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# Climatic implication in bioactive compounds and fatty acids profile of olive oil derived from new olive (*Olea europaea* L.) genotypes in Central Italy

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## Abstract

Given the current context of climate change, new olive genotypes may offer valuable variability for enhancing extra virgin olive oil (EVOO) quality in the Mediterranean region. This study evaluated five novel genotypes coming from breeding program (I77, N×N, Fs17×C, I79, N1×N3) cultivated in Central Italy. Olive oils were analyzed at two harvest times (mid-October, mid-November) over two consecutive seasons (2023 and 2024), focusing on chemical composition and its relationship to pedoclimatic conditions. Results showed that both harvest time and genotype significantly influence key oil parameters, including peroxide value, free acidity, carotenoid and chlorophyll content, α-tocopherol, and total phenolic content. Non-metric multidimensional scaling (NMDS) revealed distinct fatty acid profile for most genotypes, except I79 and Fs17×C, which showed similar profiles across years. As expected, oleacein, oleocanthal, and oleuropein predominated in I77 and Fs17×C oils. Among all genotypes, I77 consistently exhibited higher total phenolics, carotenoids, and α-tocopherols, as well as greater MUFA/PUFA and OLP indicated superior nutritional and oxidative stability potential. Correlation analyses highlighted that these key phenols appeared to be most closely associated with pedoclimatic variables, particularly temperature, solar radiation, and rainfall and that the response was genotype-dependent. Although the study spanned only two productive seasons, the consistent trends observed in phenolic response and their correlation with pedoclimatic variables suggest that genotype I77 holds strong potential for producing high-quality EVOO under variable climatic conditions. Moreover, the significant positive correlation between oleocanthal and oleacein with most pedoclimatic variables suggests environmental robustness and good nutraceutical potential of this genotype.

**Keywords:** EVOOs, Nutritional value, New olive genotypes, Climatic conditions, Fatty acids profile, Phenolic compounds



## 1 Introduction

Olive oil production holds significant economic, cultural, and nutritional importance in the Mediterranean region, particularly in Italy. With around 11 million hectares of orchards, the cultivated olive (*Olea europaea* subsp. *europaea* var. *europaea*) is predominantly grown in Mediterranean countries, which account for about 95% of the global olive cultivation area, with approximately 10% of this area located in Italy [1]. As one of the world's leading producers, Italy dedicates vast agricultural surfaces to olive cultivation, contributing substantially to the national economy and supporting rural communities by providing employment and sustaining local traditions [2]. The economic importance of this sector extends beyond primary production to include tourism, gastronomy, and exports, making Italian olive oil a hallmark of quality and authenticity in global markets. Italy possesses more than 500 olive tree varieties, accounting for approximately 42% of global diversity. This number is expected to rise as molecular analyses encompass ancient varieties that have been locally cultivated for centuries [3].

Beyond its economic role, olive oil is a fundamental element of the Mediterranean diet, a dietary pattern recognized for its numerous health benefits [4]. The inclusion of olive oil in the Mediterranean diet aligns with the United Nations Sustainable Development Goal (SDG) 3, which promotes good health and well-being by advocating sustainable and nutritious food choices. Extra virgin olive oil is renowned for its high content of monounsaturated fatty acids and for its bioactive compounds, including phenolic compounds and phytosterols [5]. The high content of unsaturated fatty acids contributes to cardiovascular health by lowering low-density lipoprotein (LDL) cholesterol levels while maintaining or increasing high-density lipoprotein (HDL) cholesterol. Phenolic compounds, including hydroxy-tyrosol and oleuropein, exhibit strong antioxidant and anti-inflammatory properties, which are crucial in reducing the risk of chronic diseases such as cardiovascular disorders, neurodegenerative diseases, and certain types of cancer [6]. Phytosterols, naturally occurring in olive oil, further enhance their nutritional value by inhibiting cholesterol absorption and contributing to overall metabolic health [5, 7, 8].

Among the chemical components of olive oil, the fatty acid profile is the one most influenced by the olive tree genotype, which in turn determines the nutritional value, flavour, and oxidative stability of the oil [9, 10]. Breeding programs involving minor cultivars can provide beneficial variability for improving olive oil quality, as well as agronomic characteristics that are functional for olive cultivation under the current scenario of climate change, which is increasingly threatening olive oil production [11, 12]. Previous olive breeding programs have aimed to improve agronomic performance, stress resistance, and oil quality through controlled crosses and open-pollinated progenies [13, 14], with early evaluations typically focused on juvenile growth and fruit traits [15]. Furthermore, significant genetic variability has been reported in progenies for both agronomic and oil quality traits, emphasizing genotype  $\times$  environment interactions [16]. Rising temperatures, shifting precipitation patterns, and prolonged drought periods pose serious challenges to olive groves, affecting yield and olive oil quality. Drought stress leads to reduced fruit size, lower oil content, and changes in the fatty acid profile, ultimately compromising the sensory and nutritional attributes of olive oil [17]. The unpredictability of extreme weather events, including heat waves and storms, further exacerbates the instability of olive production [17], underscoring the need for sustainable agricultural

practices that align with SDG 13, which calls for urgent action to combat climate change and its impact on food systems.

In response to these challenges, one promising strategy is the promotion of minor and indigenous olive varieties, which may hold key traits for resilience in a changing climate [12, 18]. These lesser-known varieties often exhibit enhanced drought tolerance, resistance to pests, and adaptability to harsh environments. By diversifying the genetic pool of cultivated olives, producers can reduce dependence on a limited number of high-yielding but climate-sensitive varieties, ensuring a more sustainable and stable olive oil supply. Moreover, oil derived from minor cultivars often possesses unique sensory profiles that appeal to niche markets, offering new economic opportunities for small-scale farmers [19–21]. Embracing genetic diversity in olive cultivation might not only strengthen the resilience of the sector but also contribute to the conservation of biodiversity, in alignment with SDG 2 (Zero Hunger) and SDG 15 (Life on Land), which emphasize sustainable food production and the protection of ecosystems. Therefore, it would be very useful to use breeding programs to take advantage of genotypes that combine high oil quality with agronomic characteristics that make them suitable for the new planting models adapted for the current climatic scenario.

