Kaya *et al. Chem. Biol. Technol. Agric.* (2024) 11:38
https://doi.org/10.1186/s40538-024-00564-2

Chemical and Biological Technologies in Agriculture

RESEARCH

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Improving organic grape production: the effects of soil management and organic fertilizers on biogenic amine levels in *Vitis vinifera* cv., 'Royal' grapes

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Abstract

Background The integration of organic viticulture practices in grape cultivation represents a pivotal advancement towards sustainable agriculture, emphasizing the importance of environmentally friendly methods that enhance soil health, grape quality, and overall ecosystem biodiversity, thereby contributing significantly to the resilience and long-term sustainability of viticultural ecosystems. This study explored the effects of soil management practices, including chisel, disc harrow, and no tillage, as well as the impact of utilizing Antep radish, broccoli, and olive blackwater as fertilizer applications, on the biochemical composition, specifically biogenic amines (BAs), in the clusters of the 'Royal' grape cultivar within a vineyard setting.

Results Throughout the three-year study, no tillage soil management consistently emerged as the most influential soil treatment for enhancing BAs in 'Royal' grape berries, especially in combination with Antep radish and olive blackwater fertilizer applications. Among fertilizer applications, the nontreated control vines consistently had the highest concentrations of critical BAs, such as putrescine, cadaverine, histamine, and dopamine, across different soil management practices. Among the soil management practices and fertilizer applications evaluated, the disc harrow soil management and olive blackwater fertilizer application generally yielded the lowest concentrations of BAs across several metrics.The PCA biplots indicated that experimental years have a similar effect on BA content in grape berries, with specific amines such as serotonin and dopamine being more affected in 2020, while cadaverine, histamine, spermidine, trimethylamine, and norepinephrine were more influenced in 2021, and putrescine, spermine, agmatine, and tryptamine in 2022.

Conclusion These findings hold significant implications for organic agriculture, emphasizing the nuanced influence of soil management practices and organic fertilizers on the BA composition of grape berries. Our results indicate the potential of tailored agricultural strategies to enhance plant health and quality, aligning with the principles of sustainability and environmental stewardship inherent to organic farming.

Keywords Antep radish, Disc harrow, Chisel, Organic agriculture, Olive blackwater, No tillage

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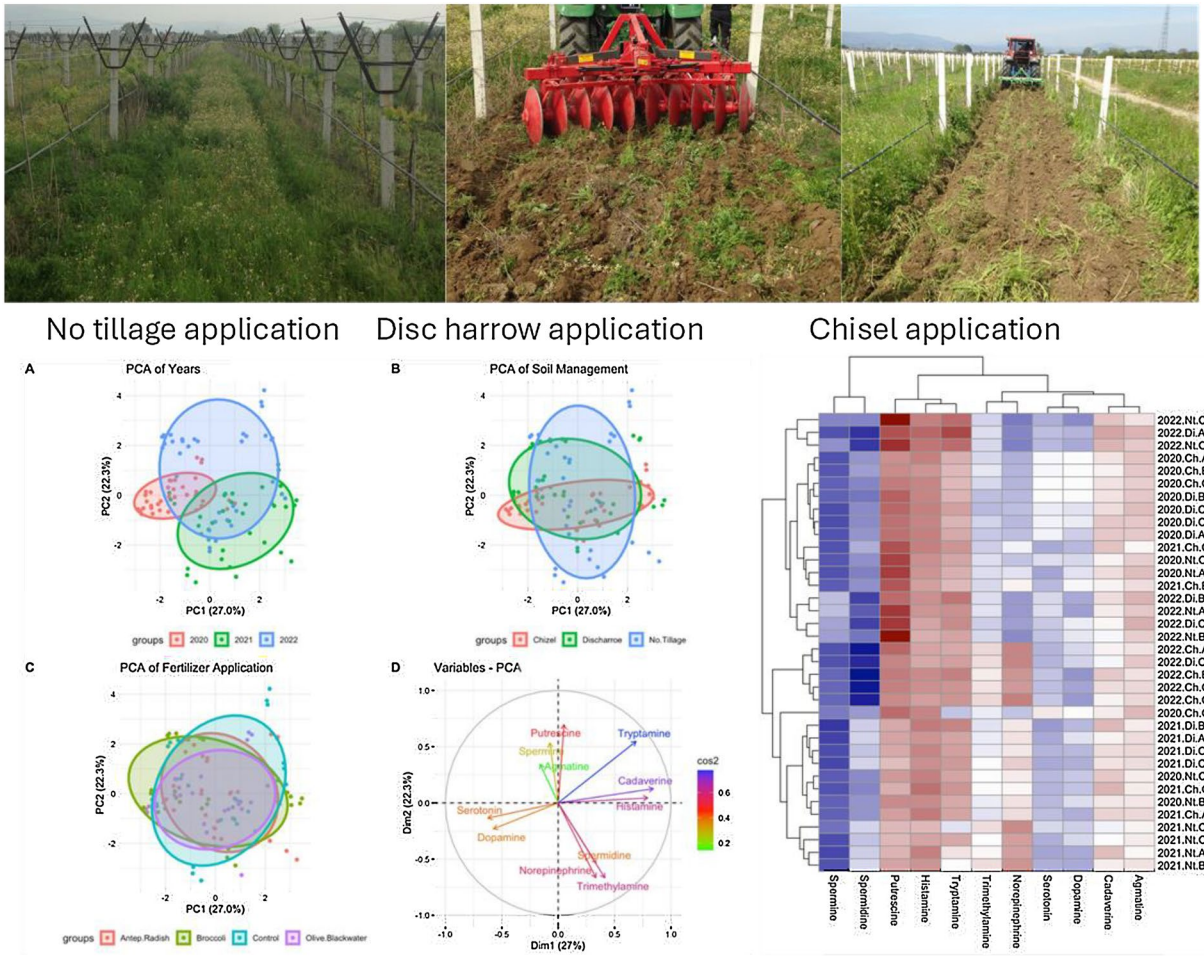
Graphical Abstract



Introduction

The prevailing adoption of conventional agricultural practices by farmers has resulted in a pronounced escalation of soil erosion and pollution rates [1, 2]. Furthermore, environmental concerns regarding the impact of conventional agriculture, such as decreased soil fertility, limited water supply, biodiversity loss, and herbicide resistance, have created ever-growing pressure for growers to incorporate increasingly sustainable practices [3]. In recent years, contemporary agricultural paradigms increasingly underscore the imperative of sustainable agronomic management systems [1, 3]. Noteworthy in this context is the farm to fork strategy articulated by the European Commission, which designated 25% of the farmland in the European Union as organically managed by 2030 [4]. This strategic initiative seeks to foster environmentally conscientious and less intensive agricultural systems, entailing substantial reductions in fertilizer applications, and pesticide usage [1]. Complementary to these objectives is an overarching commitment to biodiversity, intricately interwoven within the broader initiative entitled "Bringing Nature Back into Our Lives," which is regarded as pivotal in fortifying the resilience of European societies [4]. In this respect, recent endeavors focused on crop diversification, optimal utilization of energy, soil and water resources, and the formulation of strategies poised to counteract impending climatic exigencies [1]. Sustainable agricultural practices, as highlighted in studies by Kaya [5] and Kaya et al. [6], emphasize the need for farming methods that reduce environmental impact, enhance biodiversity, and ensure the efficient use of resources. Concomitantly, the introduction of agroecological practices that assume primacy as the inaugural

step in a transformative trajectory towards a more sustainable agriculture [7, 8].

Grapes hold a prominent position in worldwide cultivation, and the environmental impact of grape production is substantial [9]. The challenges posed by conventional agricultural methods, including soil erosion, reduced fertility, water scarcity, and the potential for biodiversity loss, are particularly pertinent to the cultivation of grapes. Organic viticulture, therefore, represents a new orientation in grape production in terms of environmental benefits, human health, and competitiveness. Organic grape management has resulted in comparable fruit quality to conventional methods but required more inputs, time, and higher costs, especially for weed control [10]. Inadequate weed management leads to yield reductions in organic vineyards [11]. The ongoing debate with soil tillage highlights its complexity. While short-term productivity may favor cultivation, continuous vineyard cultivation reduces long-term yields due to decreased organic matter and soil permeability. This emphasizes the need for a thoughtful evaluation of soil management practices in sustainable grape production. Incorporating conservation tillage and considering weed infestation impact is crucial for long-term success in vineyard management and soil health. Some evidence supports conservation tillage, including reduced and no tillage, which resulted in increased yields and reduced soil degradation [12]. The positive effects were further enhanced by adding plant waste to the soil, which contributed to improved soil health and productivity. High nitrogen and phosphorus content in cover crops has also enhanced yields and soil health [13].

When considering grapes, a crop of global significance, the implementation of these practices becomes paramount not only for the preservation of ecosystems but also for the quality and safety of the produce. Biogenic amines (BAs) in table grape and raisin have been the subject of study due to their impact on both fruit quality and consumer health [14]. For table grapes, BAs at controlled levels are indicative of grape health and nutritional value, enhancing the fruit's appeal and safety for consumption [9]. Proper management of BAs ensures that table grapes remain a desirable, high-quality food product, reflecting careful cultivation and handling practices [14]. These compounds, including histamine, tyramine, and putrescine, arise from the microbial decarboxylation of amino acids and can influence the sensory attributes of the grapes as well as pose health risks at elevated levels [15]. The concentration of BAs in table grapes was affected by various factors, such as cultivar susceptibility, agricultural practices, and post-harvest handling [16]. Research indicated that managing these factors can significantly reduce amine concentrations, thereby enhancing grape safety and quality [9]. Therefore, understanding the dynamics of BA production and accumulation in table grapes is essential for developing strategies to mitigate their presence. Integrating the concept of sustainable agricultural practices with a focus on BAs in crops, particularly grapes which hold global importance, indicates a multifaceted approach to agriculture that is crucial for environmental sustainability and food safety.

