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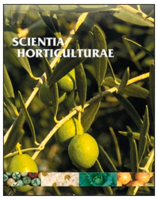

Under-vine Zulla cover crop: Effect on glycosidic aroma precursors of *Vitis vinifera* L. cv Syrah musts

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ABSTRACT

The employment of cover cropping has been developed in vineyards to mitigate soil erosion and the massive use of herbicides, but the effects on crop yields and berry quality have been little explored. Glycosidic aroma precursors constitute the aroma potential of grapes and have remarkable relevance in neutral grapes, such as Syrah. These compounds tend to be affected by environmental conditions and stressors; therefore, the influence of cover crops on aroma precursors has been studied for the first time. For this purpose, bound aroma compounds from Syrah cv. must obtained from conventional and organic vineyards, subjected to soil tillage and to the influence of different amounts of Zulla cover crop, were extracted and analysed. This experiment was carried out over three consecutive harvests (2019, 2020 and 2021). 40 aglycones were detected, mainly C_{13}-norisoprenoids and terpenes. The last harvest (2021) accounted for the highest amount of precursors, owing to the exceptional amount of isodurene, naphthalene and 2-methylnaphtalene. The year Zulla was planted (2019), treatments with lower Zulla "pressure" increased aroma precursors. In the second vintage (2020), the general impact of Zulla was negative. Decreasing the amount of aglycones with the largest amount of cover crop increased the presence of aglycones in the must. In the last harvest (2021), the two highest levels of Zulla resulted in an increase of the aromatic precursors determined in the must. These results show that the effects of the presence of Zulla are strongly vintage-dependent and probably on the development of the roots. Furthermore, the last vintage results indicate that the employment of Zulla cover crops could be favourable for varieties such as Syrah, a neutral aroma grape, where bound forms are largely responsible for the potential aroma in the resulting wines.

1. Introduction

Climate change is challenging economic systems worldwide, forcing substantial investment in order to adapt to this worrying situation, however, no sector of the economy is more reliant on climate than agriculture, which is implementing several changes in order to maintain yields, production and food quality to ensure it meets consumer demand (Carter et al., 2018). Consumers, being aware of this, increasingly demand products that are produced using ecological or eco-sustainable methods. This is the case in the winemaking industry, which has made considerable efforts to adapt wine production to ecological requirements while maintaining product quality. In this context, from 2005-2019, the certified organic vineyard surface area increased by an average of 13% per year, while the 'non-organic' vineyard area decreased by an average of 0.4% per year within the same timeframe (OIV, 2021) reflecting consumer interest. According to the OIV, organic viticulture protects consumer health and the environment and is based on three principles: soil fertility, maintaining biodiversity and pest control, in accordance with ecological cycles and processes. Thus, herbicides cannot be used to prevent the growth of vegetation between rows of vines and, therefore, tillage is presented as the most frequently used alternative to eliminate it. However, this practice has some drawbacks, such as increased soil erosion and alteration of soil microbiota, among others (Abad et al., 2020). To avoid these negative effects, the employment of cover cropping has been developed in vineyards to mitigate soil erosion, water demands, the massive use of herbicides and to reduce the need for fertilisers (Celette et al., 2009; Le Bissonnais et al., 2004; Delpuech et al., 2018; Otto et al., 2020). Moreover, the soil quality is improved (Abad

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et al., 2023). Hence, the ecological effects of cover crop are clear, but the effects on crop yields and berry quality are still opaque. Attending to plant development, cover crop competes with the grapevine for soil resources, decreasing any excessive vegetative vigour of the grapevine (Delas, 1996). Greater grapevine water stress may induce a significant decrease of grape growth and yield (Smart et al., 2006) and, therefore, of quality. These yields decreased and a slight increase in the berry quality was observed by Pou et al., (2011). Gatullo et al. (2020) observed that after the study of three consecutive vintages, only in the driest one was the yield reduced by the presence of the cover crop. Compatible with this are other studies reporting slight differences in the yield obtained using cover crops compared to the control (Ingels et al., 2005, Ferrara et al., 2021). Thus, the study of Pérez-Álvarez et al. (2015) with the grape cv. Tempranillo showed that, in the midterm (3 years), a gramineous cover crop reduced the nitrogen grapevine status and both YAN and free amino acids in the grapes, being potentially useful when a reduction in vigour and an improvement in the must quality are required. Also, this competition leads to a reduction in leaf area and may be useful in diminishing the vine water consumption at later stages (veraison and ripening), which can be of interest to ensure grape yield during dry years or to reduce irrigation necessities (Pou et al., 2011) and can also delay veraison (Parker et al., 2014). However, it has many other implications, such as a possible reduction in grape phenolic compounds (Kliewer and Dokoozlian, 2005) or an increase in herbaceous aromas in grapes and wines. Moreover, it has also been reported in table grapes that cover crops could be useful in improving soil nitrogen availability, and, consequently, contribute to a more efficient nitrogen uptake and utilisation overall (Ferrrara et al., 2021). Hence, it has been observed that the use of cover crops reduces must acidity and increases berry sugar accumulation, tartaric acid/malic acid ratio and berry skin total phenols and anthocyanin content, enhancing wine quality (Spayd et al. 2002; Monteiro and Lopes, 2007) whilst other studies reflect only few differences in juice Brix, pH, or titratable acidity (Ingels et al., 2005). In reference to the study of secondary metabolites responsible for wine quality, such as phenolic and volatile compounds, only a slight influence of cover crops on colour has been reported (Gatullo et al., 2020) or no improvement in the anthocyanins, total polyphenols and tannins content (Pou et al., 2011) has been observed. Volatile compounds are also secondary metabolites that have a major influence on wine aroma. Volatile composition of the grape is comprised of free volatile compounds, but also by non-volatile precursors, of which there are several types, glycosidic being the most abundant (Bayonove, 2003). Few authors have explored the influence of cover crops on the free aroma of grapes. Xi et al. (2011) analysed the effects of permanent green cover crops on Cabernet Sauvignon vines from China and reported that wines produced from grapes with cover crops had higher contents of volatile compounds. Conversely to these results, Coletta et al. (2021) worked with the Negroamaro grape cv. from Italy and observed that the employment of cover crops was counterproductive in terms of the amount of volatile compounds found in the grape, with tillage being the best option. The study of Yuan et al. (2015), who also described a diminution effect on aroma precursors employing grass cover crop with Pinot noir grapes, agreed with these negative results. In Spain, to date, only two studies about this subject have been published, one evaluated wines from Mencía grape cv. with permanent cover crops, concluding that this practice slightly influenced the concentration of volatiles (Bouzas-Cid et al., 2018) and, very recently, the other, carried out by our research group, analysed the volatile composition of must subjected to different levels (inter-row series) of Zulla (Hedysarum coronarium L.) a wild legume from the Mediterranean basin (Tava et al., 2021), which grows spontaneously in the area of the experiment. Here we found that the effect of the harvest on the musts volatile composition of the Syrah grape variety is more important than agronomic practices during the three years studied (Valero et al., 2022). There is no existing information about bound volatile fraction. These compounds are predominantly located on the grape skin and are comprised of an odorous molecule (volatile compound) named aglycone associated with a disaccharide. The β-glycosidic bond linking them is split during the winemaking process, by the action of yeast during the fermentation, or by acid hydrolysis during the aging process, releasing the volatile compound into the wine matrix (Bayonove, 2003). This odourless fraction in the berries has special interest because mature berries have more bound forms than free forms of these compounds (Sanchez Paloma et al., 2007, Fenoll et al., 2009). However, as far as we know, there are no studies of the analysis of precursors, despite the fact that glycosidic aroma precursors are one of the compounds that are most influenced by viticultural practices and are also highly influenced by water, sunlight, and other types of vine stressors (Hernandez-Orte et al., 2015; Hjelmeland and Ebeler, 2015; Alem et al., 2019). The importance of these aroma precursors lies in the fact that they constitute the aroma potential of grapes, and have remarkable relevance to neutral grape varieties, such as Syrah, which have no aroma themselves, and the glycosidic fraction of the wine aroma is proportionally more important than the free fraction, revealing its aromatic potential during and after the fermentation, in part owing to these molecules (López-Tamames et al., 1997; Bureau et al., 2000; Segurel et al., 2009) being indicated as essential contributors to high quality Shiraz wine (Abbott et al., 1991). Considering all these previous data we hypothesise that the employment of Zulla cover crop would produce certain stress to the vine, increasing aroma precursors in the grape must for vinification. To validate or refute it, firstly, Syrah cv. musts from conventional and organic vineyards (subjected to soil tillage) were compared in terms of their aroma precursors composition. Secondly, the influence of different amounts of Zulla in organic vineyards was studied. This study represents the first, analysing the effects of cover crops on the glycosidic precursors profile in grapes.