In the present paper, five new genotypes cultivated in Central Italy were evaluated for the chemical composition of their olive oils, considering different maturation stages across two consecutive production seasons (2023 and 2024). The investigated materials derive from a long-term olive breeding program conducted by the National Research Council of Italy (CNR), based on controlled and open pollination among local cultivars and selected germplasm conserved in the CNR olive collection of Tuoro sul Trasimeno (Perugia, Italy). These genotypes were previously evaluated for canopy architectural traits related to their suitability for super-high-density orchard systems [9]. Unlike previous studies that often focus on well-established cultivars, this research addresses breeding-derived materials for which specific information on oil composition is currently limited. The main chemical characteristics of the olive oils responsible for their nutritional and compositional properties (i.e., fatty acids profile, phenolic compounds, and phytosterols, among others) were evaluated and correlated to climatic parameters. This integrative approach aims to elucidate the genotype-specific responses of olive oil quality to interannual pedoclimate variability, offering new insights for cultivar selection in the context of climate change.

## 2 Materials and methods

### 2.1 Study site, olive genotype description and oil extraction

The study site is managed by the Institute for Agricultural and Forest Systems in the Mediterranean (CNR-ISAFOM). It is located in the municipality of Tuoro sul Trasimeno in the Umbria region (central Italy, 43°12′50″ N, 12°03′20″ E) at about 287 m a.s.l., and with a North-West exposure on a 5% slope. The area covers approximately one hectare. The mean annual precipitation is around 870 mm, and the mean annual temperature is  $12.9 \pm 5.7$  °C.

The genotypes examined in this study were developed through a breeding program initiated approximately thirty-five years ago. This program led to the establishment in 2006 of an olive collection at the Tuoro sul Trasimeno plantation in Italy (<https://biome>

[mory.cnr.it](http://mory.cnr.it)), which includes hundreds of olive genotypes derived from both free and controlled pollination. Five of the most promising genotypes were repropagated through self-rooting and planted in 2020 to assess their bio-agronomic performances. The new grove was spaced 3 m × 2 m, with NE-SW row orientation. In the experimental field, plants were aligned along the rows and arranged into 4 groups of 12 plants and 1 group of 7 plants (Fs17 × C), each group consisting of plants of the same genotype (Table 1). The trees were trained to a central leader and were not pruned, except for removing basal shoots below 20 cm from the ground. Soil management was carried out with tillage performed manually to avoid damage to the roots, starting in April and then once a month until September, and leaving for herbaceous species to colonize spontaneously; these were mown twice a year, and the herbage was left in place. An integrated pest and disease management strategy was implemented in 2023 and 2024, combining chemical and biological control measures. Tribasic copper sulfate was applied in August for chemical control, while spinosad (Success<sup>®</sup>, Corteva Agriscience) was used for biological control from mid-August to the end of September. The soil under the orchard was developed from fluvial and lacustrine sediments, with a neutral pH ranging from 6.6 to 7 and clay loam texture. It is classified as a loamy, mixed, mesic, Typic Haplustept [22].

Drupes were collected from five plants of each genotype listed above, yielding a representative composite sample of approximately 10 kg. Sampling was conducted over two consecutive years (2023 and 2024) at two harvesting dates: October (17/10/2023 and 16/10/2024) and November (15/11/2023 and 15/11/2024). Fruits were processed within 24 h from harvesting, and the pigmentation index was assessed (Table S1 in the Supplementary materials). No visible symptoms of olive fruit fly damage were observed. Olive oil was extracted using an Abencor laboratory mill, which simulates the industrial process on a reduced scale and consists of three main units: a hammer mill, a thermo-beater, and a paste centrifuge. The olives were ground to a paste using the hammer mill, then the paste was placed in the thermo-beater and stirred for 30 min with the water bath set at  $28 \pm 1$  °C, without adding warm water. Subsequently, vertical centrifugation for 2 min separated the oily phase, which was then collected and left to decant for 24 h. Finally, the oil was separated, placed in dark glass vials and stored at  $-18$  °C until analysis.

## 2.2 Meteorological and soil measurements

Meteorological and soil measurements were conducted to characterize the climatic conditions of the study sites and to monitor the dynamics of soil water balance in the upper soil layers. These data supported the interpretation of olive tree physiological responses and helped to investigate the influence of microclimatic variability and soil moisture

**Table 1** Identification letter of the genotypes under investigation

Breed name	ID	No. of Plants
I-77 self-pollinated	I77	12
Nucalia 1 × Nostrale di Rigali	N × N	12
Fs-17 × Cipro	Fs17 × C	7
I-79 free-pollinated	I79	12
N 1 × N 3	N1 × N3	12

availability on tree development. Special attention was given to the potential impact of these environmental parameters on fruit development, with regard to the biochemical composition of olives harvested during the experimental campaign. At the study site, an agrometeorological monitoring system was installed, comprising a central station and a single auxiliary node. Data acquisition began on May 13, 2022. The central station (Davis Vantage Pro2), equipped with a remote data acquisition and transmission system, included the "GroWeather" Integrated Sensor Suite (ISS), which provided the following direct measurements: (i) air temperature and relative humidity (via a sensor with a passive radiation shield); (ii) precipitation (via a tipping-bucket rain gauge with 0.2 mm resolution, upgraded filter, and bird deterrent); (iii) wind speed and direction (via an anemometer); and (iv) global solar radiation. Based on these direct observations, the system also computed several derived variables, including: Reference evapotranspiration (ET), THSW index (Temperature-Humidity-Sun-Wind), an estimate of thermal comfort, Wind direction distribution (Wind Rose), total and current rainfall accumulation, UV index, Sunrise and sunset times and Lunar phase. An auxiliary agrometeorological node (Meteo System EnviroMonitor), wirelessly connected to the central station and installed within the olive grove in correspondence with a specific cultivar at a distance corresponding to the maximum canopy width. The node is equipped with the following sensors: (i) a leaf wetness sensor to detect the duration and presence of moisture on the leaf surface; (ii) two DAVIS 6440 soil moisture sensors, installed at depths of 20 cm and 40 cm, for monitoring volumetric water content in the soil; (iii) one DAVIS 6470 soil temperature probe, installed at 10 cm depth, for assessing thermal conditions in the root zone.

### 2.3 Olive oil chemical characterization

Quality indices such as free acidity (FA) and peroxide value (PV) were determined according to the International Olive Council (IOC) methods [23, 24]. Chlorophylls and carotenoids were quantified according to Minguéz-Mosquera et al. [25] by measuring absorbance at 670 nm and 470 nm, respectively, on diluted oil samples (cyclohexane 1.5:5 w/v) (Perkin Elmer Lambda 10 UV-Vis spectrophotometer, Perkin Elmer, Waltham, Massachusetts, United States). Total chlorophyll and carotenoid contents are expressed in mg of pigment per kg of oil using the provided formulas:

$$\text{Total chlorophylls} = (A_{670} \cdot 10^6) / (613 \cdot 100 \cdot d) \quad (1)$$

$$\text{Total carotenoids} = (A_{470} \cdot 10^6) / (2000 \cdot 100 \cdot d) \quad (2)$$

where A is the absorbance and d is the path length of the cell (1 cm).

