pm$ 0.03 ab	9.39 $\pm$ 1.30 g
	2020	28.85 $\pm$ 5.42 ghi	2.31 $\pm$ 0.15 ab	0.09 $\pm$ 0.03 c	12.80 $\pm$ 3.03 d
Mean value		25.21 $\pm$ 5.65 c	2.30 $\pm$ 0.11 a	0.10 $\pm$ 0.03 ab	11.09 $\pm$ 2.89 d
‘Tuono’	2019	27.12 $\pm$ 3.38 ij	2.17 $\pm$ 0.11 ef	0.07 $\pm$ 0.02 f	12.67 $\pm$ 1.71 de
	2020	33.40 $\pm$ 5.22 bc	2.22 $\pm$ 0.14 de	0.07 $\pm$ 0.03 f	15.23 $\pm$ 2.59 c
Mean value		30.26 $\pm$ 5.40 b	2.19 $\pm$ 0.13 cd	0.07 $\pm$ 0.03 d	13.95 $\pm$ 2.53 b
‘Vialfas’	2019	26.59 $\pm$ 4.05 fgh	2.26 $\pm$ 0.14 cd	0.06 $\pm$ 0.03 g	13.11 $\pm$ 1.58 d
	2020	39.31 $\pm$ 5.11 a	2.35 $\pm$ 0.09 a	0.05 $\pm$ 0.03 gh	17.16 $\pm$ 2.03 a
Mean value		34.74 $\pm$ 6.93 a	2.30 $\pm$ 0.13 a	0.05 $\pm$ 0.03 e	15.54 $\pm$ 2.72 a
Average cultivars 2019		25.21 $\pm$ 5.35 b	2.21 $\pm$ 0.15 b	0.08 $\pm$ 0.04 a	11.49 $\pm$ 2.67 b
Average cultivars 2020		32.58 $\pm$ 6.91 a	2.27 $\pm$ 0.17 a	0.07 $\pm$ 0.04 b	14.58 $\pm$ 3.13 a

3.4. Pearson Correlation Heatmap among Almond Leaf Traits Analyzed

Figures 2 and 3 show the Pearson correlation heatmap between almond leaf traits analyzed at 90 and 120 DAFB, respectively. The Σ DOP was excluded from the correlation heatmap as it did not show any significant correlation with the other leaf traits.

According to Pearson correlation at 90 DAFB, almond leaf N content showed a strong positive correlation with Cu (0.808) moderate correlation with total Chl (0.616), NBI (0.594), P (0.540) and LDMC (0.527), whereas moderate negative correlations were observed with Ca (−0.593), B (−0.607) and quit low negative correlation with Anth (−0.498). Several studies have shown as the lack of N in leaves limits the growth of the plant due to a low supply of photosynthates between shoots and root system [65–67]. Deficiency of N in leaves is also reflected in a lower net photosynthesis rate and poor stomatal conductance, which reduces photosynthetic efficiency. This is due to a poor functioning of the photosynthesis system II (PSII) [68]. However, it increases the concentration of carbon dioxide in leaf tissue [69–71].

Inorganic C content at the same phenological stage showed quite high positive correlation with B (0.741), high-moderate with Mn (0.662) and moderate with Zn (0.515) as well as showing high negative correlation with Cu (−0.706) and moderate with Fe (−0.511)

P showed a high correlation with Cu (0.710) and more moderately with NBI (0.554), as well as a quite strong negative correlation with Ca (−0.768) and moderate with K (−0.539) and B (−0.505), respectively.