Research on soil tillage and organic fertilizers highlights their positive impact on grape yield and quality, with conservation tillage enhancing soil moisture and organic fertilizers boosting yield and phenolic content [17]. These studies diverge by implementing a combined minimal tillage and specific organic fertilizer strategy, uniquely adapted to local soil conditions. In this regard, previous studies have investigated various soil cultivation practices, including chisel, disc harrow, and no tillage, within vineyards. However, there exists a limited body of research concerning the allelopathic effects of Antep radish, broccoli, and olive blackwater fertilizer applications, particularly in the context of fertilizer, specifically on some biochemicals of the *Vitis vinifera* cv., 'Royal' grape berries. This investigation, therefore, examined soil management practices such as chisel, disc harrow, and no tillage in the vineyard of 'Royal' grapes, along with the impact of Antep radish, broccoli, and olive blackwater fertilizer applications on some biochemical components like BAs within the clusters of 'Royal' grapes. Thus, our study bridges a significant gap by delivering comprehensive insights into the effects of these practices on BAs concentrations in 'Royal' grapes, laying the groundwork for subsequent investigations in grapes and analogous crops.

Materials and methods

Experimental site and plant material

The research was carried out at the Viticulture Research Institute in Manisa, located at coordinates 38° 38′ 0.9.40" N, 27° 23′ 59.43" E, during the period from 2020 to 2022. The selected grape cultivars for this study were seedless ' Royal' scions grafted onto 110 R rootstock Richter 110 (110 R; *V. berlandieri* × *V. rupestris*) in 2012, trained to a system with a spacing of 3 m × 2 m. The 'Royal' cultivar obtained by natural mutation from 'Alphonse Lavallée' showcases unique horticultural traits, notably its large, winged, conical clusters weighing 400–500 g, and its exceptionally large, slightly flattened-round berries weighing around 8 g each, with a distinctive purple–black color and a slightly tannic taste. Managed with short pruning to regulate growth and enhance fruit production, this variety demonstrates good development and productivity, with yields ranging from 1000 to 1700 kg per hectare. Harvesting occurs in late August to September,

ensuring optimal berry maturity. The experiments were conducted with a randomized complete block design, comprising four replicates, each consisting of 12 vines per replicate. This cultivar was cultivated using a Y-shaped support system and a double arm training system, coupled with short pruning that leaves 2–3 buds per branch. For watering, drip irrigation was employed, delivering water directly to the roots efficiently.

Soil tillage applications

Three distinct soil tillage methods were employed in this study. Soil tillage was applied between the vine rows. Chisel application involved the use of a chisel for soil tillage. Chisel-based soil tillage applications were performed during autumn, and in April. The disc harrow application utilized a heavy disc harrow for soil tillage. Heavy-duty disc harrow applications were conducted in autumn, and again in April. Lastly, control vines grown in the experimental plots received no soil tillage.

Organic fertilizer applications

Organic fertilizers, namely Antep radish (*Raphanus raphanistrum* L.), broccoli (*Brassica oleracea* L. var. italica), and olive blackwater, were utilized in this study. Antep radish, broccoli, and olive blackwater were chosen as organic fertilizers in this study for their potential to improve soil quality and nutrient content. These fertilizers are rich in organic matter and nutrients, which can enhance soil health and support sustainable agricultural practices. In the conducted experiment, the olives were sourced from a facility specializing in black water olive production. Olive black water comprised a composite mixture that included the intrinsic sap of the olives, water used for washing the olives, additional water incorporated during processing, and moisture exuded from the olive pomace during the olive oil extraction process. Separately, broccoli and pistachio radish, encompassing roots, leaves, and consumable parts, were cultivated in a distinct agricultural site. These components were subsequently harvested, finely shredded, and then freshly applied to the soil in accordance with the experimental design. The nontreated did not receive any fertilizer application. For the Antep radish and broccoli fertilizers, the plants cultivated in different locations and processed through shredding, and subsequently integrated into the soil amidst the rows at a rate of 6 kg vine^{-1} in May. In addition to the above, olive blackwater was applied to the soil at a rate of 6 L of vine^{-1}. Specifically, the carbon contribution to the soil was calculated based on the percentage carbon content of each type of organic biomass. For broccoli with a carbon composition of 42%, 1.14 kg (2.52 lbs/A) of carbon per 6 m^2 was added to the soil. Similarly, olive blackwater, containing 48% carbon, added 1.19 kg (2.88 lbs/A) of carbon per 6 m^2 to the soil. Additionally, Antep radish, with a carbon content of 44.2%, was added 1.30 kg (2.64 lbs/A) of carbon per 6 m^2 to the soil.

Sample collection

'Royal' berries were harvested in August at their mature phase (17° Brix), selecting 30 bunches of grapes from each treatment block. From each bunch, 100-berry samples were randomly collected, ensuring the pedicel remained intact to prevent juice loss during transportation. The selected berries were carefully placed in plastic bags and transported to the laboratory, with a weight of 50 g designated for biochemical extraction, replicated three times. Following collection, grape clusters were promptly stored at 4 °C in the laboratory and later preserved at − 80 °C for subsequent analytical procedures.

Identification of biogenic amines from berries by HPLC

Chemicals and isolation of amines

The study utilized standard solutions of spermine, agmatine, spermidine, serotonin, histamine, tryptamine, dopamine, norepinephrine, cadaverine, trimethylamine, putrescine, and tyramine obtained from Sigma–Aldrich Chemie (Steinheim, Germany). For the isolation of amines from grape berries, 5 g of berries were homogenized using an Ultra-turax homogenizer (IKA T25 Ultra-Turrax®, Staufen, Germany) with 0.5 mL of 70% perchloric acid. The homogenate was then centrifuged at 10,000 rpm for 10 min, and the supernatant was recovered, filtered over a 0.22 mm membrane, and diluted with 10% perchloric acid to the initial homogenate weight. The resulting sample was further filtered over 0.45 µm and injected into the High-Performance Liquid Chromatography (HPLC) system (Waters, Milford, MA, USA).

Identification of biogenic amines from grape berries by HPLC

Identification of BAs from samples was conducted by HPLC, following a slightly modified method of Nagy et al. [18]. The BAs were separated and quantified using a reverse-phase column (Bondapak C18, 300 × 3.9 mm, 10 mm; Waters, Milford, MA, USA) coupled with a Waters Alliance Liquid Chromatograph and a Waters 474 fluorescence detector (Milford, MA, USA). Post-column derivatization (2-mercaptoethanol, o-phtalaldehyde) was employed to enhance detection. Peaks were identified using authentic standards, and calibration curves in the specified concentration ranges were used for quantitation. Sample BA contents were expressed in µg · L^{-1} fresh weight.

Statistical analysis

All descriptive analyses were performed using the agricolae package in R Studio. The significance of years, soil

tillage applications, organic fertilizer applications, and their interactions on BAs were analyzed through Multivariate Analysis of Variance (Three-Way ANOVA) in R Studio (R Core, 2013). Prior to analyses, all data were tested for normality using the chi-square test. Linear models were employed to evaluate the main effects (years, soil tillage applications, organic fertilizer applications) on BAs. Post-hoc analysis utilizing Tukey HSD was conducted with the agricolae package in R Studio. Principal Component Analyses (PCAs) for BAs datasets were carried out using ggbiplot2 within R Studio. The PCA, as a valuable analytical technique, facilitated the reduction of multidimensional data into a more interpretable format, aiding in the identification of underlying patterns and ren*0 s within the complex datasets. The heatmap was generated using the pheatmap package in R Studio, providing a visual representation of relationships and variations in the analyzed datasets [19].