$\alpha$ -Tocopherol was analyzed using a modified method based on Tura et al. [26]. A 0.15 g olive oil sample was dissolved in 5 mL of hexane and homogenized for 1 min by rotary shaking (Corning<sup>®</sup> LSE<sup>™</sup>, Merck Life Science (Pty) Ltd.). The solvent was evaporated under nitrogen, and the residue was re-dissolved in 1 mL of isopropanol. High-performance liquid chromatography (HPLC) analysis was performed on Agilent 1100 Series instrument (Agilent Technologies, Palo Alto, California, United States), equipped with an 1100 autosampler, column heater module, quaternary pump, and coupled

with a Diode Array Detector (DAD). The column was a Kinetex phenyl-hexyl column (100×4.6 mm column dimensions, 2.6 µm particle diameter, 110 Å pore diameter, Phenomenex, Torrance, California, United States) maintained at 25 °C and equipped with a pre-column of the same phase. A calibration curve was established using α-tocopherol standard solutions. The mobile phase consisted of water (Eluent A) and acetonitrile (Eluent B) with a gradient profile: 10:90 (A/B) for 6 min, 100:0 from minute 6 to 10, and 90:10 from minute 10 to 15. The flow rate was 1.8 mL/min, the injection volume was 5 µL, and the total run time was 15 min. Detection and quantification were carried out at 292 nm.

#### 2.4 Fatty acid composition

A 0.15 mL of olive oil sample was weighed and mixed with 2 mL of hexane. The transesterification was carried out by adding 0.2 mL of potassium hydroxide (2 N) solution in methanol, following the procedure outlined in the European Standard NF EN ISO 12966-2. Fatty acid methyl esters (FAMES) were then analyzed according to NF EN ISO 5508. The analysis was performed using a Varian CP3800 gas chromatograph (GC) (Walnut Creek, California, United States), equipped with a flame ionization detector (FID), and a ZB-WAX capillary column (60 m×0.25 mm i.d., 0.25 µm film thickness) made of polyethylene glycol (Phenomenex, Torrance, CA, USA). Helium was used as the carrier gas, with a column flow rate of 1.5 mL/min and a split 641 ratio of 1:100. The oven temperature program was as follows: the temperature was initially held at 140 °C for 2 min, then increased from 140 to 240 °C at a rate of 4 °C/min. The identification of FAMES was carried out by comparing their retention times with those of the Supelco 37 Component FAME Mix (Sigma-Aldrich). The FAMES were expressed as a percentage of the total fatty acid methyl esters identified.

#### 2.5 Phenolic compounds profile

The phenolic compounds in olive oil were extracted using a modified version of the method described by the International Olive Council (IOC) [27]. A sample of 2 g of olive oil was dissolved in 5 mL of an 80/20 methanol/water mixture, followed by 0.7 mL of an internal standard solution containing syringic acid. The samples were vortexed for 1 min and then centrifuged at 5000 rpm for 25 min at 4 °C. The resulting supernatant was injected into the HPLC–DAD system, described in the previous 2.2 paragraph. The separation of individual minor polar compounds was performed using a Kinetex phenyl-hexyl column (100×4.6 mm column dimensions, 2.6 µm particle diameter, 110 Å pore diameter, Phenomenex, Torrance, California, United States). The mobile phase A consisted of acidic water (H<sub>2</sub>O with 0.2% phosphoric acid), while mobile phase B was methanol. The initial solvent composition was 95% A-5% B, and it changed to 100% B after 37 min, with a flow rate of 1 mL/min. The phenolic compounds were identified and quantified at 280 nm. Phenolic compounds were quantified using an external standard calibration approach. Individual calibration curves were generated for each target compound. The following analytical standards were employed at the indicated concentrations of their respective stock solutions: hydroxytyrosol (8.3, 33, 66.7, 100, 158.3 mg/L), tyrosol (8.4, 33, 67.3, 101, 159 mg/L), oleacein (18, 36, 54, 85 mg/L), oleuropein (8.8, 35, 70, 105, 166 mg/L), oleocanthal (8.9, 36, 71, 107, 169 mg/L), pinoresinol (9.1, 36, 72, 109,

173 mg/L), and luteolin (8.3, 33, 67, 100, 158 mg/L). Identification and quantification were based on retention times and peak areas relative to the corresponding calibration curves.

## 2.6 Statistical analysis

Two-way analysis of variance (ANOVA) was applied to analyze the effects of genotype/cultivars and harvest and their interaction by year on the measured variables. The assumptions of normality and homoscedasticity of the data subjected to ANOVA were checked with the visual examination of the residuals against fitted values. The multiple comparison tests were performed with Tukey's honest significant differences with a significance level of 0.05. Non-metric multidimensional scaling (NMDS) was used as a non-parametric multivariate ordination method to explore similarities/dissimilarities among genotypes based on their fatty acid profiles. NMDS was performed using the "vegan" package in R, with dissimilarities computed using Gower's distance [24]. Before the NMDS analysis, the data were standardized by centering (subtracting the mean) and scaling (dividing by the standard deviation). To investigate the influence of climatic conditions on the phenolic composition of olive fruits, Pearson correlation analysis was conducted between selected meteorological variables and physicochemical oil quality parameters measured in the most promising olive genotypes: I77, Fs17×C, and N1×N3.

For correlation analysis, a 30-day rolling window was applied to the climatic variables to account for the cumulative effects of climatic conditions. For each sampling date, the corresponding environmental values were calculated as the average (or cumulative total, where appropriate, e.g., for rainfall) over the preceding 30 days (Table S2 of the Supplementary materials). This approach reduces the influence of short-term fluctuations and highlights potential medium-term climatic influences on physicochemical oil quality parameters.

The correlation analysis was performed separately for each genotype and was executed on three oil samples, two growing seasons and two harvesting times per season, resulting in a total of 12 independent observations. The statistical significance of each correlation coefficient was evaluated using a two-tailed t-test, with a threshold of  $p < 0.05$ . Only statistically significant correlations were considered relevant for interpretation. The statistical analyses were performed using R [28].