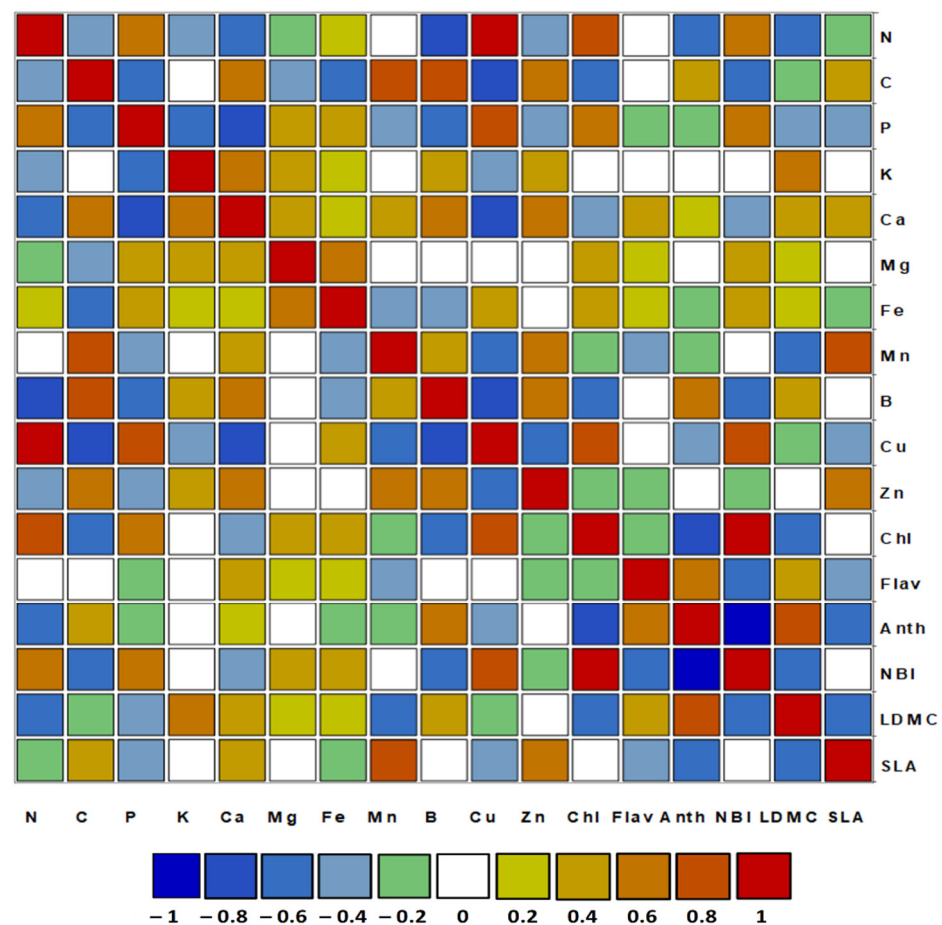


Figure 2. Heatmap of Pearson's correlation among the analyzed variables. Data from leaf diagnostics, content of total chlorophyll, flavonol, anthocyanin and nitrogen balance index and leaf biometrics recorded at 90 DAFB were compared. Positive correlations with high values as well as self-correlations are red colored while negative ones are blue colored. The other levels of correlations are indicated with different shades of colors according to the figure caption. Numerical values and the significance of correlations are shown in Supplemental Table S1. (Level of correlation: 1.0–0.8, very high; 0.8–0.6, high; 0.6–0.4, moderate; 0.4–0.2, low; 0.2–0, negligible correlation).

K at 90 DABF showed only one positive correlation with Ca of a moderate level (0.576). Studies carried out on other crops, such as sweet potato, have shown as in plants bred with K deficiency the photosynthetic rate and stomatal conductance decrease significantly and this physiological disorder was also confirmed by the analysis of Chl fluorescence [72].

Ca showed moderate positive correlations with Zn (0.544) and B (0.523), whereas it was negatively correlated with Cu (−0.650).

Mg showed only a moderate positive correlation with Fe (0.585) without showing any significant negative correlations with other leaf traits. Mg, thanks to its high mobility, tends to move to the leaves when serious reductions in photosynthesis occurs. Furthermore, Mg-free plants have difficulty in moving sucrose to the roots through the phloem and consequent Mg remobilization affects young leaves to the detriment of adult ones [65,66,73]. Mg deficiency also negatively affects the proper leaf absorption of other nutrients as confirmed by Ye et al. [74]. The authors observed, as in seedlings of *Citrus sinensis* with low leaf concentrations of Mg, that the content of Fe increased mainly in the middle and upper leaves of the shoots and simultaneously decreased in those inserted in the lower shoot portion.