Results

The BAs concentrations were influenced by the interaction of soil management practices, fertilizer applications, and years (Table 1). In 2020, grapes within the chisel soil management had higher putrescine and cadaverine concentrations when not treated with fertilizer, with values of 11.73 and 2.04 μg L^{-1}, respectively. However, in terms of cadaverine concentration, Antep Radish treatment was in the same statistical group with 1.81 μg L^{-1} with the control. In contrast, agmatine, spermine, serotonin, and trimethylamine displayed uniform concentrations across fertilizer treatments with no significant differences. In 2020, vines under chisel soil management spermidine had berries that peaked at 0.25 μg L^{-1} in broccoli ($p=0.009$), histamine peaked at 9.34 μg L^{-1} in the control and at 8.18 μg L^{-1} in the Antep radish ($p=0.001$), tryptamine peaked at 4.26 μg L^{-1} in Antep radish, and at 4.05 μg L^{-1} in the control, and dopamine peaked at 1.28 μg L^{-1} in the control and at 1.16 μg L^{-1} in broccoli. Berry norepinephrine was highest in the control at 0.38 μg L^{-1} and in broccoli at 0.34 μg L^{-1}. In 2020, using the disc harrow soil management, berries had putrescine, cadaverine, spermine, histamine and serotonin and norepinephrine levels that were similar across fertilizer treatments. Berries had significantly higher agmatine concentration, peaking at 4.35 μg L^{-1} without fertilizer ($p=0.009$). Spermine levels were uniform ($p=0.239$), while spermidine showed significant variability with high concentrations in Antep radish and the control. There are significantly differences was observed for tryptamine and dopamine, with tryptamine peaking at 7.26 μg L^{-1} in the control ($p=0.001$) and dopamine at 1.41 μg L^{-1} in broccoli ($p=0.000$). Trimethylamine peaking at 0.65 μg L^{-1} in olive blackwater and at 0.61 μg L^{-1} in Antep radish. In the no tillage category, putrescine was the most abundant, especially with the Antep radish fertilizer application (50.11 μg L^{-1}), followed by the olive blackwater fertilizer application (33.42 μg L^{-1}), with a significant difference ($p=0.0001$). Cadaverine showed the highest concentration in the control (4.11 μg L^{-1}), and Antep radish fertilizer application (3.61 μg L^{-1}, $p=0.001$). Agmatine peaked with the Antep radish fertilizer application (5.80 μg L^{-1}), ($p=0.0001$). Spermine, spermidine, trimethylamine and norepinephrine did not show significant differences among treatments. However, histamine was found in higher concentrations in the control (13.15 μg L^{-1}) and Antep radish fertilizer application (12.19 μg L^{-1}, $p=0.0001$). For tryptamine, serotonin, and dopamine, significant differences were noted, with the highest levels in Antep radish fertilizer application (7.98 μg L^{-1}, $p=0.027$), olive blackwater (0.88 μg L^{-1}, $p=0.015$), and Antep radish fertilizer applications (1.31 μg L^{-1}, $p=0.0001$) respectively. In the 2020 study, the non-fertilizer control was effective in increasing the tryptamine concentration in all soil management treatments, while the Antep radish treatment was effective in increasing the trimethylamine content in both disc harrow and no-tillage soil management conditions. However, broccoli application provided high dopamine value under both chisel and disc harrow soil management conditions. Interestingly, olive blackwater, which had lower means for all BAs contents (except trimethylamine) compared to the other fertilizer treatments, was the most effective treatment in terms of increasing serotonin content under no-tillage conditions. Taken together, the non-fertilizer control under chisel and disc harrow soil management conditions and the Antep radish treatment under no-tillage conditions had higher averages in terms of BAs contents. In addition, chisel tillage gave the best results for the no-fertilizer control and broccoli treatment, while no-tillage for Antep radish treatment and disc harrow and no-tillage conditions for olive blackwater gave the best results.

In 2021, our study on the impact of different fertilizer applications using chisel, disc harrow, and no tillage soil managements revealed distinct BAs concentration patterns and statistical significances (Table 1). Under the chisel soil management, the control showed the highest concentration of putrescine (43.03 μg L^{-1}), with a significant difference ($p=0.000$), followed by broccoli fertilizer application (28.61 μg L^{-1}). Cadaverine levels were notably high in the control and olive blackwater fertilizer application (6.70 and 6.40 μg L^{-1}, respectively), indicating significant differences ($p=0.000$). Agmatine was most abundant in broccoli (3.18 μg L^{-1}), Antep radish (3.01 μg L^{-1}) and the control (2.83 μg L^{-1}) fertilizer applications, with olive blackwater fertilizer application

Table 1 Effect of fertilizer application on BAs contents (μg·L^{-1}) in 'Royal' grape cultivars depending on soil management and years of research

BAs	Fertilizer Application	Soil Management								
		2020			2021			2022		
		Chisel	Disc Harrow	No Tillage	Chisel	Disc Harrow	No Tillage	Chisel	Disc Harrow	No Tillage
Putrescine	Olive Blackwater	9.21 ± 0.6b	14.91 ± 0.4 ns	33.42 ± 2.1b	6.72 ± 0.1c	7.92 ± 0.6a	6.83 ± 0.3 ns	13.92 ± 0.7b	12.82 ± 1.2c	39.72 ± 2.9c
	Antep Radish	7.15 ± 0.1c	14.83 ± 0.7	50.11 ± 2.1a	7.71 ± 0.2c	8.01 ± 0.2a	7.32 ± 0.4	21.93 ± 0.6a	19.81 ± 1.2b	28.91 ± 9.5c
	Broccoli	8.12 ± 0.2c	19.66 ± 1.2	10.43 ± 0.5c	28.61 ± 1.2b	8.62 ± 0.1a	7.31 ± 0.8	20.91 ± 1.7a	12.23 ± 0.4c	89.00 ± 2.1b
	Control	11.73 ± 0.2a	19.12 ± 5.1	8.4 ± 0.4c	43.03 ± 0.8a	5.73 ± 0.1b	6.12 ± 0.9	12.3 ± 10.9b	32.82 ± 1.6a	116.83 ± 4.0a
	Significance	0.000	0.451	0.000	0.000	0.001	0.583	0.000	0.000	0.000
Cadaverine	Olive Blackwater	1.12 ± 0.1c	3.32 ± 0.5 ns	1.82 ± 0.2b	6.40 ± 0.3a	3.42 ± 0.2b	2.12 ± 0.4b	2.86 ± 0.3b	1.55 ± 0.1b	4.41 ± 0.2a
	Antep Radish	1.81 ± 0.1ab	3.33 ± 0.3	3.61 ± 0.1a	3.42 ± 0.1b	2.33 ± 0.1b	6.31 ± 0.2a	2.19 ± 0.1b	5.32 ± 0.5a	1.57 ± 0.1b
	Broccoli	1.50 ± 0.2bc	2.38 ± 0.0	2.28 ± 0.2b	2.26 ± 0.1c	6.41 ± 0.6a	2.27 ± 0.1b	5.82 ± 0.5a	1.95 ± 0.1b	1.30 ± 0.1b
	Control	2.04 ± 0.0a	2.66 ± 0.2	4.11 ± 0.3a	6.70 ± 0.5a	3.08 ± 0.2b	1.55 ± 0.3b	2.28 ± 0.1b	1.34 ± 0.1b	4.44 ± 0.4a
	Significance	0.003	0.145	0.001	0.000	0.000	0.000	0.000	0.000	0.000
Agmatine	Olive Blackwater	2.51 ± 0.2 ns	3.42 ± 0.1b	3.52 ± 0.1b	2.40 ± 0.1b	3.71 ± 0.1 ns	2.83 ± 0.1a	3.19 ± 0.4bc	4.07 ± 0.2 ns	3.18 ± 0.3 ns
	AntepRadish	2.91 ± 0.2	3.36 ± 0.3b	5.80 ± 0.3a	3.01 ± 0.2a	3.27 ± 0.2	2.42 ± 0.1b	2.77 ± 0.2c	4.12 ± 0.1	2.65 ± 0.2
	Broccoli	2.90 ± 0.1	3.73 ± 0.1b	2.32 ± 0.1b	3.18 ± 0.1a	2.71 ± 0.3	2.62 ± 0.1a	3.70 ± 0.1a	3.85 ± 0.2	3.13 ± 0.2
	Control	3.02 ± 0.1	4.35 ± 0.0a	2.27 ± 0.1b	2.83 ± 0.2ab	3.36 ± 0.1	2.42 ± 0.0b	3.52 ± 0.3ab	4.60 ± 0.1	2.73 ± 0.1
	Significance	0.281	0.009	0.000	0.024	0.083	0.004	0.009	0.080	0.106
Spermine	Olive Blackwater	0.09 ± 0.0 ns	0.07 ± 0.0 ns	0.10 ± 0.0 ns	0.10 ± 0.0 ns	0.11 ± 0.0 ns	0.11 ± 0.0b	0.13 ± 0.0ab	0.10 ± 0.0c	0.21 ± 0.0c
	Antep Radish	0.07 ± 0.0	0.08 ± 0.0	0.12 ± 0.0	0.12 ± 0.0	0.08 ± 0.0	0.10 ± 0.0b	0.11 ± 0.0b	0.07 ± 0.0d	0.40 ± 0.0a
	Broccoli	0.08 ± 0.0	0.11 ± 0.0	0.12 ± 0.0	0.12 ± 0.0	0.08 ± 0.0	0.09 ± 0.0b	0.19 ± 0.0a	0.36 ± 0.0a	0.30 ± 0.0b
	Control	0.10 ± 0.0	0.08 ± 0.0	0.11 ± 0.0	0.15 ± 0.0	0.10 ± 0.0	0.19 ± 0.0a	0.15 ± 0.0ab	0.28 ± 0.0b	0.23 ± 0.0c
	Significance	0.217	0.239	0.677	0.309	0.259	0.006	0.043	0.000	0.000
Spermidine	Olive Blackwater	0.15 ± 0.0b	0.20 ± 0.1bc	0.24 ± 0.0 ns	0.25 ± 0.0b	0.74 ± 0.0 ns	0.79 ± 0.0 ns	0.03 ± 0.0 ns	0.05 ± 0.1 ns	0.05 ± 0.0c
	Antep Radish	0.19 ± 0.0b	0.24 ± 0.1a	0.26 ± 0.0	0.30 ± 0.0b	0.87 ± 0.0	0.94 ± 0.1	0.03 ± 0.0	0.04 ± 0.0	0.08 ± 0.0bc
	Broccoli	0.25 ± 0.0a	0.15 ± 0.0c	0.27 ± 0.0	0.30 ± 0.0b	0.97 ± 0.0	0.90 ± 0.0	0.03 ± 0.0	0.05 ± 0.1	0.16 ± 0.0ab
	Control	0.18 ± 0.1b	0.22 ± 0.1a	0.24 ± 0.0	0.68 ± 0.0a	0.90 ± 0.0	0.92 ± 0.0	0.04 ± 0.1	0.05 ± 0.0	0.23 ± 0.0a
	Significance	0.009	0.022	0.139	0.012	0.291	0.348	0.590	0.546	0.006
Histamine	Olive Blackwater	6.07 ± 0.4b	10.45 ± 0.7 ns	9.39 ± 0.0b	18.03 ± 0.9a	13.78 ± 1.1b	9.05 ± 0.7b	10.59 ± 0.8b	6.63 ± 1.1b	12.42 ± 0.3a
	Antep Radish	8.18 ± 0.4a	11.00 ± 0.5	12.19 ± 0.3a	13.70 ± 0.5b	10.55 ± 0.5c	17.66 ± 0.5a	8.04 ± 0.3c	14.23 ± 0.2a	6.29 ± 0.6b
	Broccoli	6.73 ± 0.4b	10.46 ± 0.5	10.23 ± 0.4b	8.93 ± 0.4c	18.52 ± 0.9a	9.07 ± 0.4b	15.41 ± 0.6a	6.97 ± 0.7b	5.13 ± 0.2b
	Control	9.34 ± 0.2a	12.21 ± 0.7	13.15 ± 0.5a	19.87 ± 0.8a	11.91 ± 1.1bc	6.12 ± 0.3c	8.64 ± 0.7bc	5.30 ± 0.3b	13.18 ± 0.2a
	Significance	0.001	0.204	0.000	0.000	0.001	0.000	0.000	0.000	0.000