## 3 Results and discussion

### 3.1 Chemical olive oil quality parameters

The result of two-way ANOVA revealed that all the tested effects (genotype, harvest time, and their interaction) were always statistically significant (Table 2). Specifically, the peroxide values were always higher in 2023 than in 2024, and in November compared to October in 2023. However, this pattern did not hold for 2024, when higher values were often recorded in October. Moreover, in 2024 the differences between October and November were not consistent across varieties, unlike in 2023. The I79 genotype showed the highest peroxide values in 2023, followed by the N×N in the same year and harvest time. Peroxide value ranged between 2.7 and 12.6 with a mean value of 6.1 meq O<sub>2</sub> kg<sup>-1</sup> oil. Although still within EVOO limits, elevated PVs in specific genotypes and harvest times suggest increased susceptibility to oxidation, possibly due to genotype-specific

**Table 2** Chemical oil quality parameters derived from five new genotypes relative to two years (2023–2024) and two harvest periods (October and November)

Genotype	Harvest date	Peroxide value		Free acidity	
		2023	2024	2023	2024
		meq O <sub>2</sub> kg <sup>-1</sup> oil		% Oleic acid	
I79	October	7.4 d	7.1 a	0.49 be	0.30 ce
	November	12.6 a	4.4 b	0.58 bc	0.47 a
N×N	October	9.2 cd	3.5 c	0.65 b	0.37 ac
	November	11.0 ab	3.9 bc	0.84 a	0.19 ef
Fs17×C	October	6.7 de	2.9 de	0.54 bd	0.44 ab
	November	8.1 cd	3.4 cd	0.61 bc	0.21 df
I77	October	5.5 f	4.5 b	0.40 de	0.16 f
	November	6.9 de	2.7 e	0.47 ce	0.16 f
N1×N3	October	6.0 ef	2.9 de	0.35 e	0.28 cf
	November	9.3 bc	4.5 b	0.51 be	0.33 bd
Two-way ANOVA					
Genotype		<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
Harvest time		<0.001 ***	<0.025 *	<0.001 ***	<0.014 *
Genotype*harvest time		<0.001 ***	<0.001 ***	0.308	<0.001 ***

Values represent means ± the standard errors (n=3). Different letters indicate significant differences within each year

enzyme activity or harvest conditions or storage conditions. Overall, the chemical quality of all the analysed oils was within acceptable standards (Table 2), showing values conform to the quality parameters defined for the “extra virgin olive oil” category under Regulation EEC/2568/1991 [29]. Free acidity (expressed as oleic acid) was always lower than 0.8 g/100 g, except for N×N, where in November 2023, the value reached 0.84%. Although this increase was limited, it is worth considering in light of the role of free acidity as a key parameter for EVOO classification. Elevated values reflect hydrolytic degradation, poor fruit quality, or processing conditions. High free acidity compromises both the sensory quality and storage stability of olive oil. Overall, all tested oils show good quality in terms of freshness and proper handling of olives before milling. Despite the two-way ANOVA results, which delineated the influence of genotype, harvest time, and their combination, on chemical olive oil quality parameters, the differences observed could be also ascribable to other abiotic factors, such as temperature or humidity [30] or biotic fruit damage [31]. Indeed, as reported in the literature, differences in free acidity and peroxide values can be associated with the activity of exogenous and endogenous olive lipase and lipoxidase, which take place in intact fruit cells during maturation or in the olive paste during oil extraction [30].

With respect to the content of carotenoids, chlorophylls, and α-tocopherol all factors were statistically significant (Table 3). With few exceptions (N1×N3 and I77), carotenoids consistently showed higher content in the oils collected in October than those collected in November for both tested years. An exception was I77 in 2024, where values were slightly higher in November. The I77 genotype exhibited the highest carotenoid content, while the lowest was recorded in the N×N oil in November 2023. Genetically, carotenoid biosynthesis and accumulation are known to vary among cultivars and genotypes due to differences in the expression of key enzymes involved in the metabolic

**Table 3** Carotenoids, chlorophylls, and  $\alpha$ -tocopherol amount in oil derived from five new genotypes relative to two years (2023–2024) and two harvest periods (october and november)

Genotype	Harvest date	Carotenoids		Chlorophylls		$\alpha$ -Tocopherol	
		2023	2024	2023	2024	2023	2024
		mg kg <sup>-1</sup> oil		mg kg <sup>-1</sup> oil		mg kg <sup>-1</sup> oil	
I79	October	5.0 b	3.3 c	0.9 ef	3.7 d	405.2 a	442.1 b
	November	2.2 f	2.1 d	0.8 ef	3.9 d	334.3 b	340.9 e
N×N	October	4.5 c	3.9 b	0.6 f	4.2 cd	304.1 c	447.7 b
	November	1.5 i	2.2 d	1.0 df	3.5 d	283.3 cd	392.9 cd
Fs17×C	October	3.8 d	4.2 b	1.2 ce	4.2 bd	340.9 b	520.5 a
	November	1.8 h	3.9 b	1.6 bc	5.4 bc	344.7 b	518.6 a
I77	October	7.5 a	5.6 a	3.9 a	5.5 b	392.9 a	425.1 bc
	November	1.9 gh	5.7 a	1.0 df	10.4 a	383.5 a	415.6 bd
N1×N3	October	2.1 fg	5.2 a	1.9 b	4.4 bd	272.0 d	383.5 d
	November	2.58 e	3.3 c	1.5 bd	5.5 b	243.6 e	325.8 e

Values represent means  $\pm$  the standard errors (n=3). Different letters indicate significant differences within each year. Genotype, harvest date and their interaction were equally significant ( $p < 0.001$ )