2. Materials and methods

2.1. Materials and chemicals

Dichloromethane, ethanol, and methanol were supplied by Merck (Darmstadt, Germany) and ethyl acetate by Fluka (Buchs, Switzerland). Sodium dihydrogen phosphate 1-hydrate and citric acid were purchased from Panreac (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). 4-Methyl-2-pentanol, used as internal standard (IS) was purchased from Merck (Darmstadt, Germany) and sodium chloride from Panreac (Barcelona, Spain). An alkane solution (C10–C40) was used to calculate the linear retention index (LRI) (Fluka; Madrid, Spain)). Chromabond® HR-P cartridges (MACHEREY-NAGEL, Düren, Germany), with 200 mg of resin, were purchased from Merck VWR. 50/30 μm DVD/CAR/PDMS (1 cm) SPME fibre was supplied by Supelco (Bellefonte, USA).

2.2. Samples

The study was conducted over a period including three consecutive vintages, 2019, 2020, and 2021. The samples of this study were musts from *Vitis vinifera* L. cv Syrah manually harvested grapes from two vineyards (conventional and organic), located in the IFAPA Centre “Rancho de La Merced”, in the Jerez winegrowing region, Spain (36:45:29N, 06:00:58W, 35 m altitude) and are the same as those employed by Valero et al. (2022) for free volatile profile determination. The vineyards were planted in 2014 and the vines grafted on 140-Ru rootstocks with a planting distance of 2.5 × 1.5 m, a North-South row orientation, and the trained system was single cordon double Guyot. The soil called “Albariza”, characteristic of this zone is a Leptosol (IUSS Working Group WRB, 2022) with a clayey texture (69% clay, 20% silt and 9% sand), with pH of 8.0 and organic matter content of 1.16%. These two vineyards were separated by a distance of 2 km and are included in region V according to the climatic classification of viticultural regions by Amerine & Whinkler (1944). No irrigation was administered in the vineyards. Climatological data was taken from the vineyard weather station (Table S1). As a control, a vineyard with bare soil by tillage was employed (SC). The conventional vineyard was treated with agricultural chemicals used as herbicide, insecticide, nematicide and fungicide. The organic vineyard (SE) was treated only with micronised sulphur and copper oxychloride as fungicide. This same organic vineyard was used for the experiments with Zulla (Hedysarum coronarium L.) cover crops which were planted (20 Kg/Ha) in lanes as described in Fig. 1 in October 2018, and were maintained until March 2019.

At this time, it was cleared and a week later the lanes were tilled to avoid the cover ground competing with the vineyard. In the second and third years (2019-20 and 2020-21), the cover crop was allowed to grow spontaneously, with no new planting of Zulla, and the same process of clearing and soil tillage was carried out. Bunches were always collected from the same plant at a similar ripening stage (approx. 24 °Brix). Thus, every year, 15 grape samples were collected, 12 grape samples in the organic vineyard (SE), for every cover crop density (3 samples for every different density) and 3 grape samples in the conventional vineyard (SC). The different cover crop levels studied were cover crop on one side and tillage on the other side of the row (LZ), cover crop on both sides of the row (ZZ), cover crop on two rows on both sides of the sampled row (4Z) and tillage on both sides of the vine row as a control (LL). Approximately 2 kg of grapes, stems included, were harvested in aseptic conditions from each sampling point and placed directly into sterile bags, which were transported to the laboratory in portable refrigerators with plastic ice blocks. At the laboratory, grapes were squeezed by hand in the plastic bags, opened and about 50 mL of juice was poured into a glass vial and immediately frozen at -20°C for further analyses (Valero et al., 2022).

2.3. Extraction of glycosidically bound aroma compounds by SPE

The night before the extraction, must samples were thawed in the refrigerator at 5°C and were centrifuged at 4000 rpm for 10 minutes. For the extraction and analysis of precursors, the method employed by Ubeda et al. (2017) was followed with some modifications. Thus, 10 mL of the supernatant were passed through 3 mL Chromabond® HR-P cartridges (MACHEREY-NAGEL, Düren, Alemania) containing 200 mg of resin previously conditioned (5 mL dichloromethane + 5 mL methanol + 5mL of milliQ water) employing a Visipred SPE vacuum manifold (Supelco, Bellefonte, PA, USA). Once the glycosidic aroma precursors were adsorbed into the resin, 10 mL of Milli-Q water were passed through the cartridge to eliminate interferences and vacuum dried. Subsequently, 10 mL of dichloromethane were employed to eliminate free volatile compounds and finally the precursors were eluted with 10 mL of a solution with ethyl acetate:methanol 9:1 (v/v) collected in a conical flask, and subsequently removed with a rotary evaporator (IKA, Staufen, Alemania). To recover the precursors, 10 mL of citrate buffer were used (0.2 M, pH 2.5) and were transferred to a 20 mL screw cap vial. The oxygen contained in the headspace (HS) of the vial was removed with a nitrogen stream prior to acid hydrolysis which was carried out by incubating for 1 hour at 100 C° in a stove (Selecta, Barcelona, Spain) releasing the aglycones to the matrix. After this stage, vials were tempered to subsequently proceed to the analysis.

2.4. Analysis of volatiles released from precursors by HS-SPME-GC-MS

For the analysis of aglycones, 7.5 mL of hydrolysate sample were placed in a 20 mL SPME vial and 2.25 g of sodium chloride were subsequently added to the sample joined to 10 µL of 4-methyl-2-pentanol (1045 mg/L) as internal standard. The vials were kept at 20 °C in a thermostatised autosampler tray until analysis. For this purpose, an Agilent 8890 gas chromatograph coupled to an Agilent 5977B simple quadrupole mass spectrometer (Agilent, Santa Clara, CA, US) equipped with a multipurpose autosampler MPS Robotic Pro LS (Gerstel, Müllheim an der Ruhr, Alemania) was used. Samples were incubated at 45°C for 10 minutes at 300 rpm and the fibre was subsequently exposed to the vial headspace for 40 minutes (Ubeda et al., 2017). Desorption of the compounds adsorbed to the fibre was performed for 3 minutes in the injection port at 250 °C, on splitless mode at 50 mL/min. A J&W CPWax-57CB 50 m x 0.25 mm and 0.20 µm film thickness (Agilent, Santa Clara, CA, US) was used. Helium was employed as carrier gas at 1 mL/min. The oven temperature programme was as follows: started at 35°C for 2 minutes, increasing by 5°C/min to 50°C, followed by a temperature ramp at 2°C/min to 200°C, maintained for 1 minute. Finally, the temperature was raised by 5°C/min to 220°C, for 2 min. Detection of volatile compounds was performed on full scan mode at 70 eV and a mass registration from 29 to 300 m/z. The MS quadrupole, source, and transfer line temperatures were maintained at 150 °C, 230 °C and 280 °C, respectively.

2.5. Identification and quantitation of volatile compounds

Compound identification was performed using the NIST Mass Spectral Search program for the NIST/EPA/NIH EI and NIST Tandem Mass Spectral Library (v.2.3, 2017) (Gaithersburg, MD, US). LRI values were calculated by injecting an n-alkanes mixture (C10–C40) under identical conditions as the samples. Subsequently, identification was performed at three levels. Identification was carried out in accordance with the matching of the linear retention indices (LRIs), and the LRIs from the literature obtained with authentic standards and their mass spectra, with those from the compounds NIST library (A). The second level was for the compounds that match with the NIST library and the LRIs from the literature (B). The third level was for the compounds that match with the NIST library, however their LRI does not coincide with the data found in the literature (C). Data were expressed as relative peak area values with respect to the internal standard (4-methyl-2-pentanol).

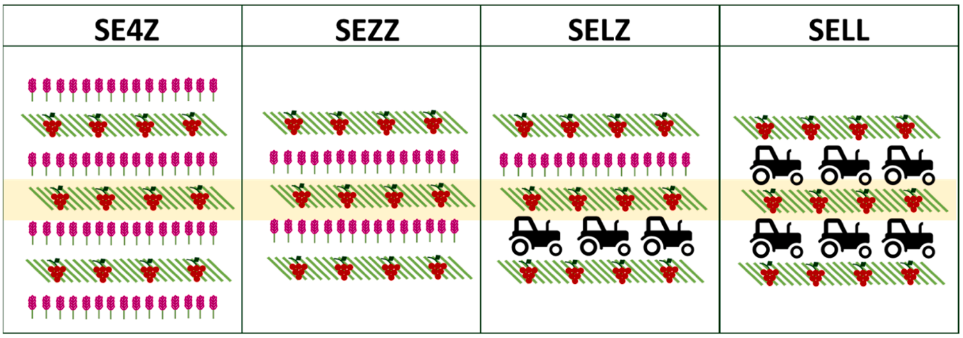

Fig. 1. Diagram of the treatments carried out in the organic vineyard. (SELZ) cover crop on one side and tillage on the other side of the row; (SEZZ) cover crop on both sides of the row; (SE4Z) cover crop on two rows on both sides of the sampled row; (SELL) tillage on both sides of the vine row as a control.