Table 1 (continued)

BAs	Fertilizer Application	Soil Management								
		2020			2021			2022		
		Chisel	Disc Harrow	No Tillage	Chisel	Disc Harrow	No Tillage	Chisel	Disc Harrow	No Tillage
Tryptamine	Olive Blackwater	0.30 ± 0.0c	4.61 ± 0.3c	6.65 ± 0.5bc	10.78 ± 0.3b	10.8 ± 0.3b	8.37 ± 0.6a	9.24 ± 0.7bc	7.84 ± 0.6c	14.89 ± 0.4b
	Antep Radish	4.26 ± 0.1a	5.57 ± 0.2b	7.98 ± 0.2a	7.53 ± 0.4c	7.75 ± 0.4b	3.32 ± 0.1b	7.26 ± 0.6c	23.14 ± 0.9a	6.94 ± 0.3c
	Broccoli	3.65 ± 0.1b	4.92 ± 0.3bc	6.16 ± 0.4c	5.52 ± 0.4d	15.67 ± 0.4a	1.58 ± 0.1c	19.41 ± 0.7a	10.78 ± 0.8b	6.36 ± 0.4c
	Control	4.05 ± 0.1a	7.26 ± 0.4a	7.39 ± 0.2ab	13.96 ± 0.4a	9.80 ± 0.4b	1.24 ± 0.1c	9.98 ± 0.7b	8.47 ± 0.6bc	19.28 ± 0.6a
	Significance	0.000	0.001	0.027	0.000	0.000	0.000	0.000	0.000	0.000
Serotonin	Olive Blackwater	1.25 ± 0.1 ns	1.10 ± 0.2 ns	0.88 ± 0.2a	0.54 ± 0.0 ns	0.55 ± 0.0 ns	0.59 ± 0.0b	0.58 ± 0.0c	0.51 ± 0.0b	0.40 ± 0.0c
	Antep Radish	1.00 ± 0.0	1.17 ± 0.1	0.46 ± 0.0b	0.55 ± 0.0	0.45 ± 0.0	0.50 ± 0.0c	0.53 ± 0.0c	0.40 ± 0.0c	0.65 ± 0.1a
	Broccoli	1.07 ± 0.2	1.48 ± 0.0	0.49 ± 0.0b	0.59 ± 0.0	0.40 ± 0.1	0.43 ± 0.0c	0.85 ± 0.0a	0.60 ± 0.0a	0.53 ± 0.0b
	Control	1.36 ± 0.1	1.19 ± 0.0	0.51 ± 0.0b	0.60 ± 0.0	0.48 ± 0.0	0.98 ± 0.0a	0.72 ± 0.0b	0.49 ± 0.0b	0.44 ± 0.0c
	Significance	0.172	0.186	0.015	0.167	0.224	0.000	0.001	0.003	0.000
Trimethylamine	Olive Blackwater	0.62 ± 0.1 ns	0.65 ± 0.0a	0.86 ± 0.2 ns	1.87 ± 0.4 ns	1.44 ± 0.0b	2.25 ± 0.6 ns	2.69 ± 0.2 ns	2.95 ± 1.2a	0.69 ± 0.0 ns
	Antep Radish	0.61 ± 0.1	0.61 ± 0.0ab	1.24 ± 0.1	1.06 ± 0.0	1.37 ± 0.0b	2.52 ± 0.1	3.29 ± 0.2	0.65 ± 0.0b	0.71 ± 0.0
	Broccoli	0.78 ± 0.0	0.54 ± 0.0b	1.14 ± 0.2	0.94 ± 0.0	1.34 ± 0.0b	2.80 ± 0.2	3.13 ± 0.1	0.61 ± 0.0b	0.76 ± 0.0
	Control	0.68 ± 0.0	0.51 ± 0.0b	1.53 ± 0.1	1.15 ± 0.2	2.1 ± 0.2a	3.26 ± 0.3	3.28 ± 0.2	0.56 ± 0.1b	0.80 ± 0.0
	Significance	0.261	0.053	0.118	0.055	0.004	0.265	0.080	0.050	0.112
Dopamine	Olive Blackwater	0.76 ± 0.0c	1.0 ± 0.0b	0.79 ± 0.0bc	0.66 ± 0.0c	0.65 ± 0.0b	1.09 ± 0.0a	0.38 ± 0.0b	0.85 ± 0.0a	0.32 ± 0.0b
	Antep Radish	1.05 ± 0.0b	0.67 ± 0c	1.31 ± 0.0a	0.46 ± 0.0d	1.16 ± 0.0a	0.55 ± 0.0c	0.94 ± 0.0a	0.34 ± 0.0b	0.24 ± 0.0c
	Broccoli	1.16 ± 0.1ab	1.41 ± 0a	0.87 ± 0.1b	1.63 ± 0.0a	0.67 ± 0.0b	0.43 ± 0.0d	0.4 ± 0.0b	0.28 ± 0.0b	1.01 ± 0.0a
	Control	1.28 ± 0.0a	1.0 ± 0.1b	0.68 ± 0.0c	0.83 ± 0.1b	0.53 ± 0.0c	0.88 ± 0.0b	0.34 ± 0.0b	0.87 ± 0.0a	0.25 ± 0.0c
	Significance	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Norepinephrine	Olive Blackwater	0.31 ± 0.0b	0.4 ± 0.0 ns	0.59 ± 0.0 ns	2.08 ± 0.2 ns	2.9 ± 0.6 ns	10.23 ± 4.2 ns	9.08 ± 1.0b	12 ± 5.9a	0.17 ± 0.0 ns
	Antep Radish	0.32 ± 0.0b	0.5 ± 0.1	1.31 ± 0.8	2.87 ± 0.2	2.9 ± 0.4	10.48 ± 2.1	13.51 ± 2.6a	0.2 ± 0.0b	0.22 ± 0.0
	Broccoli	0.34 ± 0.0ab	0.5 ± 0.0	2.46 ± 0.6	2.23 ± 0.4	3.0 ± 0.2	9.64 ± 0.8	13.37 ± 1.8a	0.2 ± 0.0b	0.22 ± 0.0
	Control	0.38 ± 0.0a	0.6 ± 0.0	2.21 ± 0.9	1.96 ± 0.2	4.4 ± 0.5	13.3 ± 0.9	13.76 ± 0.8a	0.2 ± 0.0b	0.19 ± 0.0
	Significance	0.054	0.293	0.267	0.164	0.112	0.726	0.038	0.053	0.477

Significance level at $p \leq 0.05$ was detected for the applications and BAs factors (Duncan test). Different letters in columns represent statistical differences

showing the lowest concentration ($p = 0.024$). Spermidine concentration was significantly higher in the control (0.68 μg L^{-1}, $p = 0.012$), while spermine did not exhibit significant differences ($p = 0.309$). Histamine levels were markedly different across treatments ($p = 0.0001$), with the highest concentration in the olive blackwater fertilizer application and the control (both above 18.00 μg L^{-1}). Tryptamine also showed significantly differences in Chisel ($p = 0.0001$), peaking in the control group (13.96 μg L^{-1}). Serotonin and norepinephrine levels remained consistent across treatments ($p = 0.167$ and $p = 0.164$, respectively), and trimethylamine presented a near-significant difference ($p = 0.055$). Dopamine showed significant differences ($p = 0.0001$), with the highest concentration in broccoli (1.63 μg L^{-1}). Using the disc harrow soil management, histamine was most abundant in broccoli fertilizer application (18.52 μg L^{-1}, $p = 0.001$), followed by olive blackwater fertilizer application (13.78 μg L^{-1}) and the control (11.91 μg L^{-1}). Tryptamine peaked significantly in broccoli fertilizer application (15.67 μg L^{-1}, $p = 0.0001$) compared to olive blackwater, Antep radish and control. Putrescine and Cadaverine showed significantly differences highest in broccoli fertilizer application (8.62 μg L^{-1} for putrescine, $p = 0.001$; 6.41 μg L^{-1} for cadaverine, $p = 0.0001$) while control for Putrescine (5.73 μg L^{-1}) and Cadaverine (3.08 μg L^{-1}) were least in Disc Harrow. Agmatine and serotonin, norepinephrine, spermine, and spermidine did not demonstrate significant differences across treatments. Dopamine and trimethylamine showed mixed results, with dopamine highest when Antep radish fertilizer was applied (1.16 μg L^{-1}, $p = 0.000$) and trimethylamine in the control (2.1 μg L^{-1}, $p = 0.004$). In the no tillage soil management, cadaverine and agmatine exhibited notable variations, with the highest cadaverine concentration when Antep radish fertilizer was applied (6.31 μg L^{-1}, $p = 0.0001$) and agmatine with olive blackwater (2.83 μg L^{-1}, $p = 0.004$) and broccoli (2.62 μg L^{-1}). Spermine showed significant differences ($p = 0.006$), peaking in the control (0.19 μg L^{-1}), whereas spermidine did not show significant variations ($p = 0.348$). Histamine levels varied significantly, highest in Antep radish (17.66 μg L^{-1}, $p = 0.0001$). Tryptamine and serotonin also showed significant differences, with tryptamine most abundant with the olive blackwater fertilizer application (8.37 μg L^{-1}, $p = 0.000$) and serotonin in the control (0.98 μg L^{-1}, $p = 0.0001$). Dopamine exhibited significant variation ($p = 0.0001$), highest with the olive blackwater fertilizer application (1.09 μg L^{-1}).