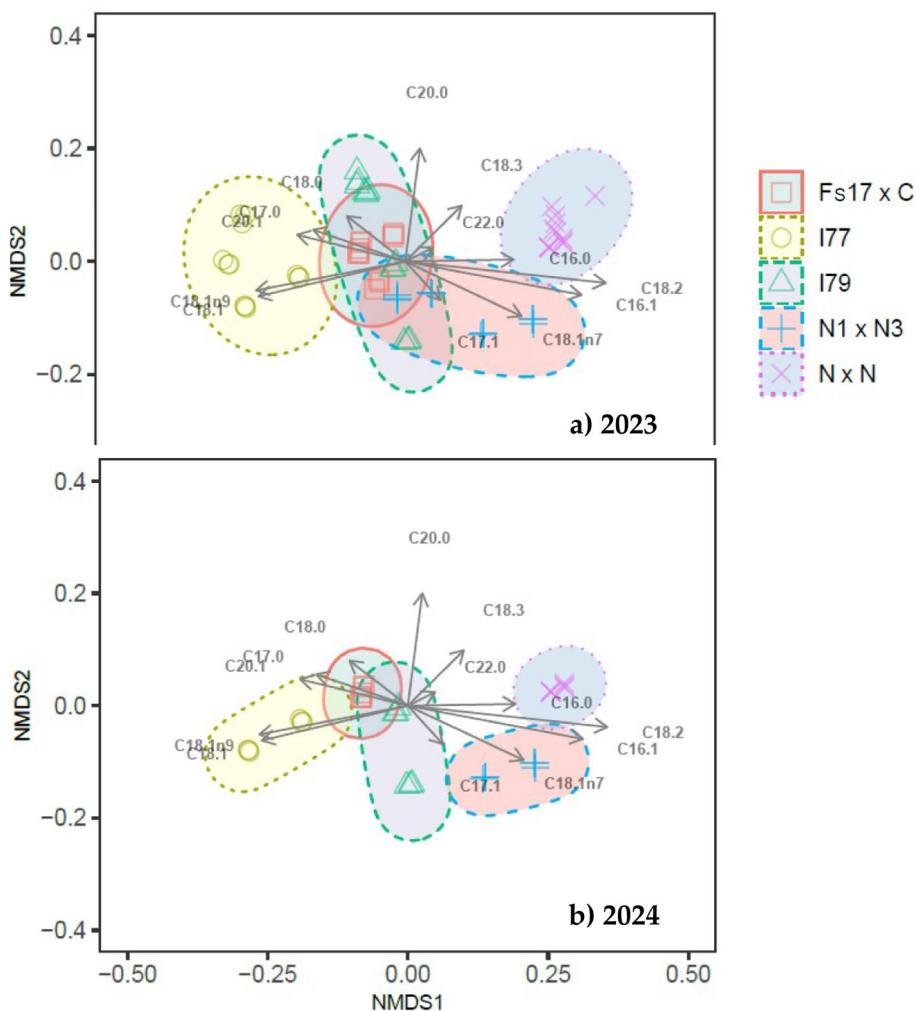
pathway (e.g., phytoene synthase, lycopene  $\beta$ -cyclase) [32]. These intrinsic differences may explain the baseline variability in carotenoid content across the genotypes analyzed. Regarding oil chlorophyll content, the highest value (10.4 mg kg<sup>-1</sup> oil) was recorded in 2024, particularly in the oil coming from the I77 genotype in November (Table 3). Finally, the I77 and I79 genotypes showed the highest values of  $\alpha$ -Tocopherol in October 2023, while Fs17×C in both months of 2024. According to our results and literature [33, 34], oils from early harvests and specific genotypes (e.g., I77) are likely to have better oxidative stability and nutritional profile due to higher antioxidant pigment content.

Despite variations across genotypes and years, all tested oils meet international standards for extra virgin olive oil. The I77 genotype consistently stands out for its high content of carotenoids, chlorophyll, and tocopherol, suggesting that it may offer both better nutritional quality and longer shelf-life. Meanwhile, I79 showed signs of oxidation in 2023, pointing to a need for optimized harvest timing or processing to maintain quality.

### 3.2 Fatty acid profile of oils from new genotypes

Olive oil is widely recognized as one of the best sources of dietary fatty acids due to its well-documented health benefits [35, 36]. These benefits are largely attributed to its unique fatty acid composition, which can vary among genotypes and harvest years. The NMDS (Non-metric Multidimensional Scaling) plots based on the fatty acid profiles of the oils (stress = 0.07 and 0.06 for 2023 and 2024, respectively) revealed clear distinctions among the genotypes analyzed, although no differences were observed between the two years (Fig. 1a, b).

The I77, I79, N×N and N1×N3 showed a complete separation of the ellipses, which means a different fatty acid profile of these genotypes. This dissimilarity occurred along the NMDS1 axis, and this separation appeared to be mainly driven by C18:2 and C16:1 along the positive semi-axis and C18:1 n9 and C18:1 along the negative semi-axis. As was expected, the predominant fatty acid is oleic acid, especially for the I77 genotype that presented the highest contents (74–77%) (Table S3 of Supplementary materials),



**Fig. 1** Two-dimensional non-metric multidimensional scaling (NMDS) plots of the fatty acids profile of five new genotypes relative to two productive years, 2023 (a) and 2024 (b). Circle lines in the NMDS plot are 95% confidence ellipses

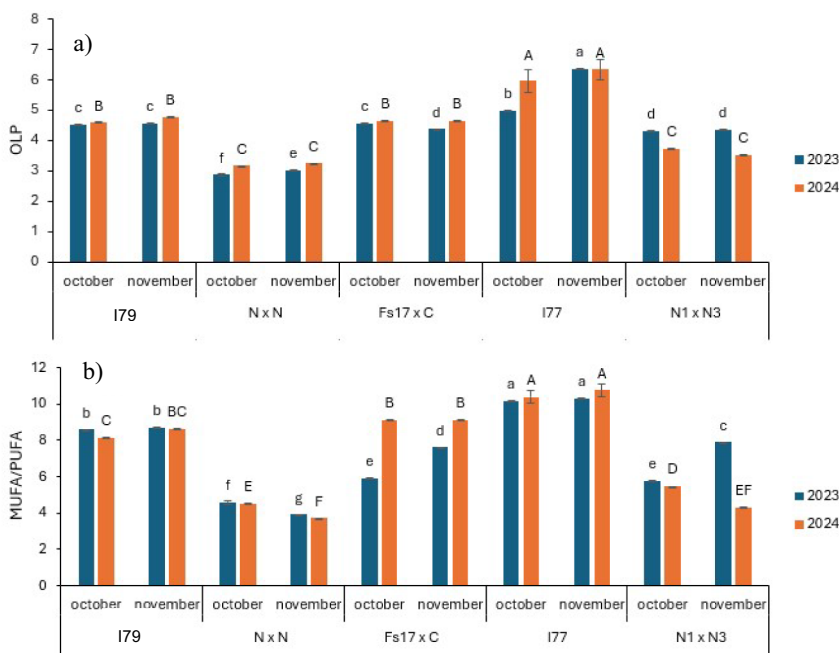
very similar to the ones reported by Farinelli and Tombesi [37] for the *Frantoio* and *Lec-cino* varieties registered in the Umbria region, with values of 76.4% and 75.6%, respectively. Conversely, N×N showed higher values of linoleic acid in both years with respect to the other genotype (Table S3 of Supplementary materials). The NMDS plots also showed a complete overlap of the Fs17 × Cipro and I79 ellipses, which means a similar fatty acid profile of the oil derived from these two genotypes. Accordingly, a significant effect of genotype on fatty acid composition has also been observed in previous studies conducted in different olive breeding programs in Tunisia [38–40], Italy [41, 42], and Spain [43, 44], where most fatty acids, except for C18:3, were shown to be strongly genotype-dependent.