2.6. Statistical analyses

The values of the relative peak area of the diverse volatile compounds found in each agronomic treatment were subjected to analysis of variance (ANOVA) (Tukey's test) ($p>0.05$)) using INFOSTAT software (FCA, Universidad Nacional de Córdoba, Argentina). The Principal Component Analyses (PCA) were performed using SIMCA® Multivariate Data Analysis software 14.1.0.2047 (Umetrics Umea, Sweden). Pareto scaling was the method selected to normalise the data of the technique before the PCA analysis. Main effects ANOVA was also performed using Statsoft Statistical, version 7.0 (Statsoft, Tulsa, OK).

3. Results and discussion

A total of 40 aglycones were detected, 13 norisoprenoids, 11 terpenes, 7 aldehydes, 4 alcohols, 2 ketones and 3 non-identified compounds. This distribution was expected, given that C_{13}-norisoprenoids and terpenes are the glycosidically-bound precursors most usually present in the grape and in higher amounts, and are mainly accumulated in the berry skin during the ripening process. The number of compounds found in each harvest was slightly different, hence, in 2019 and 2020 there were 36 precursors, and 32 in the 2021 harvest (Table 1). It is important to consider the variability between the 2019 and 2020 harvests and that of 2021.

As can be observed at the end of Table 1, the 2021 vintage accounted for a much higher amount of total volatile precursors, contrary to the results obtained in the free volatile fraction of the same samples (Valero et al., 2022). This is mainly because in 2021 an outstanding amount of isodurene, naphthalene and 2-methylnaphtalene was observed.

The results are organised firstly by comparing the effect of the organic and conventional crop, with both subjected to soil tillage, and, secondly, by analysing the results of the glycosidic precursors composition of the musts from the organic vineyard grapes grown with different proportions of Zulla cover crop.

Regarding the compounds observed, the 2019 and 2020 harvests were similar; however, the 2021 harvest was the most different, principally owing to the presence of the above-mentioned amounts of isodurene and naphthalenes. On the contrary, in 2021 a smaller number of terpenes was found, with rose oxide, neo-allo-ocimene, terpinen-4-ol, β-cyclocitral, myrcenol, and α-terpineol present only in the 2019 and 2020 harvests.

Moreover, some of the compounds found in Syrah must have been detected in grapes for the first time as far as we know. This is the case of the 2,5,8-Trimethyl-1,2-dihydronaphthalene, 2-methyl-1-decanol and undecanol.

3.1. Comparison of conventional and organic crop

The volatile precursors composition of musts of the Syrah grape variety obtained from conventional and organic culture (SC and SELL) during three vintages was compared. As can be observed in Fig. 2, the total amount of terpenes present in the must from the conventional cultivation vineyard was higher than in the must from the organic crop, but only significant in the first harvest.

These significant differences were due to the high concentration of isodurene, cyclocitral, myrcenol, menthol, ocimenol, humulene and L-α-terpineol. These last two compounds also presented significantly higher contents in the must from the conventional crop in the 2020 harvest (Table 1). Aldehydes presented the same tendency, with more total contents in the musts from conventional cultivation vines, but only significant in the first harvest, as well as the ketones group, being, in this case, significant in the 2019 and 2020 harvests. For the alcohols, no significant differences were found between the two vineyard systems. Only in the case of norisoprenoids did the trend differ from the rest. The first year the must from the conventional vineyard accounted for a significantly higher amount of these compounds than from the organic vineyard, in the second year a significantly higher quantity of norisoprenoids was found in the organic must, mainly due to TDN and naphtalene, and finally, in the last studied year, the presence of these isoprenoids in the must from both vineyards was quite similar, with no significant differences between them. Very little research has been carried out on the comparison of wine composition of organic vs conventional crops. Moyano et al., (2009) observed that sherry wines from Pedro Ximenez grapes from organic crops showed, in general, a lower concentration of the determined volatile compounds. With respect to the analysis of free volatile compounds in musts, specifically of these same samples, Valero et al. (2022) observed that, in general, in the 2019 and 2020 harvests the total volatile compounds of every group were higher in the organic crop compared to the conventional crop. This is the inverse result to that of the aroma precursors, and a possible explanation could be a higher level of glycosylation of volatile compounds in the conventional crop vs organic, in response to chemicals applied to the vine. In the last year, 2021, this tendency was not so clear, and no significant differences were found in the total volatile compounds of every group sum between conventional and organic crops. This divergent behaviour among vintages was reflected in the results of multivariate statistical analysis (Fig. 2). Thus, in the PCA, the first two principal components were able to explain a 96.1% of the total variance of the data with PC1 separating the samples from the 2019 and 2020 harvests from the samples of the 2021 harvest. However, no differences concerning the vineyard system was observed, although there seems to be more distance between the musts from different harvests in the case of the conventional crop (Fig. 3).

Moreover, according to the main effect ANOVA, the influence of harvest was more important than the crop type for a total of 28 compounds (Tables S2A and S2B). These results are in accordance with Valero et al. (2022) who concluded that an important influence of the harvest year on agronomic practices did exist. This strong dependence of the glycosides on the vintage, especially terpenes and C_{13}-norisoprenoids, has been previously observed by other authors (Koundouras et al., 2009).

3.2. Study of Zulla cover crop effect in organic culture

As described above, the control employed did not have Zulla and used tillage to avoid the growth of other plants near the vine. The Zulla treatments are described in Fig. 1 and include 3 treatments with different quantities (pressure/influence) of Zulla. The presence of Zulla produced significant differences in several volatile compounds from the musts obtained from the grapes grown in the organic vineyards (Table 1). As can be observed in Fig. 2, the tendencies in the influence of every treatment assayed in the different chemical groups described a very similar pattern within each vintage. Therefore, the Zulla "pressure" affected most of the compounds every year in a very similar way (except ketones). However, the effect of each treatment was not the same with respect to the control treatment. At the end of Table 1, the global effects of every Zulla treatment can be observed. Thus, in the first harvest (2019), a light Zulla pressure (LZ) significantly increased the total precursors present in the must. The next level of Zulla (ZZ) applied also increased precursors production, but not significantly, however, the higher level of Zulla (4Z) implied an important decrease in the volatile precursors determined versus tillage, but without significance. It is important to consider that, as previously mentioned, during this first harvest, the Zulla was planted and maintained until March 2019. At that point, the cover crop was cleared and a week later the lanes were tilled to avoid the cover ground competing with the vineyard. Therefore, the effect of the Zulla was perhaps still low. In the second and third years (2020 and 2021), the cover crop was allowed to grow spontaneously, with no new planting of Zulla. In the second year, 2020, the effect was different with respect to the preceding year. In this case, the presence of Zulla was significantly counterproductive for the aromatic precursors in all conditions. The musts from the last harvest presented significantly

Table 1

Glycosidic aroma precursors in Syrah grape must from the 2019, 2020 and 2021 harvests grown under different agronomic conditions.