In 2022, our analysis of BAs concentrations across various fertilizer applications showed notable variations (Table 1). For the chisel soil management, putrescine was most abundant in Antep radish (21.93 μg L^{-1}) and broccoli fertilizer application (20.91 μg L^{-1}) which was significantly higher than in the control group (12.3 μg L^{-1}). Cadaverine peaked in broccoli (5.82 μg L^{-1}), and agmatine was highest in broccoli fertilizer application (3.70 μg L^{-1}) and the control group (3.52 μg L^{-1}). Spermine showed the highest concentration with the broccoli (0.19 μg L^{-1}), the control (0.15 μg L^{-1}) and olive blackwater fertilizer application (0.13 μg L^{-1}), while spermidine and trimethylamine did not exhibit significant differences across treatments. Histamine levels were markedly higher with the broccoli fertilizer application (15.41 μg L^{-1}), and a notable variation across the groups. Tryptamine was significantly higher in broccoli (19.41 μg L^{-1}), and serotonin also peaked with the broccoli fertilizer application (0.85 μg L^{-1}). Dopamine was most abundant with the Antep radish fertilizer application (0.94 μg L^{-1}). The highest concentrations of norepinephrine were obtained from the control (13.76 μg L^{-1}), Antep radish (13.51 μg L^{-1}) and broccoli fertilizer application (13.37 μg L^{-1}) ($p = 0.038$). Under the disc harrow soil management, putrescine again emerged as the most abundant BAs, with the highest levels in the control (32.82 μg L^{-1}) followed by Antep radish fertilizer (19.81 μg L^{-1}). Cadaverine was notably higher in Antep radish fertilizer (5.32 μg L^{-1}) compared to the control (1.34 μg L^{-1}) and other fertilizer treatments. Spermine showed the highest concentration in broccoli fertilizer application (0.36 μg L^{-1}), while spermidine and agmatine did not exhibit significant differences across treatments. Tryptamine and histamine showed the highest concentrations in Antep radish fertilizer application, with significant values. Serotonin peaked in broccoli fertilizer application (0.60 μg L^{-1}), and dopamine was highest in the control (0.87 μg L^{-1}) and olive blackwater fertilizer application (0.85 μg L^{-1}). Trimethylamine and norepinephrine varied, with the highest concentration in olive blackwater fertilizer application (2.95 μg L^{-1} and 12 μg L^{-1}, respectively)). In the no tillage soil management, putrescine was most abundant in the control (116.83 μg L^{-1}), followed by broccoli fertilizer application (89.00 μg L^{-1}). Berry cadaverine levels were high with both the control vines and the olive blackwater fertilizer application (4.44 μg L^{-1} and 4.41 μg L^{-1}, respectively). Histamine showed the highest concentrations with the control (13.18 μg L^{-1}) and olive blackwater fertilizer application (12.42 μg L^{-1}). Spermidine peaked in the control (0.23 μg L^{-1}) and broccoli fertilizer application (0.16 μg L^{-1}), while spermine was highest with the Antep radish fertilizer application (0.40 μg L^{-1}). Tryptamine showed the highest levels in the control (19.28 μg L^{-1}), while dopamine was most abundant when broccoli fertilizer was applied (1.01 μg L^{-1}). The highest concentration of serotonin was obtained from Antep radish fertilizer

application (0.65 μg L^{-1}) ($p = 0.000$). There were no significant differences between treatments in agmatine, trimethylamine and norepinephrine values.

Putrescine has showed significant differences for all soil management treatments and years, except Antep radish fertilizer applications in 2021. No tillage soil management with Antep radish and when olive blackwater fertilizer was applied, and chizel soil management in the control (22 μg L^{-1}) had highest values in 2020, while it was found that treatments disc harrow in olive blackwater, Antep radish and broccoli fertilizer applications and chizel in the control in 2021 along with the no tillage and disc harrow soil management the control, and chizel in broccoli and Antep radish had showed highest values significant in 2022. Berry cadaverine concentrations for all soil management treatments and all fertilization applications were significant for all years except for 2020. No tillage with Antep radish (3 μg L^{-1}), broccoli (2 μg L^{-1}) fertilizers and the control (4 μg L^{-1}) had highest values in soil managements, however, disc harrow in olive blackwater (3.5 μg L^{-1}) fertilizer application soil management had significantly differences among fertilizers in 2020. There was significant differences in soil managements in 2021. For example, no tillage soil management with Antep radish (6.1 μg L^{-1}), disc harrow soil management with broccoli (6.2 μg L^{-1}), chizel soil management for the control (7 μg L^{-1}) and olive blackwater (6.5 μg L^{-1}) had greater values in 2021. There was the same senerio in 2022, where disc harrow and chizel soil management had significant values in Antep radish (5.1 μg L^{-1}) and broccoli (5.2 μg L^{-1}), expect in 2022. However, no tillage soil management was significant for the control (4.2 μg L^{-1}) vines and when olive blackwater fertilizer (4.2 μg L^{-1}) was applied. The agmatine concentration was the highest with no tillage soil management when Antep radish (5.9 μg L^{-1}) was applied in 2020, while disc harrow soil management was the treatment with higher value in the control (4.2.6 μg L^{-1}). In 2021, chizel and disc harrow were displayed greater values in the control and Antep radish fertilizer applications, while disc harrow and no tillage soil management was greatest in olive blackwater fertilizer application and chizel and no tillage soil management had highest values in broccoli fertilizer application. Disc harrow soil management was demonstrated significant values in Antep radish (4.1 μg L^{-1}) and olive blackwater (4.0 μg L^{-1}) fertilizer applications in 2022, althouht chizel soil management had highest values in the control and broccoli fertilizer application with 4.1 μg L^{-1}. In 2020, spermine did not differ between fertilizers in all three tillage methods. In spermidine, disc harrow gave the best result in control and Antep radish fertilizer application in 2020, while chizel soil management had the highest values in broccoli fertilizer application. In 2021, chizel soil management had the highest values in the control treatment, while in 2022, no-tillage management gave the best result in the control and broccoli fertilizer treatment. Histamine has greatest values in chizel and no tillage soil management in control and Antep radish fertilizer application in 2020. On the other hand, in 2021, the highest values were obtained from the chizel tillage method in the control and olive blackwater fertilizer application, while no-tillage fertilization in Antep radish and disc harrow in broccoli were obtained from the tillage method. In 2022, while no tillage soil management had significant values in control and olive blackwater fertilizer application, it was disc harrow soil management in Antep radish fertilizer and chizel in broccoli fertilizer application in 2022. In 2020, all tillage treatments had high averages in the control without fertilizer application, while chisel and no-tillage treatments gave the highest averages in Antep radish. In 2021, Chizel tillage in the control, disc harrow tillage in broccoli fertilizer and no-tillage in olive blackwater fertilizer application gave the highest averages, while it was disc harrow in Antep radish fertilizer application and no-tillage soil management in the control and chizel soil management in broccoli fertilizer in 2022. About serotonin, no-tillage in olive blackwater fertilizer application gave the highest averages in 2020. In 2022, chizel and disc harrow soil management had significant values in broccoli fertilizer application, while it was no-tillage soil management in Antep radish fertilizer application. There were no differences in broccoli, Antep radish and olive blackwater fertilizer applications in 2021; however, no tillage soil managements were found to be significant in control in 2021. For trimethylamine, in 2020, disc harrow tillage in olive blackwater, no-tillage treatment in the control and broccoli fertilizer application, and disc harrow and no-tillage treatment in Antep radish gave the highest averages, while it was chizel soil management in olive blackwater and disc harrow soil management in the control, in 2021. In 2022, disc harrow soil management has greatest values in olive blackwater fertilizer, while chizel soil management was significant in the control and Antep radish fertilizers. About dopamine, disc harrow and chizel soil managements were significant in broccoli fertilizer application, while it was no tillage soil management in Antep radish fertilizer application and chizel soil management in control, in 2020. In 2021, no tillage soil management had significant values in olive blackwater fertilizer application; however, chizel soil management was significant in broccoli and Antep radish fertilizer applications. Disc harrow soil management was significant in control and olive blackwater fertilizer application, while it was chizel soil management in Antep radish fertilizer and no tillage soil management in broccoli

fertilizer, in 2022. For norepinephrine, in 2020, chizel soil management was significant in the control and broccoli fertilizers, while in 2021, the differences between fertilizer treatments were not statistically significant. Finally, disc harrow soil management has highest values in olive blackwater fertilizer, while chizel soil management in the control, broccoli and Antep radish fertilizer had greatest values, in 2022 (Figs. 1 and 2).