In fact, fatty acid composition can depend both on genetic factors and on environmental conditions during the development and maturity of the fruit. Numerous studies have shown that factors such as cultivar, geographical origin, fruit ripeness, harvest time, and pedo-climatic conditions can significantly modify the lipidic profile

of olive oil [45–47]. Our findings are consistent with previous research, particularly that of Gagour et al. [36], who also reported significant genotypic variability in fatty acid composition, with oleic and linoleic acids being the primary discriminating components. Concerning the (oleic acid/linoleic acid + palmitic acid) OLP ratio, which is an important indicator of oil quality and nutritional value [48], the highest values were recorded in 2024 with respect to 2023 for both harvest times and all genotypes, except for N1 × N3 genotype, which showed higher OLP ratios in 2023 (Fig. 2a).

In 2024, the I77 genotype exhibited the highest OLP ratio at both harvest dates, followed by Fs17 × C and I79, with the same values. Additionally, N × N and N1 × N3 had similar values. Conversely, in 2023, there were differences between harvest time in all genotypes except for I79 and N1 × N3 (Fig. 2a). Our results showed that the I77 genotype harvested in November was the only one that reached an OLP ratio value equal to 6, which is generally considered acceptable in an EVOO, but it lies at the lower end of the desirable range, especially for high-quality cultivars. A higher OLP ratio is desirable, as it indicates not only improved oxidative stability but also a more health-promoting fatty acid profile and potentially greater resistance of the genotype to environmental stresses that affect oil composition.

In addition to OLP, the MUFA/PUFA ratio serves as a good descriptor of the fatty acid profile of olive oil and its potential health benefits. Indeed, this ratio, together with the phenol’s concentration, can be a good indicator of the oil’s oxidative stability. Olive oils with a higher MUFA content are more stable, with better resistance to oxidation, which is important for maintaining the oil’s quality over time [49]. Our results showed that the highest values were found in the I77 genotype and the lowest



**Fig. 2** Variation of OLP index (a), and monounsaturated (MUFA), polyunsaturated (PUFA) fatty acids ratio (MUFA/PUFA); (b) in oil derived from five new genotypes relative to two productive years (2023–2024) and two harvest periods (October and November). Different lowercase letters indicate significant differences in 2023, whereas different uppercase letters indicate significant differences in 2024 at the  $p$ -level < 0.05

in N×N in both tested years (Fig. 2). The highest MUFA/PUFA ratio (10.8) reached by I77 genotype in November 2024 was comparable to that of *Leccino* EVOO from the Umbria region with an approximate value of 9.9 [37].

### 3.3 Phenol composition of oils from new genotypes

The phenol composition of olive oil provides useful information on its quality because phenols significantly affect the organoleptic evaluation and nutritional value of the final product [36]. Table 4 showed that in October, the total phenols concentration was higher than in November, with the exception of Fs17×C and N1×N3 in 2023, where the higher values were registered in November. The oil extracted in October from the I77 and Fs17×C genotypes recorded the highest total phenol content in 2023 and 2024 (853.7 and 934.7 mg kg<sup>-1</sup>, respectively), whereas the lowest levels were consistently observed in N×N in November across both years (139.2 and 122.5 mg kg<sup>-1</sup>, respectively).

The high total polyphenol content in I77 was mainly attributed to the oleacein content, although some significant variations between harvest periods occurred (Table 4).

Oleacein is considered one of the most powerful antioxidants found in olive oil, particularly in EVOOs. It is a polyphenolic compound, specifically a hydroxytyrosol derivative, and is known for its strong antioxidant and anti-inflammatory properties [50]. From an organoleptic perspective, olive oils with higher levels of oleacein tend to have a more bitter and pungent taste, which is considered a desirable characteristic in high-quality olive oil [51]. Conversely, in the Fs17×C genotype, the high phenolic concentration resulted from an approximately equal contribution of oleocanthal, oleacein, and oleuropein (Table 4). These bioactive compounds are known for their beneficial effects in managing various diseases [52, 53]. Our results were in accordance with those reported by a study of Rózańska et al. [54] analyzing EVOO samples from the *Leccino*, *Frantoio*, and *Moraiolo* cultivars from the Umbria region and found comparable levels of oleacein (ranging from 137.0 to 254.0 mg kg<sup>-1</sup>). As reported by Kafkaletou et al. [55], the significant differences in phenolic content among genotypes, aside from the harvesting period, could also be influenced by chemical and enzymatic changes during olive ripening. These differences may also be associated with factors such as fruit position within the canopy and the quality of irradiance. In particular, the upper layers of the canopy, which receive higher irradiance, show an increased accumulation of phenolic compounds [56, 57].