				SC			SELL			SELZ			SEZZ			SE4Z		
Compounds	LRI	ID	HY	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A
Terpenes (T)																		
cis-Rose oxide (T1)	1349	A	2019	8.53	1.43	ab	10.09	2.17	b	8.72	0.84	b	11.72	0.90	b	3.30	0.80	a
			2020	17.59	5.57	a	10.42	3.09	a	15.17	0.63	a	12.36	2.01	a	8.25	1.39	a
			2021	nd	nd		nd	nd		0.05	0.01		nd	nd		nd	nd	
Neo allo-ocimene (T2)	1358	B	2019	21.37	2.89	d	15.57	0.54	cd	13.06	1.98	bc	9.00	0.42	ab	5.12	0.15	a
			2020	10.07	3.73	ab	18.40	2.47	b	6.79	1.44	a	4.65	0.86	a	10.29	2.70	ab
			2021	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
Nerol oxide (T3)	1465	B	2019	22.89	5.70	b	22.48	1.63	b	13.57	4.29	ab	6.98	0.49	a	4.91	0.24	a
			2020	20.64	3.14	b	25.85	0.61	b	8.83	0.95	a	5.77	0.69	a	7.47	2.07	a
			2021	36.27	3.69	c	8.83	3.81	a	9.77	0.57	a	12.59	0.06	ab	20.32	1.25	b
Isodurene (T4)	1473	B	2019	357.36	20.76	b	199.41	34.65	a	431.42	4.11	b	596.36	13.64	c	217.80	14.47	a
			2020	205.50	31.06	a	238.64	62.99	a	170.63	34.95	a	124.34	1.53	a	129.92	3.68	a
			2021	8228.17	1648.42	ab	5689.62	998.67	a	4245.98	283.61	a	10025.21	1313.00	b	7241.83	135.84	ab
Terpinen-4-ol (T5)	1605	A	2019	0.94	0.10	a	3.69	0.03	d	3.03	0.30	c	2.18	0.04	b	1.03	0.13	a
			2020	4.92	0.22	c	3.19	0.00	b	2.05	0.33	a	1.53	0.09	a	1.72	0.49	a
			2021	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
β-Cyclocitral (T6)	1616	B	2019	13.22	0.24	c	8.16	1.16	ab	5.52	1.40	a	9.77	0.21	bc	6.13	1.35	ab
			2020	9.86	1.84	bc	11.86	1.28	c	5.97	1.07	ab	4.02	0.71	a	6.67	0.19	ab
			2021	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
Myrcenol (T7)	1619	B	2019	54.06	3.65	d	24.20	0.08	b	35.03	3.29	c	20.49	0.77	ab	10.99	2.04	a
			2020	45.25	16.94	a	37.75	1.42	a	19.82	4.34	a	10.70	3.05	a	26.03	7.38	a
			2021	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
Menthol (T8)	1646	A	2019	12.53	0.71	b	7.10	1.10	a	8.55	0.90	a	12.00	0.23	b	6.66	0.80	a
			2020	9.55	1.29	b	6.58	0.75	ab	7.00	1.18	ab	3.96	0.17	a	5.30	0.84	a
			2021	92.83	10.15	ab	94.93	1.98	ab	85.50	11.24	a	142.31	3.70	c	121.82	11.10	bc
α-Humulene (T9)	1663	A	2019	60.04	5.38	d	28.93	0.48	bc	39.41	3.39	c	22.86	1.14	ab	12.87	0.54	a
			2020	104.43	4.07	c	41.83	0.28	b	23.49	6.06	ab	12.39	3.77	a	30.32	8.75	ab
			2021	13.05	0.97	b	18.17	3.02	b	3.80	2.00	a	2.74	1.12	a	15.76	1.49	b
Ocimenol (T10)	1687	B	2019	51.37	0.97	d	22.43	0.37	b	33.60	1.61	c	18.51	1.84	b	9.72	1.95	a
			2020	76.96	21.43	b	37.54	3.81	ab	20.90	3.92	a	10.40	2.71	a	27.06	8.75	a
			2021	24.35	3.21	b	8.15	1.14	a	21.29	1.76	b	21.87	4.34	b	25.85	4.50	b
L-α-Terpineol (T11)	1708	A	2019	69.74	1.30	b	42.90	0.87	a	42.05	5.95	a	46.87	1.77	a	39.29	0.14	a
			2020	74.02	16.23	b	29.72	3.36	a	21.24	3.66	a	14.21	0.13	a	15.70	5.57	a
			2021	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
Norisoprenoids (N)																		
Vitispirane A (N1)	1517	A	2019	988.72	42.72	b	395.91	33.76	a	959.12	289.45	b	559.58	35.04	ab	309.79	47.44	a
			2020	423.60	108.40	a	507.08	91.62	ab	250.65	6.05	a	256.78	60.59	a	747.72	7.55	b
			2021	413.64	16.59	b	529.12	52.52	b	250.45	23.85	a	519.72	2.47	b	460.99	39.55	b
Vitispirane B (N2)	1520	A	2019	677.39	15.98	b	302.97	22.79	a	706.52	196.26	b	388.24	27.65	ab	215.04	27.80	a
			2020	313.95	79.98	a	361.97	66.69	ab	186.54	11.70	a	176.39	41.66	a	523.81	5.66	b
			2021	390.48	34.45	b	482.37	63.90	b	217.53	16.65	a	435.28	18.17	b	371.49	39.37	ab
α-Ionene (N3)	1545	B	2019	220.63	8.62	c	125.67	17.59	ab	170.18	31.90	bc	152.86	11.17	abc	83.26	10.71	a
			2020	36.41	7.14	a	210.03	17.01	c	96.52	22.92	b	30.68	8.93	a	62.16	6.84	ab
			2021	116.84	6.37	ab	159.19	18.60	b	62.64	6.58	a	77.76	24.19	a	67.24	5.93	a
Naphthalene (N4)	1725	A	2019	361.18	46.17	a	270.49	36.19	a	415.11	94.17	a	518.46	30.74	a	307.72	97.06	a
			2020	83.51	1.22	b	433.03	17.66	c	32.63	1.04	a	30.09	2.37	a	53.43	1.52	ab
			2021	24410.25	1594.00	a	19528.04	6479.96	a	19907.57	1017.05	a	39893.14	976.47	b	30488.71	272.71	ab
TDN (N5)	1731	A	2019	3316.72	220.06	b	2775.62	325.86	ab	3046.05	119.40	b	2910.12	71.00	ab	1760.38	506.52	a
			2020	1764.15	49.16	ab	3737.89	535.49	c	1568.75	205.90	ab	1161.96	111.21	a	2755.05	491.10	bc
			2021	385.99	4.44	a	748.97	61.99	ab	400.21	67.83	a	792.83	77.79	b	758.55	176.21	ab
β-Damascenone (N6)	1826	A	2019	225.39	6.86	c	64.29	0.84	a	104.04	13.78	b	85.68	0.42	ab	56.45	8.40	a
			2020	179.03	41.06	b	178.33	9.84	b	127.66	39.22	ab	57.16	18.59	a	89.12	0.94	ab

(*continued on next page*)

Table 1 (*continued*)