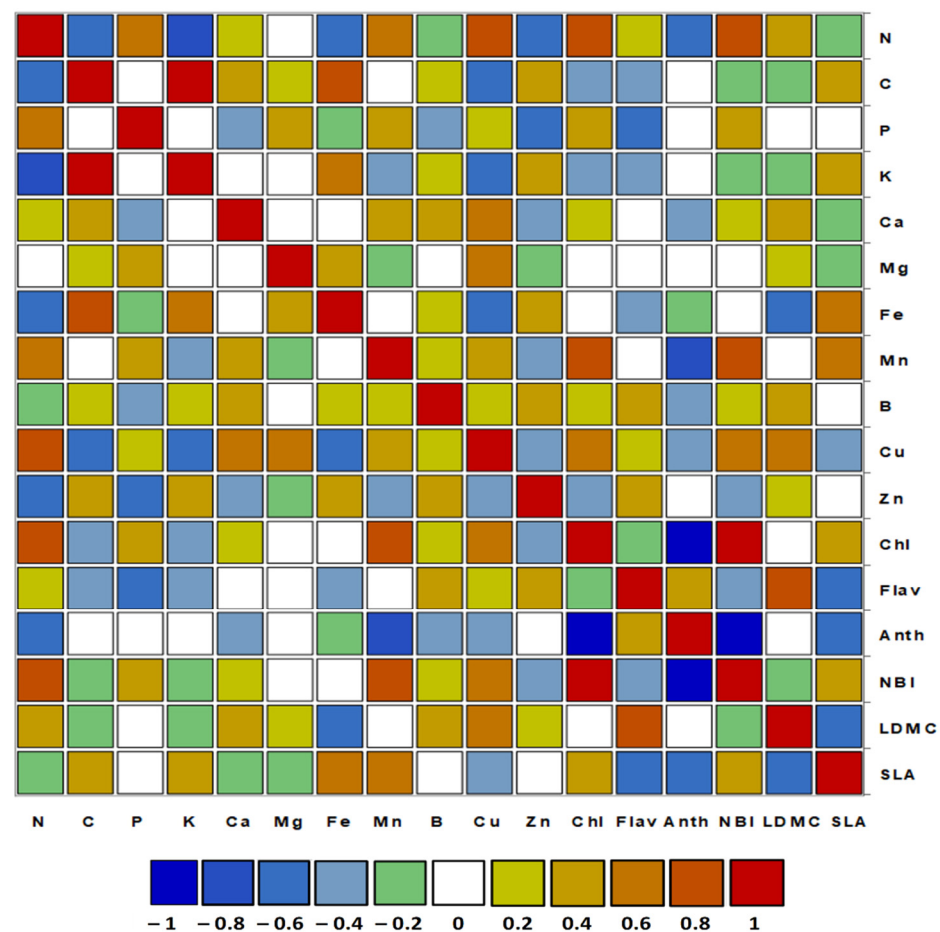


Figure 3. Heatmap of Pearson's correlation among the analyzed variables. Data from leaf diagnostics, content of total chlorophyll, flavonol, anthocyanin and nitrogen balance index and leaf biometrics recorded at 120 DAFB were compared. Positive correlations with high values as well as self-correlations are red colored while negative ones are blue colored. The other levels of correlations are indicated with different shades of colors according to the figure caption. Numerical values and the significance of correlations are shown in Supplemental Table S2. (Level of correlation: 1.0–0.8 very high; 0.8–0.6 high; 0.6–0.4, moderate; 0.4–0.2, low; 0.2–0: negligible correlation).

Fe, as well as Zn, Flav and NBI when considered main traits at 90 DAFB showed no significant correlation with all other leaf traits analyzed (Figure 2; Supplemental Table S2). Nevertheless, several studies demonstrated that P interferes with Zn absorption and often Zn deficiencies are due to the effect of high P levels in the soil [75–78].