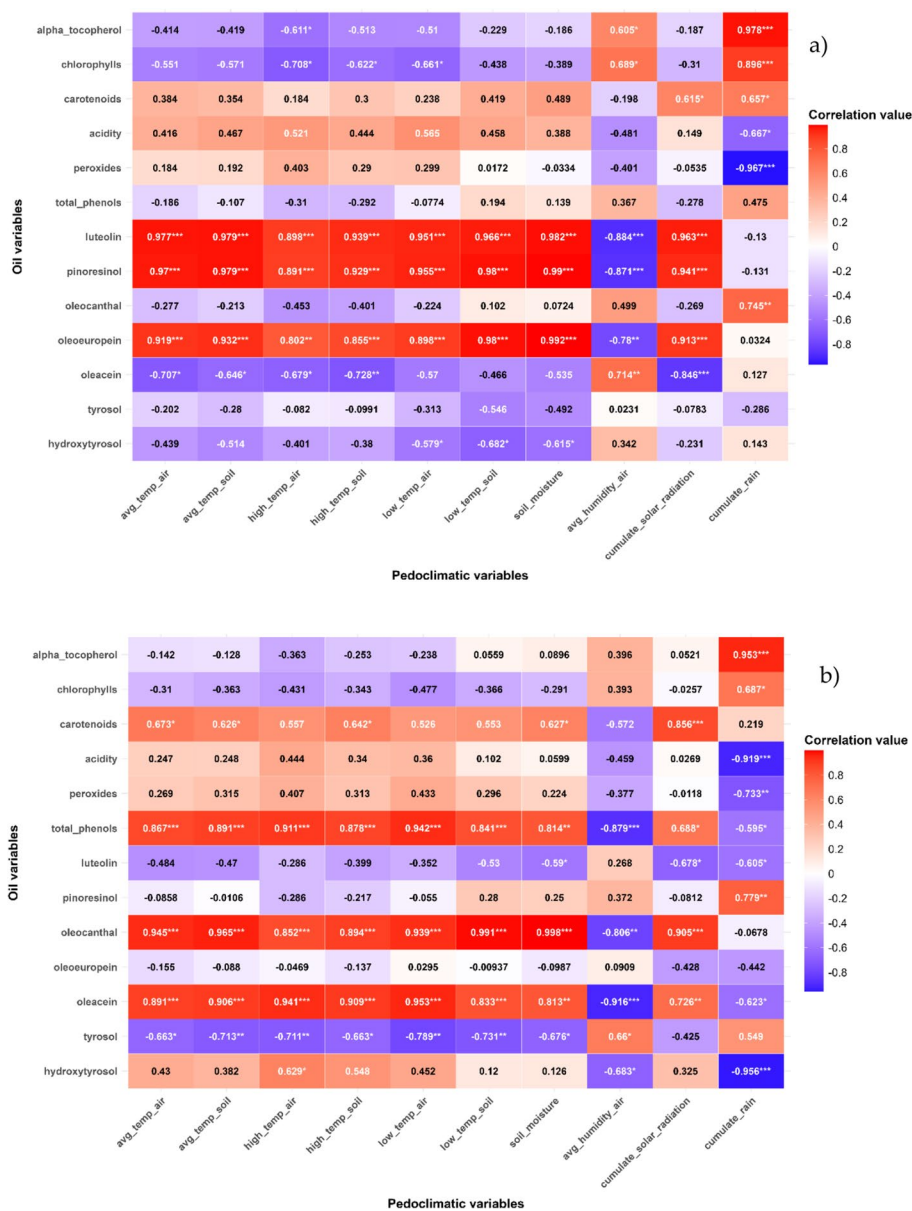
### 3.4 Relationship between oil composition and pedoclimatic variables

Among the various nutritional attributes of olive oil, the content of total and specific polyphenols has emerged as the parameter most strongly influenced by the timing of harvest and the pedoclimatic conditions of the olive orchard's production area [58, 59]. In the present study, we aimed to highlight the potential relationship between several olive oil parameters, including phenolic compound content, and the average pedoclimatic conditions recorded during the month preceding the harvest period when fruits maturation occurred, over two years of experimentation. However, the observed correlations should be interpreted with caution, as they are based on a limited number of observations and are intended to provide exploratory insights. Based on the total

**Table 4** Phenolic concentrations in oil derived from five new genotypes relative to two years (2023–2024) and two harvest periods (October and November)

Genotype	Harvest date	HTYR		TYR		OLEAC		OLEU		OLEOC		PIN		LUT		TOTAL PHENOL	
		2023	2024	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
		mg kg <sup>-1</sup>															
I79	Oct	3.8 g	0.2 l	8.3 df	6.0 F	166.0 e	87.7 D	85.1 f	54.9 F	131.8 c	149. B	7.0 d	9.0 EF	11.5 f	13.8 E	413.6 g	321.3 E
	Nov	2.5 h	21.2 B	7.7 ef	26.6 B	92.9 f	15.7 G	94.6 ef	56.4 F	56.9 e	39.2 E	6.5 de	6.0 GH	16.6 c	17.1 D	277.7 i	182.3 H
NXN	Oct	16.7 a	4.3 G	8.2 ef	9.3 E	57.8 g	37.4 F	186.9 b	164.8 C	NA	44.6 DE	17.2 b	10.0 E	13.8 e	13.1 E	300.6 h	283.6 F
	Nov	2.8 h	0.3 l	8.6 de	9.7 DF	NA	5.9 H	102.2 e	55.9 F	NA	25.2 F	4.7 e	5.1 H	20.9 b	20.5 B	139.2 j	122.5 l
Fs17XC	Oct	14.7 c	6.4 E	14.8 a	6.8 F	60.4 g	330.3 A	232.7 a	207.3 B	68.3 d	339.8 A	42.6 a	33.0 B	13.4 e	11.1 F	447.0 f	934.7 A
	Nov	11.3 e	32.1 A	12.2 b	18.4 C	461.3 b	234.4 B	41.2 g	36.7 G	177.5 a	152.7 B	13.5 c	10.6 DE	7.2 h	7.0 G	724.1 b	491.94 D
I77	Oct	15.3 b	3.3 H	9.6 cd	11.2 D	594.9 a	327.0 A	90.3 ef	105.3 D	132.3 c	115.2 C	6.0 e	12.3 D	5.4 i	4.5 l	853.7 a	578.8 B
	Nov	12.0 d	7.6 D	10.4 c	17.2 C	342.6 c	56.4 E	147.9 c	69.9 E	57.5 e	46.9 D	7.2 d	7.3 FG	10.4 g	6.0 H	587.9 d	211.2 G
N1XN3	Oct	2.0 i	5.4 F	7.2 f	17.1 C	175.1 d	110.9 C	121.7 d	243.5 A	166.6 b	117.6 C	7.0 d	48.8 A	15.0 d	18.4 C	494.6 e	561.8 C
	Nov	4.3 f	17.9 C	5.2 g	34.7 A	344.4 c	6.2 H	46.7 g	112.4 D	172.3 ab	49.3 D	16.7 b	18.2 C	36.5 a	40.4 A	626.2 c	279.2 F

Values represent means ± the standard errors (n=3). Different lowercase letters indicate significant differences in 2023, whereas different uppercase letters indicate significant differences in 2024 at the p-level < 0.05  
 HTYR: hydroxytyrosol; TYR: tyrosol; OLEAC: oleacein; OLEU: oleuropein; OLEOC: oleocanthal; PIN: pinoresinol; LUT: luteolin. NA = not available



**Fig. 3** Pearson's correlation heatmap for genotype **a** Fs17xC; **b** I77; **c** N1 x N3

phenolic concentration results, we focused on three genotypes that showed the highest values (Fs17xC, I77, and N1 x N3). The correlation matrix relative to Fs17xC genotype indicated that cumulative rain was highly positively correlated with chlorophyll and alpha-tocopherol content and negatively correlated with peroxide values (Fig. 3a).