				SC			SELL			SELZ			SEZZ			SE4Z		
Compounds	LRI	ID	HY	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A
			2021	168.36	2.51	ab	217.29	21.13	b	108.43	12.43	a	120.61	23.76	a	135.76	3.64	a
2-Methylnaphthalene (N7)	1845	A	2019	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
			2020	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
			2021	1835.06	23.41	a	2393.40	427.39	ab	2403.32	147.21	ab	4509.13	661.68	c	3735.09	638.65	bc
1,1,6,8-Tetramethyl-1,2-dihydro-naphthalene (N8)	1896	C	2019	38.52	0.54	b	19.64	1.88	a	32.92	0.31	b	30.88	3.00	b	18.20	4.25	a
			2020	33.24	6.57	bc	45.96	0.70	c	26.44	5.75	ab	14.32	2.36	a	19.27	0.02	ab
			2021	10.27	0.35	a	18.17	3.02	b	12.26	0.66	ab	14.68	1.23	ab	16.01	0.27	ab
β-Ionone (N9)	1947	A	2019	2.82	0.01	c	1.33	0.02	a	2.31	0.05	b	2.30	0.02	b	1.56	0.15	a
			2020	2.28	0.55	bc	3.58	0.09	c	1.87	0.35	ab	0.78	0.04	a	1.35	0.33	ab
			2021	58.99	1.52	a	56.78	14.91	a	52.04	1.71	a	65.42	4.81	a	73.16	6.77	a
2,5,8-Trimethyl-1,2-dihydronaphthalene (N10)	1985	C	2019	263.24	11.45	d	103.16	4.73	a	183.38	32.46	bc	234.72	4.17	cd	170.14	5.13	b
			2020	25.44	5.16	a	177.57	15.30	c	86.95	16.92	c	51.65	11.88	ab	60.45	14.64	ab
			2021	70.29	5.50	a	104.62	10.45	ab	77.89	7.80	ab	88.88	20.82	ab	121.81	4.12	b
Dehydro-β-ionone (N11)	2009	C	2019	30.75	1.51	c	8.13	0.17	a	19.15	0.97	b	33.11	1.30	c	11.08	1.15	a
			2020	28.13	9.77	b	16.56	2.03	ab	4.06	0.69	a	2.45	0.07	a	3.25	0.28	a
			2021	21.02	2.41	c	0.06	0.01	a	0.06	0.02	a	0.14	0.00	a	6.96	0.88	b
β-Methylionone (N12)	2143	C	2019	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
			2020	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
			2021	567.94	22.07	a	995.40	450.02	a	427.17	11.15	a	986.72	94.29	a	700.52	22.26	a
1,6-Dimethyl-4-(1-methylethyl) naphthalene (N13)	2211	B	2019	187.41	12.88	b	34.66	1.43	a	35.62	0.63	a	33.64	3.09	a	20.64	7.63	a
			2020	36.80	7.39	ab	45.72	5.36	b	32.32	2.06	ab	16.06	3.42	a	32.38	10.64	ab
			2021	33.18	0.13	a	62.78	28.33	a	23.17	4.64	a	67.51	15.58	a	44.64	2.25	a
Aldehydes (A)																		
Nonanal (A1)	1400	A	2019	18.30	0.44	b	10.78	1.64	a	17.48	0.75	b	11.86	2.87	ab	7.95	1.45	a
			2020	33.38	9.09	b	23.64	0.33	ab	26.33	1.98	ab	11.66	0.47	ab	16.58	3.97	ab
			2021	158.94	48.07	a	125.83	34.10	a	87.75	33.02	a	166.67	68.64	a	166.56	7.33	a
Decanal (A2)	1492	A	2019	19.08	0.53	d	7.39	1.44	b	10.64	0.15	c	4.67	0.32	ab	4.00	0.59	a
			2020	13.26	5.09	a	8.02	1.32	a	4.46	0.24	a	3.88	0.82	a	10.59	0.20	a
			2021	186.69	14.05	ab	180.86	4.79	ab	131.42	17.17	a	215.85	0.74	ab	289.20	56.69	b
Benzaldehyde (A3)	1507	A	2019	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
			2020	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
			2021	1074.44	327.04	a	332.77	104.70	a	506.62	86.91	a	894.01	136.46	a	674.25	248.27	a
(E)-2-Nonenal (A4)	1531	A	2019	23.05	0.15	b	9.87	0.58	a	9.48	0.01	a	10.03	1.27	a	7.35	1.56	a
			2020	9.33	0.85	ab	21.89	2.54	bc	13.44	3.63	abc	8.66	0.92	a	23.97	5.74	c
			2021	51.80	1.33	a	96.51	34.72	a	47.31	4.71	a	74.89	20.24	a	84.02	4.66	a
5-Methylfurfural (A5)	1567	A	2019	66.39	8.65	b	27.55	0.73	a	29.60	1.57	a	22.26	1.48	a	14.28	0.93	a
			2020	51.61	16.67	b	51.50	6.62	b	26.68	2.93	ab	17.41	0.14	a	30.12	2.87	ab
			2021	19.99	0.92	ab	15.44	3.55	a	13.71	0.16	a	21.23	1.68	ab	26.08	1.47	b
2,4-Dimethyl-benzaldehyde (A6)	1806	C	2019	192.06	4.16	ab	132.03	0.62	a	205.01	19.90	ab	216.47	5.70	ab	156.83	38.46	a
			2020	425.57	30.71	b	304.59	52.84	ab	306.10	81.32	ab	209.13	46.29	a	178.43	34.98	a
			2021	663.26	100.13	a	671.81	448.98	a	631.16	26.10	a	1096.67	120.46	a	551.64	32.65	a
2,4,5-trimethyl benzaldehyde (A7)	1878	B	2019	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
			2020	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
			2021	732.38	52.17	b	539.35	47.17	a	723.84	14.95	ab	924.79	36.46	c	969.78	64.85	c
Alcohols (AL)																		
2-Ethyl-1-hexanol (AL1)	1498	A	2019	121.01	14.00	b	81.02	1.35	a	74.88	6.15	a	74.91	2.09	a	60.78	4.23	a
			2020	220.83	33.07	c	80.16	15.30	ab	130.87	17.73	b	48.26	0.63	a	120.47	6.96	ab
			2021	2128.75	706.11	a	2447.21	636.25	a	2746.22	645.83	a	3069.18	147.36	a	4296.99	775.12	a
2-Methyl-1-decanol (AL2)	1808	B	2019	2.17	0.66	a	7.83	1.05	bc	2.89	0.37	a	10.72	1.13	c	5.79	1.06	ab
			2020	9.46	2.06	b	21.10	1.58	c	4.12	0.01	a	16.36	0.73	c	17.81	1.04	c
			2021	27.19	7.69	a	314.64	178.40	a	14.33	1.55	a	264.20	10.89	a	42.56	4.86	a
Undecanol (AL3)	1883	B	2019	4.37	0.04	a	34.94	4.38	b	12.12	1.43	a	60.27	2.11	c	32.91	1.39	b
			2020	18.27	0.29	a	65.36	5.66	c	12.49	0.79	a	40.84	0.37	b	66.82	10.91	c
			2021	130.24	7.29	a	128.85	49.49	a	139.23	2.54	a	309.86	15.15	b	202.09	40.94	ab

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Table 1 (*continued*)

				SC			SELL			SELZ			SEZZ			SE4Z		
Compounds	LRI	ID	HY	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A	Mean	±SD	A
Dodecanol (AL4)	1980	A	2019	20.42	2.08	a	19.32	2.90	a	20.44	4.38	a	41.67	1.33	b	20.90	2.56	a
			2020	41.11	3.91	b	40.27	2.05	b	33.85	7.93	ab	17.46	1.06	a	23.60	1.46	a
			2021	543.22	18.99	a	580.30	256.95	a	492.37	27.80	a	913.67	91.46	a	937.20	153.19	a
Ketones (K)																		
2,6-Dimethyl-4-heptanone (K1)	1164	B	2019	51.75	1.42	c	23.51	4.53	ab	18.38	2.58	ab	31.61	6.06	b	11.32	2.63	a
			2020	61.35	4.25	c	10.50	2.28	ab	14.02	3.45	ab	29.55	1.26	b	15.64	0.90	a
			2021	18.64	0.49	a	15.09	4.06	a	26.27	1.02	ab	35.05	6.42	b	25.37	0.69	ab
2-Undecanone (K2)	1594	A	2019	58.33	4.20	c	16.62	3.23	ab	16.71	2.55	ab	22.12	1.06	b	8.82	0.91	a
			2020	5.17	0.90	a	6.76	1.06	a	5.82	1.55	a	2.72	0.22	a	3.50	1.00	a
			2021	128.57	17.08	ab	114.09	12.36	ab	89.00	7.77	a	195.20	0.57	b	180.15	42.33	b
Unknown (U)																		
n.i. (m/z 159, 174) (U1)	1408		2019	156.89	1.25	c	77.41	20.10	ab	104.02	4.77	b	78.34	7.19	ab	40.99	6.09	a
			2020	71.86	17.19	ab	110.79	24.01	b	47.65	7.84	a	19.75	3.23	a	38.84	4.09	a
			2021	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
n.i. (m/z 159, 174) (U2)	1640		2019	75.90	7.58	a	56.98	8.12	a	72.47	1.21	a	56.07	0.62	a	61.77	11.34	a
			2020	141.01	9.75	c	78.70	11.73	b	48.35	15.21	ab	20.60	4.95	a	31.28	3.86	a
			2021	nd	nd		nd	nd		nd	nd		nd	nd		nd	nd	
n.i. (m/z 159, 174) (U3)	1697		2019	108.49	1.74	c	59.62	5.12	b	57.98	2.30	b	72.82	4.77	b	39.15	5.11	a
			2020	66.25	11.65	b	101.57	7.15	c	37.35	9.79	ab	19.78	5.53	a	27.16	4.23	a
			2021	44.50	4.27	ab	76.85	18.47	b	34.95	1.18	a	42.87	5.64	ab	44.02	4.86	ab
TOTAL PRECURSORS SUM			2019	7449.60	376.06	c	4791.65	211.29	ab	6613.80	210.68	c	6132.89	148.39	bc	3558.89	765.00	a
			2020	4424.52	158.94	bc	6704.83	407.63	d	3235.32	434.59	ab	2397.69	109.48	a	5089.25	432.56	c
			2021	41936.36	2774.20	ab	36056.93	7367.74	a	33206.81	2181.18	a	64998.15	812.73	c	51859.41	344.29	bc

LRI: Experimental linear retention index values estimated by linear regression. ID: identification of the compound; A. mass spectrum and LRI agreed with standards; B. mass spectrum agreed with mass spectral database and LRI agreed with the literature data; C. mass spectrum agreed with mass spectral database. HY: Harvest year. Sample codes of grape musts: SC: conventional vineyard. Organic vineyard: SELL: tillage on both sides of the vines row; SELZ: cover crop on one side and tillage on the other side of the vines row; SEZZ: cover crop on both sides of the vines row. SE4Z: cover crop on two rows on both sides of the sampled row. nd: non-detected compound. Mean: mean values. SD: standard deviation. A: ANOVA test results (Different lower case letter in the same row indicates significant differences according to Tukey's test ($p>0.05$) between crop treatments of the same harvest).