Figure 2f depicts a hierarchical clustering heatmap illustrating the relative concentrations of BA in grape samples across soil managements, fertilizer applications and years. BAs are clustered at the bottom of the heatmap, revealing similarities and dissimilarities between them. Putrescine, histamine and tryptamine emerge as closely related, indicating similar concentration patterns across samples. Conversely, compounds like spermine and spermidine exhibit lower concentrations in these same samples, as shown by the deep blue color. Grape samples, labeled with soil managements, fertilizer applications and years, are vertically clustered on the right. It shows that the year 2022 was strongly correlated among soil management and fertilizer applications, while 2021 and 2020 were separated in the heatmap depending on the amount of the BA. Figure 3A–C presents PCA biplots of grape berries to visually represent relationships and variances among various BA components, including years (A), soil managements (B) and fertilizer applications (C). The most effected variables which oversaw the feasible grouping of the samples were recognized by the plots. The biplot graph explains 49.3% of the total variance. In Fig. 3A, where the trial years are shown while the red dots representing the year 2020 are close to each other, indicating that the practices for this year have similar values; the blue dots representing the year 2022 show a very wide distribution, indicating that the differences

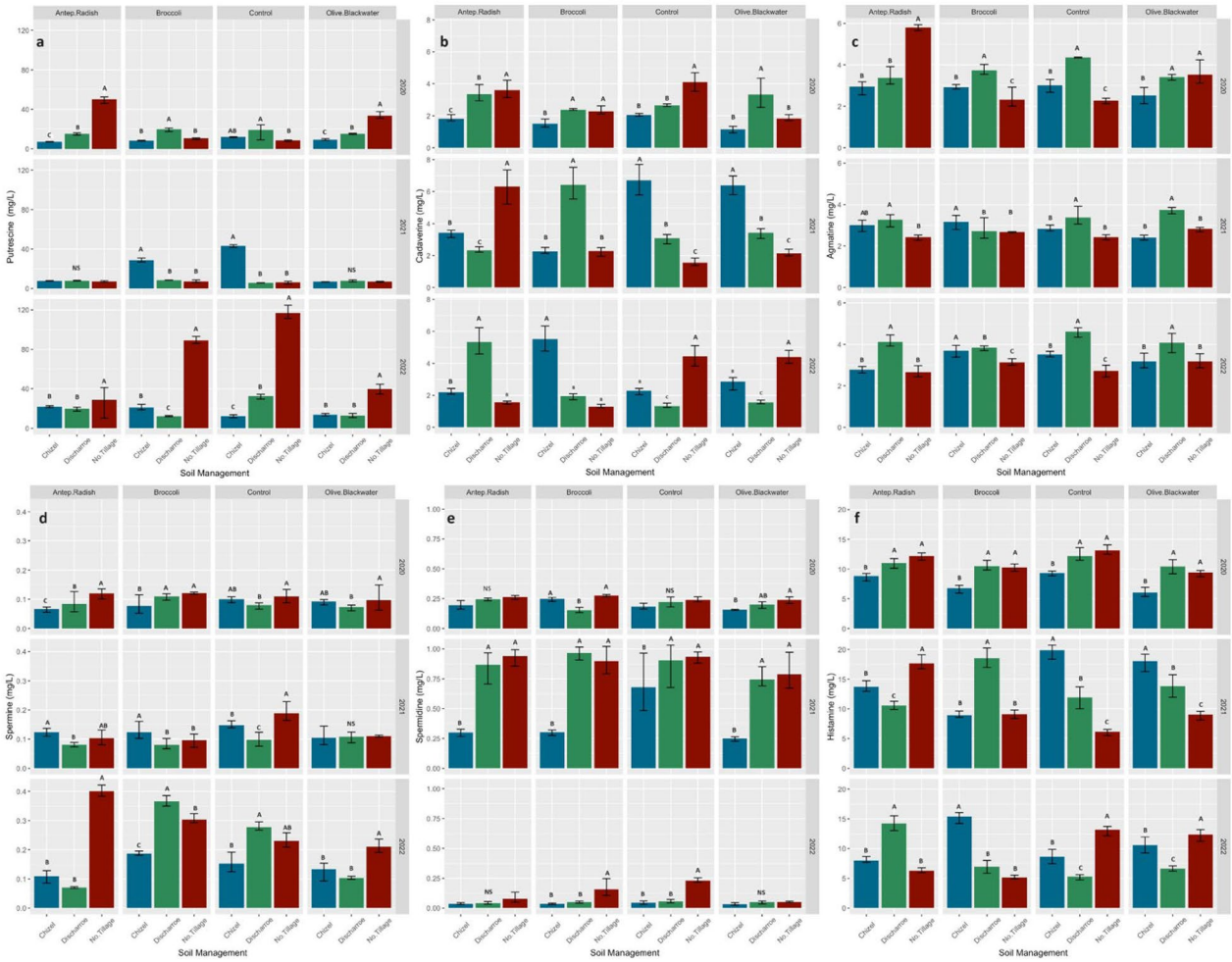


Fig. 1 Effect of soil management on BAs contents (μg·L^{-1}) in 'Royal' grape cultivars depending on fertilizer application and years of research. Significance level at $p \leq 0.05$ was detected for the applications and stress factors (Duncan test). Different letters on bars represent statistical differences (**a**: Putrescine, **b**: Cadaverine, **c**: Agmatine, **d**: Spermine, **e**: Spermidine, **f**: Histamine)

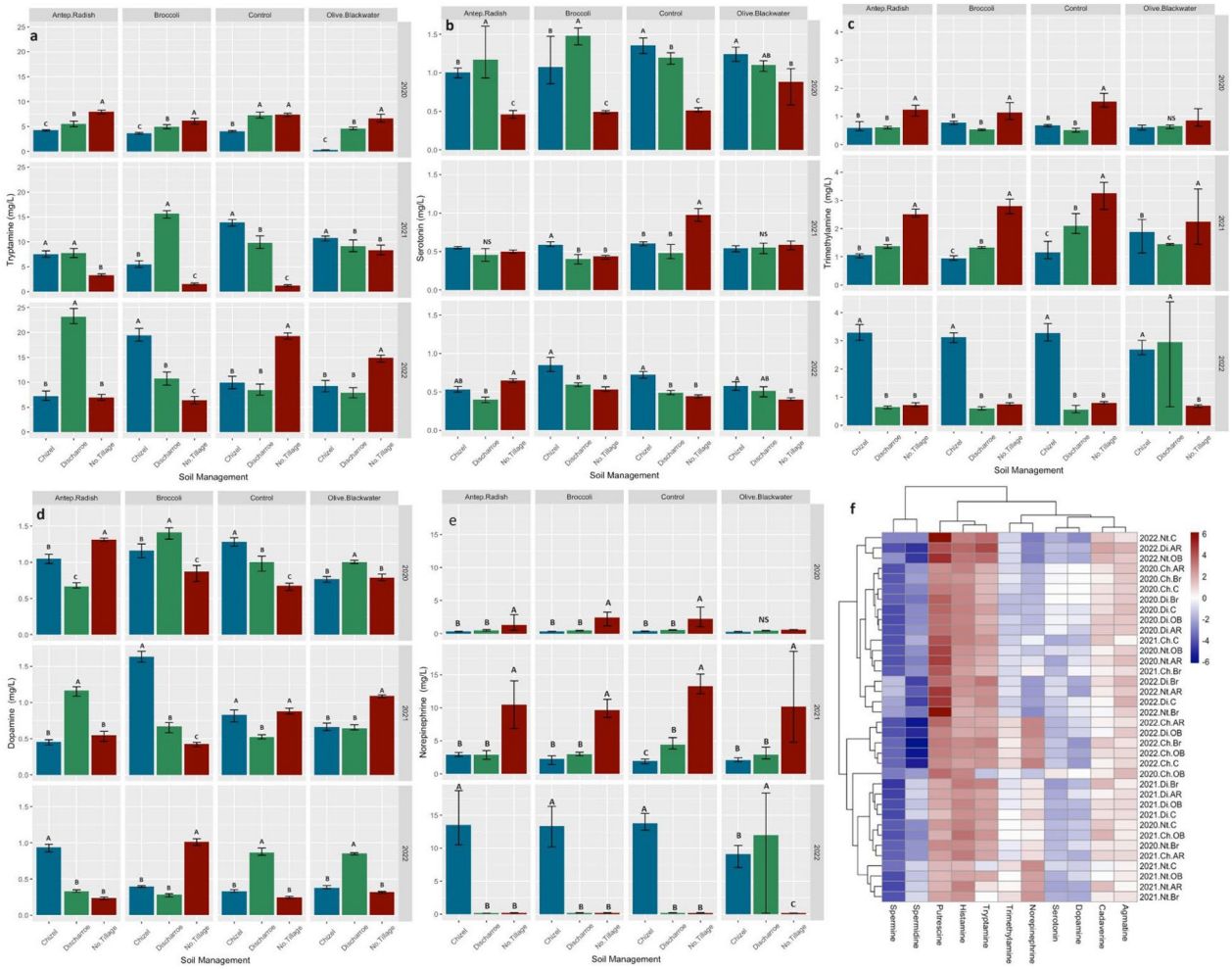


Fig. 2 Effect of soil management on BAs contents (μg·L^{-1}) in 'Royal' grape cultivars depending on fertilizer application and years of research and a hierarchical clustering heatmap demonstrating the relative concentrations of BAs. Significance level at $p \leq 0.05$ was detected for the applications and stress factors (Duncan test). Different letters on bars represent statistical differences (**a**: Tryptamine, **b**: Serotonin, **c**: Trimethylamine, **d**: Dopamine, **e**: Norepinephrine, **f**: hierarchical clustering heatmap)

between the practices for this year are high. On the other hand, the green dots representing the year 2021 showed a distribution between 2020 and 2022. When we look at the relationships between the variables of the years and the BAs, it is seen that 2020 has a higher effect on serotonin and dopamine; 2021 has a higher effect on cadaverine, histamine, spermidine, trimethylamine and norepinephrine; and 2022 has a higher effect on putrescine, spermine, agmatine and tryptamine content. However, all three circles intersect to a large extent. These results suggest that experimental years have a similar effect on the BA content of grape berries. In Fig. 3B, where tillage methods are shown, the red circle representing the chizel tillage method is distributed along the horizontal axis of PC1. This shows that the chizel method provides the greatest contribution to serotonin, dopamine, cadaverine and histamine content. On the other hand, the no-tillage method was widely distributed along the vertical axis of PC2, indicating that the method had a greater effect on putrescine, trimethylamine and norepinephrine content than the other tillage methods. The disc harrow method showed a distribution between the other two methods. However, the blue circle almost covers the green circle and the green circle almost covers the red circle. This shows that chizel tillage has the lowest contribution to the total variance, while no-tillage represents a significant portion of the total variance. In Fig. 3C, where fertilization treatments are shown, red dots represent the Antep radish fertilizer, green dots represent broccoli, purple dots represent olive blackwater and blue dots represent control groups. However, all four circles in the graph overlap each other to a great extent. This shows that the