Moreover, among the phenolic compounds, luteolin, pinoresinol, and oleuropein were highly positively correlated with all pedoclimatic variables except cumulative rain and negatively correlated with average air humidity. This last result could be because low air humidity causes stomatal closure to reduce water loss, affecting water status and reducing CO<sub>2</sub> assimilation. The resulting physiological stress triggers the plant to produce more phenolic compounds, which act as antioxidants and protectants, helping the olive tree adapt to challenging pedoclimatic conditions. Conversely,

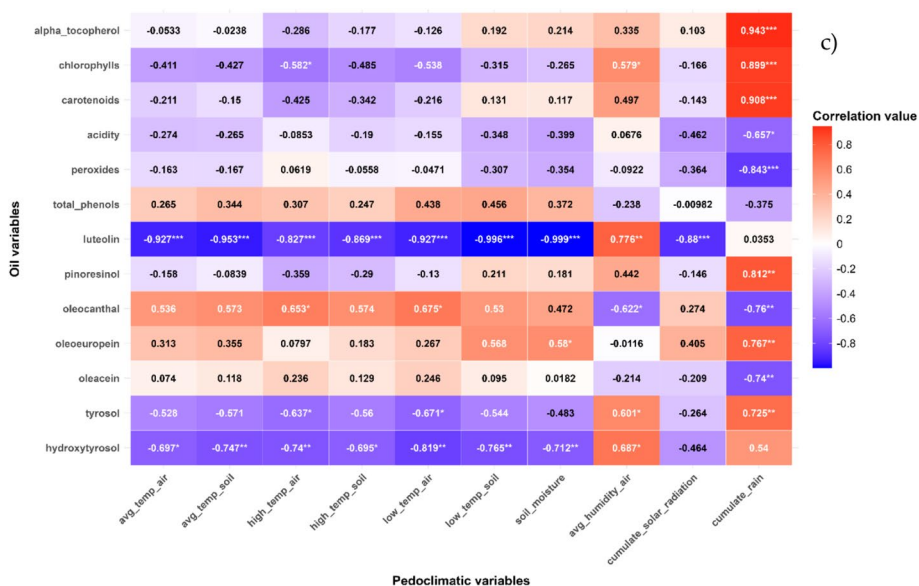


Fig. 3 continued

the I77 genotype showed a significantly positive correlation of  $\alpha$ -tocopherol with cumulative rain and of carotenoids with solar radiation (Fig. 3b). Moreover, total phenols, oleocanthal, and oleacein concentrations were positively correlated with all pedoclimatic variables and negatively correlated with the average air humidity. Finally, acidity and hydroxytyrosol concentration were negatively correlated with cumulative rain (Fig. 3b).

N1  $\times$  N3 genotype, on the other hand, showed a strong positive correlation of alpha-tocopherol, chlorophyll, and carotenoids with cumulative rain and, differently from the Fs17  $\times$  C genotype, a significantly negative correlation of luteolin concentration with all the pedoclimatic variables except the average air humidity (Fig. 3c). These results confirm that the relationship between the concentrations of certain olive oil parameters, including phenolic compounds, and the pedoclimatic conditions depends on the phenological phase of the olive tree and on genotype. Indeed, the products of secondary metabolism like phenols are synthesized at the earliest stages of ripening principally as a physiological defense against drought and high temperatures [60].

These results highlight the complex interaction between genotype-specific responses and environmental stimuli in shaping the nutraceutical properties of olive oil. The marked differences among genotypes, particularly in how specific compounds such as luteolin, oleocanthal, and tocopherols respond to variations in rainfall, temperature, solar radiation, and humidity, underline the importance of tailored agronomic practices and harvest timing strategies. Notably, the inverse correlations observed between phenolic content and average air humidity may suggest a stress-induced synthesis mechanism, aligning with the hypothesis that phenolic accumulation functions as a protective response to abiotic stress [60, 61].

Based on these observations, we hypothesize that lower humidity and higher thermal and solar radiation conditions may act as abiotic stressors that stimulate the

biosynthesis of polyphenolic compounds [62, 63], particularly in drought-adapted genotypes. Conversely, increased rainfall and moderate temperatures may promote the accumulation of chlorophylls and tocopherols [64], contributing to oxidative stability but potentially reducing polyphenol content. This is in accordance with our results that showed lower values of peroxide value and free acidity in 2024, which was characterized by higher average cumulative rainfall (Table S2 of the Supplementary materials). This hypothesis would suggest that olive oil quality, in terms of both antioxidant content and oxidative resistance, is dynamically regulated by the balance of stress signaling pathways activated by climatic conditions.

The contrasting patterns in Fs17×C and N1×N3 further support the role of genotype as a key modulator in this dynamic, justifying genotype-specific approaches for maximizing both yield and quality.

#### 4 Conclusions

A specific experimental setting was designed and tested to investigate how micro-climatic and pedological conditions influence the bioactive compounds and fatty acid profile of oil from different new olive genotypes potentially suitable for adaptation to changing climate scenarios. Overall, the results demonstrated clear differences among the genotypes studied in terms of chemical and nutritional quality. These differences highlight the importance of tailoring harvest strategies to the specific phenological and compositional traits of each genotype.

Among the studied genotypes, I77 consistently showed higher levels of key phenolic compounds, a higher MUFA/PUFA ratio and greater  $\alpha$ -tocopherol content, as well as more stable relationships with pedoclimatic factors. Although the dataset covers only two seasons, the repeated trends provide a solid basis for identifying I77 as a promising candidate for producing high-quality EVOO.

These findings underline the relevance of genotype-environment interactions and support the need for continued evaluation of new genotypes under changing climatic conditions, with particular attention to traits linked to oil quality and environmental resilience.

#### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44274-026-00709-0>.

Supplementary file 1.

#### Author contributions

Conceptualization, M.B.; Formal analysis, L.M., A.M., A.D., E.O.; data curation, L.M., A.M., E.O., T.B.; writing—original draft preparation, L.M., M.C., M.B., F.F., M.R.; writing—review and editing, L.M., M.C., M.B., L.C., F.F., M.R.; supervision, M.B.; project administration, M.B.; funding acquisition, M.B. All authors have read and agreed to the published version of the manuscript.

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#### Data availability

The data used to support the findings of this study are included within the article. Any other data can be available upon request from the corresponding author or first author.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

Mirko Cucina declares they are an Editorial Board Member of Discover Environment and confirms that they were not involved in the handling or decision-making of their own submission.

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