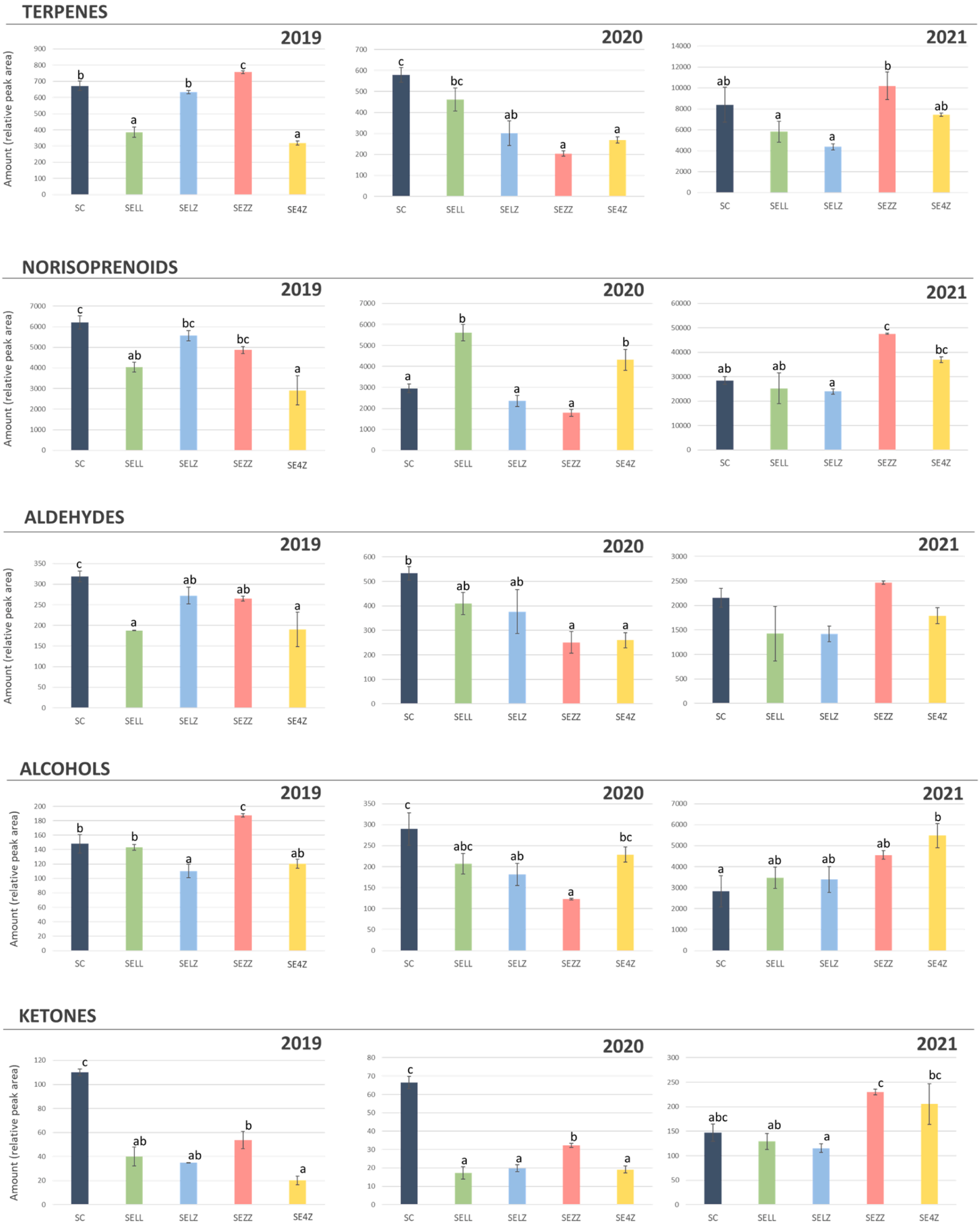

Fig. 2. Total amount of volatile compounds determined in the three consecutive harvests (2019, 2020 and 2021) of the main chemical families in all conditions tested (SC, SELL, SELZ, SEZZ, SE4Z). Data are expressed as relative area.

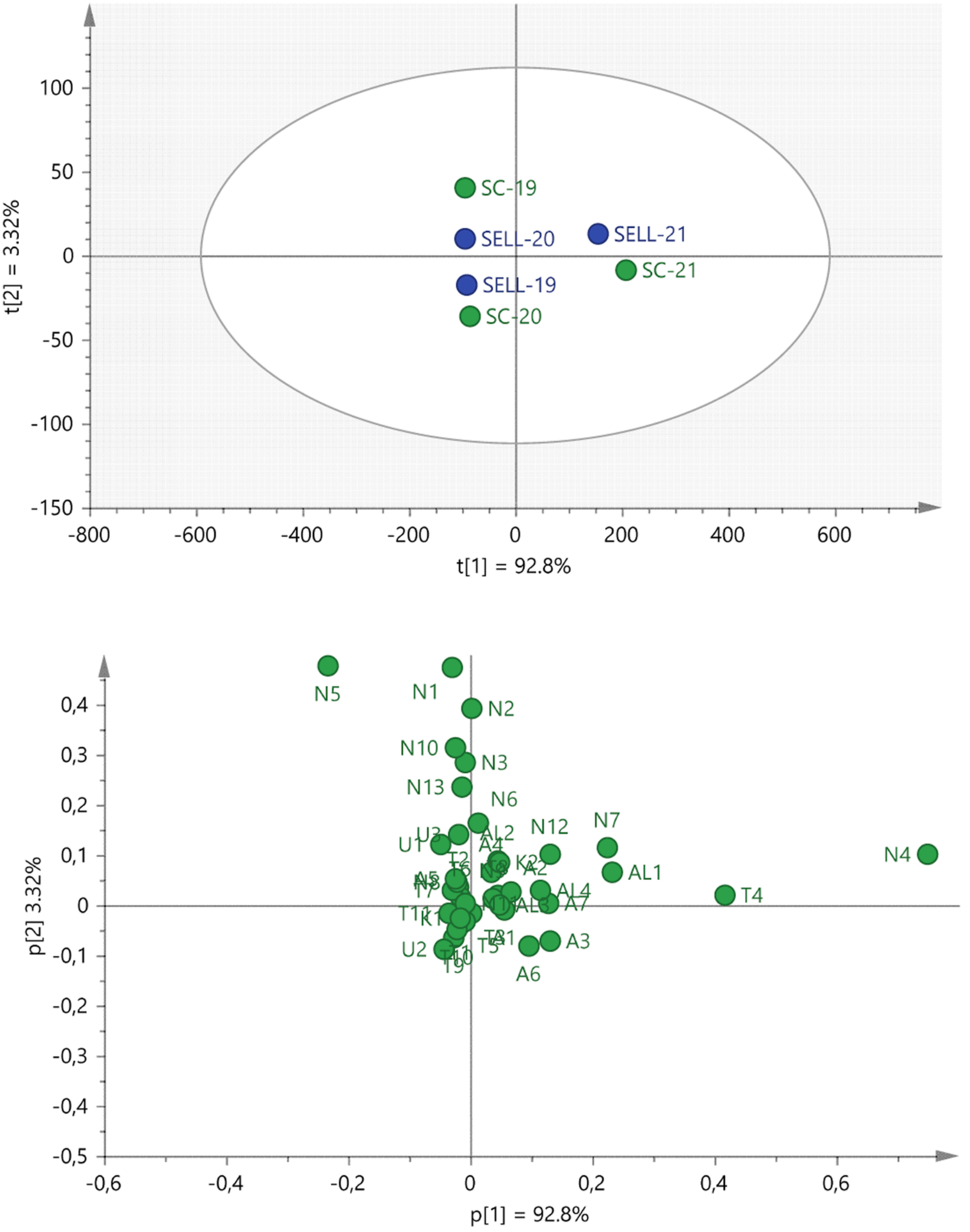

Fig. 3. Principal Component Analysis (PCA) showing the data scores (a) and loadings (b) biplot on the plane of the first two principal components (PC1 against PC2) comparing the samples of conventional vs. organic crop of the three vintages (2019, 2020 and 2021) including all the precursors determined as variables.

higher amounts of precursors when the highest pressure of Zulla was applied (SEZZ and SE4Z). In the third year this cover crop was possibly starting to settle and the vine was being stressed by the competition of nutrients and water and reacting by storing these compounds in their glycosylated form. Previous studies have pointed out that the insertion of a permanent cover crop in vineyards in a water-limited environment would generate both water and nitrogen stress in the grapevine (Celette and Gary, 2013). Water competition can lead to water deficits in the vine. The influence of water deficit has been previously observed in Cabernet Sauvignon and Merlot grapes and wines (Koundouras et al., 2009; Qian et al., 2009; Song et al., 2012; Talaverano et al., 2018). Furthermore, water deficit reduces canopy size, which subsequently increases cluster exposure to sunlight (Intrigliolo and Castel, 2011) and this is a key external factor that has been demonstrated to increase glycosidically bound aroma compounds in grapes (Marais et al., 1992; Meyers et al., 2013). This effect is mainly due to the increase of C_{13}-norisoprenoids in grapes, which mostly come from the β-carotene oxidative degradation. With more sunlight exposure, more carotenoids are present in the berry and consequently more C_{13}-norisoprenoids (Baumes et al., 2002). Considering that, despite competition for nutrients, the employment of cover crops has been shown to enrich the soil in nitrogen and carbon, with potential effects on the berry quality of *Vitis vinifera* grapes and table grapes (Gatullo et al., 2020; Ferrara et al., 2021).