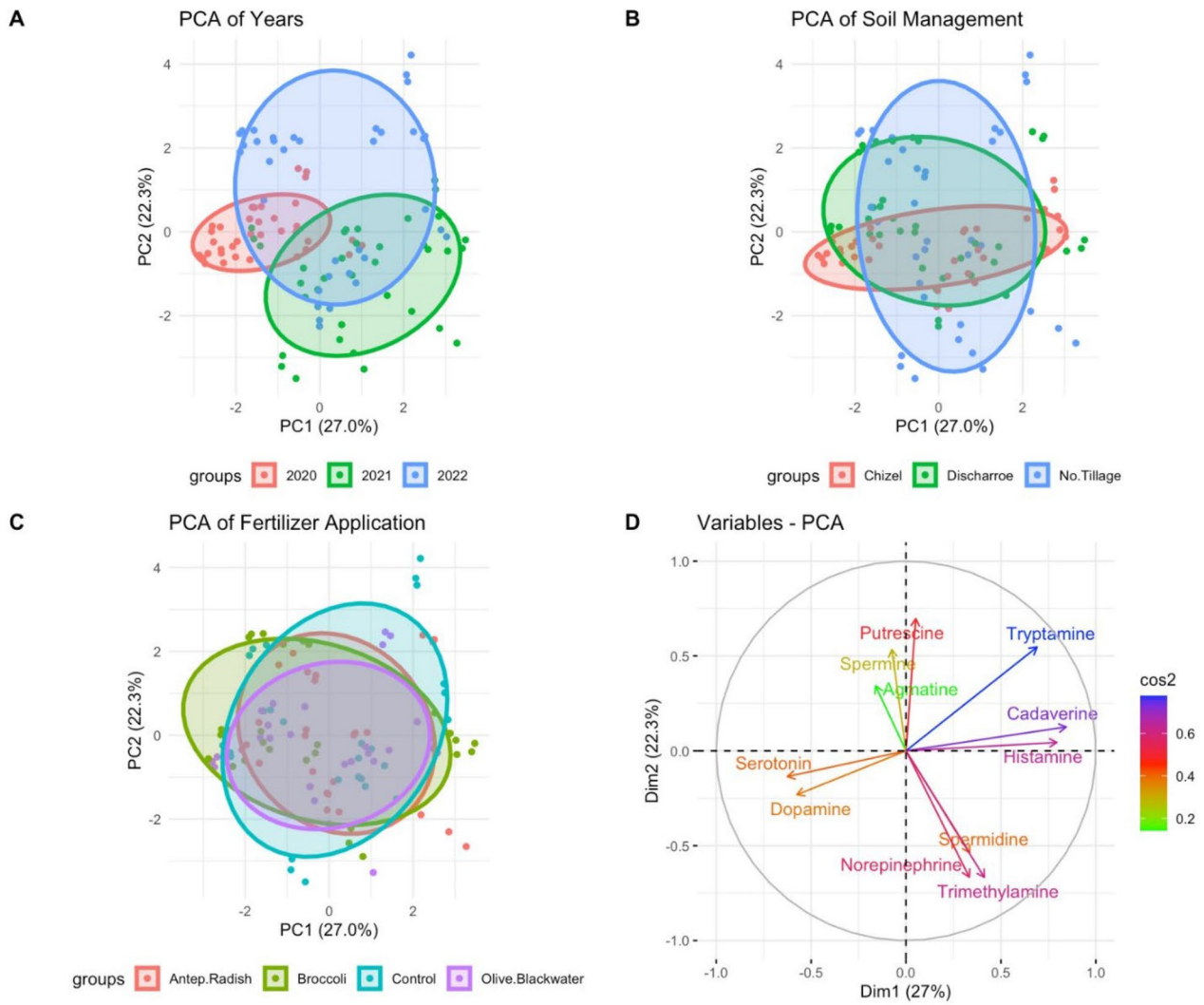


Fig. 3 PCAs biplot of berries colored by research years (**A**), soil managements (**B**), fertilizer applications (**C**) and BAs (**D**) are demonstrated

fertilization methods used are correlated with each other and have similar variance structures. According to the Biplot in Fig. 3D, the tryptamine, cadaverine and histamine contents loading on the positive axis of the first principal component (Dim1), which explains 27% of the total variance, showed a strong positive correlation with each other, while the serotonin and dopamine contents loading on the negative axis of the same component showed a high negative correlation. The second component (Dim2), which explained 22.3% of the total variation, showed a strong positive correlation with putrescine, spermine and agmatine on the positive axis, and a negative correlation with spermidine, trimethylamine and norepinephrine on the negative axis. These patterns provide valuable insights into the distribution of BA in grape samples and their relationships.

Discussion

Our 2020 findings reveal significant differences in BAs concentrations, highlighting the intricate relationship between various soil management and fertilizer applications and the chemical composition of grapes (Table 1). The variation in BAs concentrations due to different fertilizer applications and soil management suggests that vineyard management practices could significantly influence the BAs content in grapes, which is crucial for berry quality and the vines' stress responses. These results align with previous research demonstrating the significant impact of agricultural practices on the chemical composition of crops, including grapes [20]. Based on the 2020 results, the elevated concentrations of BAs such as putrescine, cadaverine, histamine, tryptamine, and dopamine in the control group

across various soil managements corroborate findings by Pérez-Álvarez et al. [21], who also found that standard fertilization regimes could effectively increase BAs levels in crops. This similarity suggests a potentially universal response of plants to standard fertilization practices, irrespective of the crop type or soil management approach employed. The notable increase in putrescine, cadaverine, agmatine, histamine, tryptamine, trimethylamine and dopamine concentrations under the no tillage soil management, particularly with Antep radish fertilizer application, indicates a potentially pivotal synergy between specific crops and soil management practices in optimizing BAs content. This observation suggests that the no tillage soil management may create a conducive environment for BAs synthesis, possibly due to its impact on soil health, including enhanced microbial activity and improved nutrient cycling, which are crucial for the biosynthesis of BAs in plants [22]. In contrast, the disc harrow soil management's role in influencing BAs concentrations, though not consistently yielding the highest levels, shows its capacity to induce significant variability in BAs such as agmatine, tryptamine, and dopamine across different crops. This variability could be attributed to the soil management's impact on soil aeration and disturbance, which might affect root growth patterns, soil microbial communities, and the availability of precursors necessary for BAs synthesis [23]. Given the absence of direct studies on the subject, we can hypothesize that the observed differences in BAs concentrations between the no tillage and disc harrow soil managements, and among various crops, might stem from the intricate interactions between soil physical properties, microbial ecology, and plant physiology. The no tillage soil management, by minimizing soil disturbance, could favor the establishment of a stable and diverse microbial ecosystem that supports the synthesis of specific BAs. Meanwhile, the physical soil disruption caused by the disc harrow soil management might transiently alter microbial communities and nutrient availability, leading to variable BAs synthesis outcomes. These assumptions are grounded in the broader understanding of soil–plant interactions, where soil management practices are known to significantly impact plant growth, health, and biochemical composition through changes in soil structure, moisture retention, and microbial dynamics [24]. Given the absence of direct studies on the subject, we can hypothesize that the observed differences in BAs concentrations between the no tillage and disc harrow soil managements, and among various crops, might stem from the intricate interactions between soil physical properties, microbial ecology, and plant physiology. As highlighted by Duchene et al. [25], the no tillage soil management, by minimizing soil disturbance, could favor the establishment of a stable and diverse microbial ecosystem that supports the synthesis of specific BAs. This perspective is supported by the notion that less disturbed soils tend to exhibit enhanced microbial diversity, which plays a pivotal role in nutrient cycling and plant health [26]. Meanwhile, the physical soil disruption caused by the disc harrow soil management might transiently alter microbial communities and nutrient availability, leading to variable BAs synthesis outcomes, as suggested by Nogales et al. [27], who noted that soil tillage practices significantly influence root zone environments and microbial assemblages. These dynamics, in turn, impact the availability of precursors required for BAs synthesis. These assumptions are grounded in the broader understanding of soil–plant interactions, where soil management practices are known to significantly impact plant growth, health, and biochemical composition through changes in soil structure, moisture retention, and microbial dynamics, a concept widely accepted in the literature on agroecology and plant science [28, 29].