Mn showed high positive correlation with SLA (0.696), whereas it was negatively correlated with LDMC (−0.529) and Cu (−0.500) in a moderate way in both cases.

B was only positively correlated with Zn (0.579) and highly negatively correlated with Cu (−0.786), whereas Cu was positively correlated with Chl, LDMC and NBI to a high-moderate extent (0.622, 0.621 and 0.610, respectively).

Analyzing the almond leaf eco-physiological parameters that showed significance in the Pearson correlation matrix (Supplemental Table S2), when considered as main parameter, Chl was very strongly positively correlated with NBI (0.950) and highly negatively correlated with Anth (−0.775), whereas Anth was strongly negatively correlated with NBI (−0.819) and LDMC was negatively correlated with SLA (−0.550) in a moderate way.

The heatmap determined at 120 DAFB over the two research years is shown in Figure 3. N had high positive correlation with Chl (0.721), NBI (0.664), Cu (0.634), and more moderately with Mn (0.558), whereas it was highly negatively correlated with K (−0.702) and moderately with C (−0.590).

Inorganic C content at 120 DAFB showed very high positive correlation with K (0.838) and high correlation with Fe (0.649) without any negative correlation with the other leaf traits.

K showed positive correlation with Fe (0.505) and negative correlation with Cu (−0.539) to a moderate entity in both cases.

While Fe in mid-July showed only one moderate negative correlation with Cu (−0.532), this last micronutrient showed, at the same time, only a moderate positive correlation with Chl (0.521).

Mn was positively correlated with Chl and NBI (0.700 and 0.672, respectively) and negatively with Anth (−0.695) in a high manner for all cases.

Leaf eco-physiological parameters which showed significance in the Pearson correlation matrix at 120 DAFB (Supplemental Table S3), when considered as main parameters, were Chl, Flav, Anth and LDMC. The first one was very strongly positively correlated with NBI (0.972) also at this time of investigation, whereas it was highly negatively correlated with Anth (−0.862). Flav showed a high positive correlation with LDMC (0.650) and negative correlation with SLA (0.580), whereas Anth was very highly correlated with NBI (−0.892) and moderately with SLA (−0.493), in a negative way in both cases. Furthermore, LDMC was moderately negatively correlated with SLA (−0.500).

Finally, P, Ca, Mg, B, Z and NBI did not show any significant correlation with all other leaf traits analyzed, when considering the main traits at 120 DAFB.

4. Conclusions

The analysis of the data acquired during the trial revealed differences in leaf nutrient content due to both almond cultivar and phenological stage of the plants.

The cultivars ‘Lauranne’[®] Avijor’, ‘Penta’[®], ‘Tuono’ and ‘Vialfas’ showed high stability in leaf diagnostic in the study environment. Furthermore, the Σ DOP expressed as mean of cultivars at 90 DAFB was quite stable during the two research years, whereas at 120 DAFB it was significantly higher in 2020.

The correlation of the analyzed leaf traits also highlighted the reciprocal influences between some leaf nutrients, including those between certain nutrients and eco-physiological leaf parameters. This assessment is in accordance with the finding obtained through the Pearson correlation heatmap that confirmed that almond leaf N content had a medium–high positive correlation with NBI either at 90 or 120 DAFB. This eco-physiological index, which can be easily investigated using proper field instruments, may be used for a preliminary screening of the plant’s nutritional status.

The findings of this study also help to develop novel environmentally friendly almond nutrition and fertigation strategies supported by proper cultivar-site-specific foliar diagnostics which also consider punctual nutritional requirements during seasonal growth, as reported in Supplemental Table S1.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae8090822/s1>, Supplemental Table S1: Proposed references of almond leaf macro- and micronutrients content; Supplemental Table S2: Pearson correlation matrix at 90 DAFB; Supplemental Table S3: Pearson correlation matrix at 120 DAFB.

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