Analysing the results in detail by chemical group to see the effects of the cover crop by year, it can be clearly observed that the initial planting of Zulla in the first vintage, 2019, had a positive effect on the production of terpene precursors with light cover crop influence (SEZZ) (Fig. 2). Glycosidic terpenes are of great importance to non-aromatic varieties, such as Syrah, as they confer a characteristic varietal aroma on wines (Maicas and Mateo, 2005). Hence, from the 11 terpenes, myrcenol (fresh floral/lavender/citrus), ocimenol (citrus/lemon) and isodurene (camphor-like odour) were significantly higher in SELZ musts, with isodurene being the most impacted (Table 1). When more Zulla was planted close to the vine, SEZZ, the significantly higher terpenes with respect to the control were menthol (mint) and, again, isodurene, which tripled its content. The increment of this compound was massive with respect to the control (SELL), which is tilled. This compound has been found in traditional Msalais wine and in wine from goji berries (Yuan et al., 2016; Zhu et al., 2019). The highest Zulla applied in this vintage, SE4Z, not only did not produce increases, but significantly reduced with respect to the control in most cases. In the next vintage, 2020, most of the terpenes precursors determined showed non-significant differences from the control sample, undergoing a significant decrease only in nerol oxide (floral), terpinen-4-ol (lavender/turpentine), and β-cyclocitral (floral). Therefore, it was the harvest in which the terpenes were most affected by the use of Zulla cover crop in the vineyard. Conversely, in 2021 among the 5 terpenes detected, ocimenol and nerol oxide precursors increased significantly with the higher Zulla presence, SE4Z. This last year the SEZZ musts also increased the ocimenol, isodurene, and, in this case, the menthol precursors presence significantly. Isodurene specifically stood out, doubling its quantity with respect to the control. It is possible that the biological establishment of Zulla plants this second year triggered some stress and competition for nutrients and water but not at an aggressive level that produces negative effects on the berry, but rather at a tolerable level capable of allowing growth, but generating the necessary stress to glycosylate these compounds. Talaverano et al. (2018) observed over two consecutive vintages that medium water stress in Cabernet sauvignon grapes gave rise to wines richer in terpenes than the wines obtained from grapes with low and high-water stress.

Within the isoprenoids group, C_{13}-norisoprenoids are the other family of compounds that are found predominantly in glycoside form. The most representative compounds from this group in the wine aroma are β-ionone, β-damascenone, vitispirane, and 1,1,6-trimethyl1,2-dihydro naphthalene (TDN) (Parker et al., 2017). Terpenes have a similar chemical structure to norisoprenoids, therefore, their parallel behaviour was predictable. During the first year, Zulla treatments generally accounted for lower amounts of norisoprenoids in the musts, but did not always show significance. Thus, vitispirane isomers (floral/fruity/woody) and β-damascenone (apple/plum/raisin) were found in higher significant quantities when lower amounts of Zulla cover crop were used, SELZ. Moreover, SELZ and SEZZ presented higher amounts of the C_{13}-norisoprenoids 1,1,6,8-Tetramethyl-1,2-dihydro-naphthalene, β-ionone (violet/floral/woody) and dehydro-β-ionone. Finally, in all Zulla treatments in 2019, 2,5,8-Trimethyl-1,2-dihydronaphthalene increased significantly with respect to the control (Table 1). A completely inverse result was obtained after the analysis of 2020 samples, since in this harvest all Zulla treatments negatively affected the precursors profile of the musts, with the exception of the Zulla application, SE4Z, where vitispiranes were determined in higher amounts than in the control, however, without significance. In the last year of evaluation, as well as the situation of terpenes, the vines surrounded by the most abundant Zulla produced grape musts richer in C_{13}-norisoprenoids than the control (SELL) and SELZ treatment (Fig. 2). Among the 13 detected norisoprenoids, only α-ionene and β-damascenone decreased in these treatments significantly, compared to the control. Hence, the naphthalene (pungent like/mothballs) peak observed in 2021 musts was the highest signal of the study, showing the highest amounts, and in SEZZ doubled its presence in the musts. If we look in more detail at norisoprenoids that are key aromas in wine, we can evaluate the suitability of Zulla as cover crop for wine production. Thereby, TDN (kerosene/gasoline), which is a varietal and impact aroma compound in wines such as Riesling, and vitispiranes, which are also key volatile compounds, did not present differences with respect to the control samples, which in turn is a remarkable result since it informs that the use of these cover crops would have no effect on them, accounting for all the ecological advantages. However, compounds such as β-damascenone significantly decreased every time Zulla was present (Table 1). This compound has been described as a volatile compound that does not have a contribution *per se*, but its presence extols the fruitiness of the wines (Escudero et al., 2007; Pineau et al., 2009) and plays a relevant role in the aroma of neutral grapes, such as Syrah (Ferreira and Lopez, 2019). Our results coincide with those obtained by Yuan et al. (2015) who observed a diminution of this compound in most years when employing cover crops in the vineyard. Something remarkable to consider was the behaviour of β-ionone and its related compounds, β-methylionone and dehydro-β-ionone. These compounds, such as β-ionone, are important since they are characteristic odorants of Syrah wine, conferring on this wine its typical woody/violet aroma (Reynolds, 2010). They usually occur in wines around or above their detection threshold of 90 ng/L (Kotseridis et al., 1999; Sabon et al., 2002). Thus, it was found that the musts with high Zulla pressure, SEZZ and SE4Z, presented higher amounts of β-ionone with respect to the control, however these were not statistically significant. Nevertheless. its derivative dehydro-β-ionone (woody aroma) drastically increased in SE4Z musts. In the case of the tentatively identified β-methylionone (woody, floral, violet), the Zulla treatments SEZZ and SE4Z did not reflect a significant difference with respect to the control with tillage treatment in the 2021 harvest.

The trends observed with respect to alcohols were the same as those described for terpenes and C_{13}-norisoprenoids (Fig. 2), considering that 2-ethyl-1-hexanol is classified as an unfavourable/undesirable attribute in wine due to its “green” or “grassy” notes. In the first harvest, 2019, the presence of Zulla on all levels showed similar amounts in the musts, while in the second year, 2020, despite not being significant, it can be observed that SELZ and SE4Z musts presented higher amounts. Concerning the last vintage, 2021, again, despite not being statistically different, it can be clearly seen that the Zulla presence in the soil produced musts with higher amounts of 2- ethyl-1-hexanol in its glycosidic form (Table 1). The vines with Zulla growth near them are expected to be water-stressed plants at some point. Song et al. (2012) investigated the effects of deficit irrigation on C6-bound aroma compounds in Merlot grapes and, despite not being statistically significant, a similar tendency to the increase was observed in hexanol with irrigation deficit.

Regarding the ketones group, the trend observed was similar in the three harvests, with SEZZ accounting for the highest amount of these compounds, although it was only significant in 2020 and 2021. This may be due to the content of 2,6-dimethyl-4-heptanone. Finally, among the 7 aldehydes determined, only two of them appeared in the 2021 vintage, benzaldehyde and 2,4,5-trimethylbenzaldehyde and in both cases higher amounts were observed in grapevines with a higher abundance of Zulla around them, and was statistically significant only in the case of 2,4,5-trimethylbenzaldehyde.

Results obtained regarding the influence of Zulla in the glycosidically bound volatile compounds were also analysed using a multivariate statistical approach. When the PCA was performed, the two principal components, PC1 and PC2, explained 97.2% of the data variance. The results revealed that PC 1 grouped the samples according to the vintages (Fig. 4).

Thus, harvests 2019 and 2020 had more similarities between them and were placed on the left side of the plane (negative PC1 axis) and 2021 vintage in the right side (positive PC1 axis). The loadings plot shows that naphtalene, isodurene and 2-ethyl-1-hexanol were strongly related to the last harvest. The important effect of the vintage on the Zulla treatments did not allow for observation of any tendency when all the vintages were analysed at the same time. In fact, the main effect ANOVA results showed higher significant differences for harvest factor than for treatment factor in the case of 33 precursors (Table S2). Therefore, each vintage was analysed separately and made it clear that vintages 2019 and 2020 were quite similar, with PC1 separating the control, SELL and the highest Zulla treatment, SE4Z, from the Zulla treatments with lower amounts, SELZ and SEZZ. Moreover, PC2 seemed to group must samples by the amount of Zulla (Fig. 5a and b). In the last vintage analysed (Fig. 5c), the samples were more separated according to the effect of Zulla treatment than previous ones, therefore, PC1 grouped samples according to the Zulla “pressure”, explaining an 88% cumulative variance.