Our findings from 2021 to 2022 reveal a nuanced understanding of how different fertilizer applications such as olive blackwater, Antep radish, broccoli fertilizer applications, and the control affect BAs concentrations in 'Royal' grape cultivar (Table 1). We observe that the soil management of fertilizer application can significantly influence the accumulation of BAs, with each soil management showcasing a unique pattern of BAs concentrations across 'Royal' grape cultivar. This aligns with existing literature that suggests agricultural practices, including fertilizer application, play a crucial role in the biochemical pathways that lead to BAs synthesis in plants [26, 30]. We assume that the distinct BAs concentration patterns observed under each fertilizer application are indicative of the complex interactions between the soil microbiome, plant physiology, and the specific characteristics of the fertilizer applications. For example, chisel soil management, which involves tillage, might aerate the soil more effectively, thereby influencing the microbial decomposition processes that affect BAs production. This hypothesis is supported by the high levels of putrescine and cadaverine observed in the control group and olive blackwater fertilizer application, in the chisel soil management in 2021, suggesting that soil aeration and disturbance may enhance the conditions favorable to produce these amines. Our findings also highlight that specific crop, like broccoli fertilizer applications, consistently show higher levels of several BAs across both years and soil managements, suggesting a crop-specific tendency towards BAs accumulation. This is consistent with

previous studies that have indicated that genetic factors and the inherent metabolic pathways of different plant species significantly influence BAs accumulation [31]. For instance, the consistent accumulation of histamine and tryptamine in broccoli fertilizer application, regardless of the tillage applied, could be attributed to the plant's specific metabolic pathways that favor the synthesis of these amines. Comparing our results with the literature [32], we find that the variability in BAs concentrations across different treatments and crops is a common theme. However, our study contributes new insights into how these variations manifest under different fertilizer applications. The literature on the impact of these applications on BAs concentrations, especially in crops like the 'Royal' grape cultivar mentioned hypothetically, is limited. Therefore, our research fills a gap by providing detailed data on how such practices affect BAs concentrations in other crops, offering a basis for further exploration in grapes and similar crops. Given the limited studies on this subject, we hypothesize that environmental factors, such as soil type, moisture content, and temperature, combined with the specific characteristics of each fertilizer application, contribute to the observed BAs concentration patterns. These environmental conditions, alongside the physiological and genetic predispositions of each crop, likely play a critical role in influencing BAs synthesis and accumulation.

Considering the complex patterns observed regarding the impact of different soil management treatments (chisel, disc harrow, and no tillage) on BAs concentrations in the 'Royal' grape cultivar over multiple years, our delved into how these agricultural practices shape the metabolic landscapes of berries (Figs. 1, 2). This nuanced interplay between soil management techniques and the resultant BAs concentrations indicated the intricate dynamics at berries. Our hypothesis, pointing towards the specific influence of soil management on putrescine accumulation, is informed by significant differences noted across almost all treatments and years. This hypothesis aligns with the literature suggesting that putrescine's biosynthesis and accumulation are highly responsive to changes in agricultural practices, potentially reflecting alterations in soil aeration, microbial activity, or nutrient availability [21, 22]. Continuing this thread, the consistent significance of cadaverine levels across all soil management treatments and years underpins our assumption that cadaverine's presence may serve as an indicator of soil and crop health, resonating with findings from Dijkstra et al. [23] and Das et al. [24], who documented similar sensitivity to agricultural practices. The variable responses of agmatine and spermine to different soil management practices further our hypothesis that these biogenic amines might be influenced by specific aspects of the soil environment or crop genotype responses, a notion compatible with the observations made by Guo et al. [32] and Gupta et al. [26]. The notable impact of no tillage treatments on histamine and tryptamine concentrations, in particular, supports our hypothesis regarding the potential benefits of reduced soil disturbance on BAs accumulation. This is in line with the broader discourse on sustainable agriculture practices as discussed by La Torre et al. [33], highlighting the complex biological processes at play in BAs metabolism in response to varying agricultural interventions. Our findings on the varied significant levels of serotonin, trimethylamine, and dopamine across different treatments and years lead us to hypothesize that these variations are influenced by a confluence of factors, including soil management practices, the specific crop being cultivated, and possibly, the environmental conditions of each growing season. This perspective is supported by literature, particularly the work of Rienth et al. [34], which explores the multifaceted influences on plant metabolic profiles. Given the limited number of studies on the impact of soil management on BAs concentrations, especially concerning crops like the 'Royal' grape cultivar, our research adds a valuable layer to the existing body of knowledge. This contribution is particularly significant in the context of developing sustainable and effective agricultural practices, a goal that our findings support, and the literature corroborates. Notably, the organic fertilizations applied to Antep radish and broccoli fertilizer applications, and the insights gained from studying olive blackwater fertilizer application, provided invaluable data for enhancing grape quality and health, underpinning the importance of tailored agricultural strategies in viticulture.

The elucidation of BAs concentration patterns in grape samples through hierarchical clustering Heatmap and PCA biplots in Figs. 2f, 3A–D respectively, indicated the intricate relationships between soil management, fertilizer applications, and the metabolic profiles of berries of 'Royal' grape over multiple years. Our findings, directed by the observed clustering and PCA results, posits that specific BAs such as putrescine, histamine, and tryptamine share similar biosynthetic pathways or environmental stimuli responses, which are influenced by the agricultural practices employed. This result is compatible with existing literature that links BAs synthesis in plants to stress responses and soil management practices [32]. The heatmap analysis from our study on the 'Royal' grape cultivar provided a nuanced view of how different soil management strategies and fertilizer applications impact BAs concentrations. The clustering of putrescine, histamine, and tryptamine, indicative of their similar concentration patterns across various samples, suggests a specific regulatory mechanism at play within the grape

cultivar's metabolic response to environmental and agricultural inputs. This pattern contrasts with the notably lower concentrations of spermine and spermidine, showing a possible differential regulation of BAs metabolism that might be influenced by the cultivar's genetic makeup, soil conditions, or the type of fertilizer used. Our results support the hypothesis that agricultural practices exert a selective impact on BAs synthesis, with certain BAs potentially acting as biomarkers for the grape's physiological stress or nutritional status. This is in line with the literature, such as the findings by Oliveri et al. [35], which suggest that variations in BAs concentrations can reflect the plant's response to its environment. Specifically, the increased levels of putrescine, histamine, and tryptamine might indicate a stress response or a particular nutritional need that varies depending on the soil management and fertilizer application. Furthermore, the PCA biplots highlighting the distinct grouping of samples based on years, soil managements, and fertilizer applications corroborate our assumption that these factors significantly influence BAs profiles. The distribution of BAs on different axes of the principal components, especially tryptamine, cadaverine, histamine, putrescine, spermine, and agmatine loaded on the positive axes of the principal components; spermidine, trimethylamine, norepinephrine, dopamine, and serotonin loaded on the positive axes of the principal components, providing visual confirmation of our hypothesis. This distribution pattern suggests inherent variations in metabolic responses to external agronomic interventions, as supported by Zee et al. [36], who found similar variations in BAs concentrations in response to different agricultural practices. The observed correlation between certain BAs and specific agricultural years, soil management techniques, and fertilizer applications through hierarchical clustering and PCA further supports our findings, suggesting a nuanced interplay between these factors and BAs metabolism. This correlation, indicative of the complex influence of environmental and management factors on BA synthesis, aligns with our hypothesis and is substantiated by literature that emphasizes the role of environmental stressors and agricultural practices in modulating BAs concentrations in grapes [29, 33, 35].

Conclusion

Our findings from this three-year study provide pioneering insights into the impact of various soil management practices and fertilizer applications on the BAs content within the 'Royal' grape cultivar. These pioneering findings, representing the first comprehensive analysis of BAs concentrations in grapes under different organic viticulture practices (Antep radish, broccoli, olive blackwater fertilizer application and chisel, disc harrow, no tillage soil management practices), hold paramount importance for advancing the scientific understanding and sustainable management of organic grape cultivation. Our results revealed significant fluctuations in BAs concentrations, notably putrescine, cadaverine, histamine, tryptamine, and dopamine, contingent upon the applications of soil management (chisel, disc harrow, no tillage) and the type of fertilizer applied (control, Antep radish, broccoli, olive blackwater). Critical observation was the consistently higher BAs concentrations under the no tillage soil management and the control, highlighting the intricate relationship between soil disturbance levels and BAs accumulation in grape crops. Our investigation indicates the significance of these findings for organic agriculture, emphasizing the potential for specific organic fertilizers and reduced tillage practices to influence the metabolic profiles of grapes. This knowledge paves the way for organic viticulturists to tailor farming practices that optimize the nutritional and biochemical properties of the grape, enhancing both produce quality and vineyard sustainability. Looking forward, our study highlights the need for further research to elucidate the mechanistic links between soil management practices, fertilizer types, and BAs synthesis in grapes. Expanding this research to encompass more grape varieties and broader geographical locations could affirm the universality of our findings. Ultimately, our research lays the foundation for innovative, evidence-based practices in organic viticulture aimed at achieving optimal grape quality and environmental sustainability.

Author contributions

Investigation, methodology and conceptualization, OK, FA, FK, TY. Draft preparation, OK, HSH, and HHV. Software and formal analysis, OK and TY Writing-original and writing-review and editing OK visualization FA, HHV, MT, and OK. All authors have read and agreed to the published version of the manuscript.

Funding

Not applicable.

Availability of data and materials

Correspondence and requests for materials should be addressed to O.K and F.A.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Received: 27 February 2024 Accepted: 7 March 2024
Published online: 12 March 2024

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