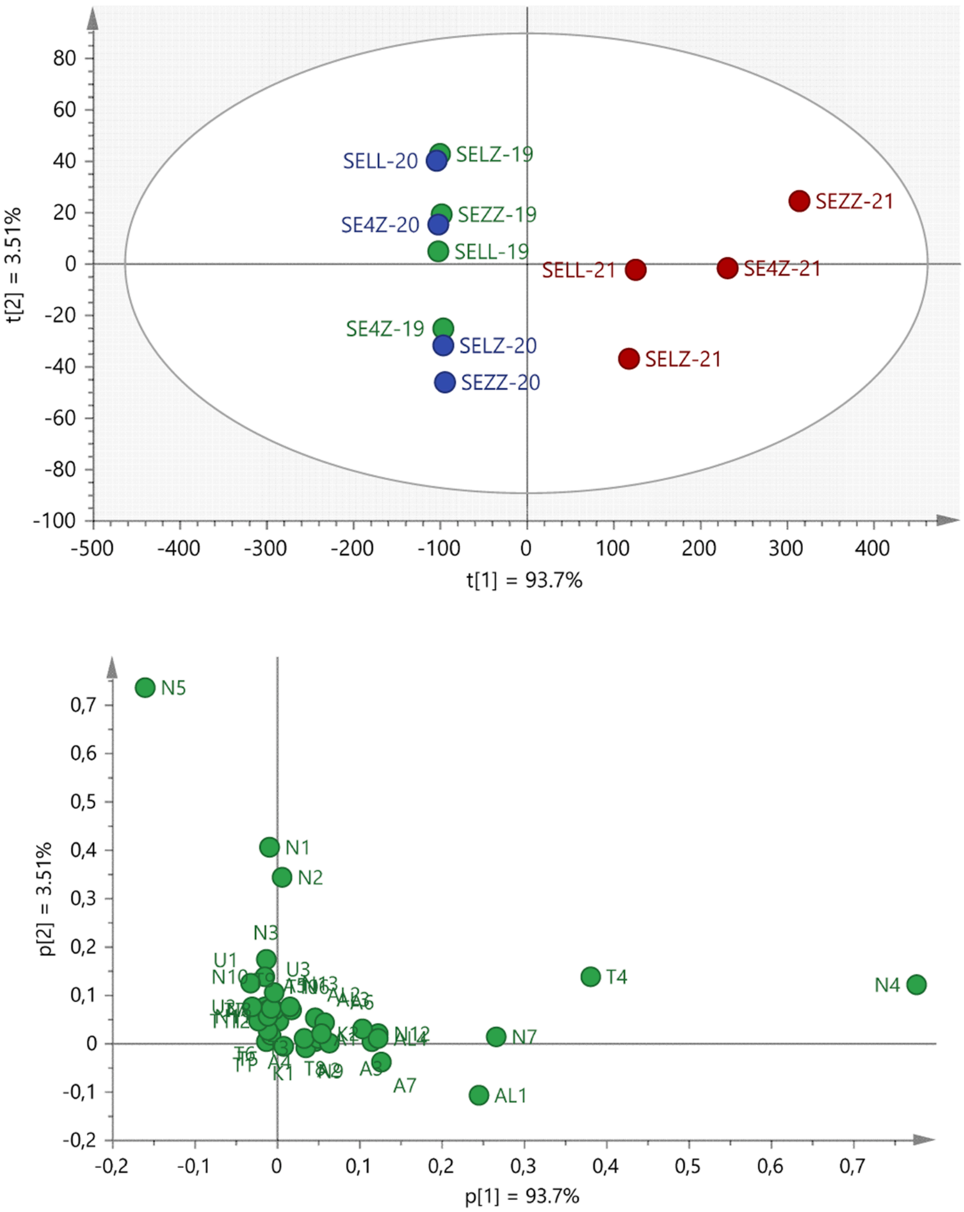

Fig. 4. Principal Component Analysis (PCA) showing the data scores (a) and loadings (b) biplot on the plane of the first two principal components (PC1 against PC2) comparing the different quantities of Zulla cover crop (SELL, SELZ, SEZZ, SE4Z) applied in the three harvests and including all the precursors determined as variables.

4. Conclusions

The restricted regulation in organic vineyards did not benefit the glycosidically bound aroma compounds, however, the employment of Zulla cover crops in some cases successfully compensated for these restrictions, increasing the compounds responsible for the potential aroma in wines. The effects of the presence of Zulla were strongly harvest dependent. In the first harvest, when Zulla was planted, treatments with lower Zulla pressure were the only ones that had a positive influence on aroma precursors. In the following harvest, where there was an advance in rooting and spontaneous growth, classical tillage management accounted for the highest amount of precursors. Finally, in the last harvest, it seemed that the steady establishment of the plant revealed positive effects, increasing aroma precursors when the two highest levels of Zulla were applied. These results point out that the employment of Zulla cover crops may be a positive vineyard implementation which, apart from its several ecological advantages, would be favourable for varieties such as Syrah, a neutral aroma grape where bound forms are strongly responsible for the potential aroma in the resulting wines.

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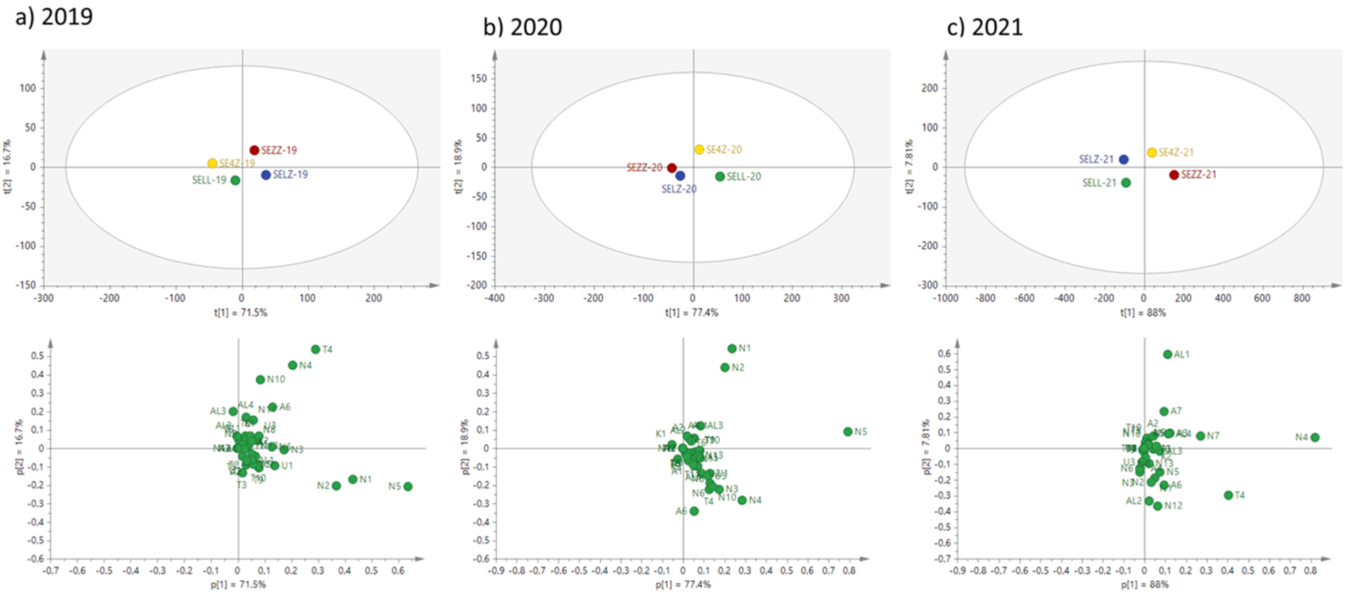

Fig. 5. Independent PCAs of every harvest (a)2019; (b)2020; (c)2021 including the different amounts of Zulla cover crop in organic vineyard as scores and all the volatile precursors as variables.

CRediT authorship contribution statement

María Pilar Segura-Borrego: Investigation, Formal analysis, Writing – original draft. **Susana Tejero:** Investigation, Formal analysis. **Belén Puertas:** Conceptualization, Methodology, Resources, Writing – review & editing, Project administration, Funding acquisition. **Eva Valero:** Conceptualization, Methodology, Writing – original draft, Supervision, Project administration, Funding acquisition. **Cristina Ubeda:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, Supervision. **María Lourdes Morales:** Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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