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SEMİH TANGOLAR

SIMONE PRIORI

SERPİL TANGOLAR

METİN TURAN

ERHAN AKÇA

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Local organic and inorganic amendments to improve table grape production in Mediterranean vineyards of southern Türkiye

Semih TANGOLAR[1,*], **Simone PRIORI**[2], **Serpil TANGOLAR**[1], **Metin TURAN**[3], **Erhan AKÇA**[4]

[1]Department of Horticulture, Faculty of Agriculture, Çukurova University, Adana, Turkiye
[2]Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy
[3]Department of Agricultural Trade and Management, Faculty of Economics and Administrative Sciences, Yeditepe University, İstanbul, Turkiye
[4]Vocational School of Technical Sciences, Adıyaman University, Adıyaman, Turkiye

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Abstract: In the Mediterranean Region, calcareous soils are generally considered to be of low fertility. The use of large amounts of chemicals and irrigation to compensate for low productivity reduces the producer's net profit and degrades physical and chemical properties of the soils. As a result, the selection of adaptable genotypes is important in agriculturally limited lands. Grapevine is one of the leading crops in the Mediterranean Basin due to its high tolerance to abiotic conditions. However, the fact that the grapevine grows in extreme soils does not mean that such crop does not respond to a good nutritional program, especially to the natural amendments. In this research, natural organic and inorganic soil amendments were tested on "Prima" grape cultivar grown in calcareous soils of the Mediterranean Basin, with the aim of developing environmentally friendly and cost-effective nutrition recommendations. Basaltic pumice (P), cereal straw (S), commercial dried compost (DC), grapevine pruning residue (PR), and farm cattle manure (FM) were used alone or in different combinations. The combination of P+PR+FM produced the best results for yield, cluster weight, and berry weight. However, it also resulted in the highest acidity and the lowest ripening index (total soluble solids/acidity). The highest ripening index values were obtained from S+FM and PR+FM applications. The treatments did not have different effects on total sugars, organic acids, and malvidin-3 glucoside anthocyanin, a relatively abundant component in *V. vinifera* grapes. However, P+PR+FM resulted in significantly higher levels of ascorbate peroxidase and total amino acids. The highest total anthocyanins were shown by DC, P+DC, and P+PR+FM. The results of the work demonstrated that the uses of different mixtures of natural and readily available materials have positive and diverse effects on grape quantity and quality in Mediterranean calcareous soils where vine is widely cultivated.

Key words: Amino acids, antioxidant enzymes, grapevine, soil amendments, soil management, vitamins

1. Introduction

Table grape is one of the most important fruit crops in the world, with a global production of 27.3 million tons, around 36% of the total production of grape, including wine grapes and raisins[1],[2].

Vineyard efficiency has been continuously improving in recent years, as evidenced by the increase in global grape production despite the decline in global vineyard surface area[3]. This agricultural intensification must take into account the sustainable use of natural resources, such as soil, water, and fertilizers. Awareness of sustainable use of natural resources is even more important for soils with cropping limitations due to natural factors such as geology, morphology, and climate, as well as anthropic impacts and agricultural mismanagement. Çilek et al. (2020) reported that only a few places on the planet have soils without natural limitations for cultivation. In addition, one third of the world's soils have lost their

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[1] OIV (2019). International Organisation of Vine and Wine, Statistical Report on World Vitiviniculture [Online]. Website http://oiv.int/public/medias/6782/oiv-2019-statistical-report-on-world-vitiviniculture.pdf [Accessed 30 November 2022].

[2] FAOSTAT (2021). Food and agriculture data. The Food and Agriculture Organization [Online]. Website https://www.fao.org/faostat/en/#data/QCL. [Accessed 20 June 2023].

[3] OIV (2021) International Organisation of Vine and Wine, Activity Report [Online]. Website https://www.oiv.int/public/medias/8767/acitvityreporteng.pdf [Accessed 20 June 2023].

* Correspondence: tangolar@cu.edu.tr

productivity due to mismanagement practices (FAO and ITPS, 2015). Throughout history, humans have attempted to improve soil conditions on poorly agricultural lands by enhancing the physical and hydrological conditions of the soils through terracing, drainages, and irrigation, as well as improving chemical properties through fertilization and amendments. As a result, human landscapes, or anthroscapes, have been developed for sustainable agriculture (Akça et al., 2020). Kapur et al. (2019) stated that viticulture is important for food security in limited soil and water resources of the Mediterranean Basin, where table and wine grapes have been domesticated and evolved for thousands of years (Dong et al., 2023). Grapevine is one of the few plants that can thrive in a wide range of climates and soil types. As a result, it is found not only in the Mediterranean Basin but also on six different continents (Schultz, 2016), making it the most widely grown fruit on the planet. However, just because grapes are grown in different locations and under various conditions does not mean that they will not respond to optimal soil management and plant nutrition.

Costantini et al. (2018) reported that erosion is the major threat to grapevine production in Mediterranean Basin countries. In addition to erosion, nutrients other than N, P and K are not often provided to the soil or plants in grape cultivation, which has negative effects on yield and quality, especially on sites where low value-added grapes, such as table grapes, are cultivated (Tangolar et al., 2020). Although priming technology helps overcome adverse environmental conditions, such as salt stress (Montanaro et al., 2022), it has been observed that increasing the use of agricultural chemicals to address declining soil fertility in grape fields is often impractical due to chemical pollution, high costs, and market demand for organic crops (Mariani and Vastola, 2015). Another issue for grape production is the use of agrochemicals, together with nonconservative agronomic practices such as deep tillage and insufficient addition of organic matter to soils. These practices result in a decrease in soil organic carbon levels, which are already low in Mediterranean Basin soils (Francaviglia et al., 2012, 2018). Furthermore, grapes grown under rainfed conditions in many areas face additional costs, such as irrigation, due to reduced rainfall caused by climate change (Ramos and Mulligan, 2005; Masia et al., 2021; Yadav et al., 2022). Most Mediterranean vineyards are located on shallow and calcareous soils with low water retention and organic matter content, sometimes on slopes. These conditions render these soils highly fragile and scarcely resilient to erosion, nutrient deficiencies, and depletion of organic carbon (Akça et al., 2020).

In this sector, as in many other agricultural sectors, it is strategic to optimize the best practices of soil management and plant nutrition. These practices aim to increase both yield and quality while reducing costs, ultimately benefiting the economic interests of farmers. Additionally, they contribute to the maintenance or improvement of soil quality. Table grape producers have several options at their disposal to achieve these objectives (Cataldo et al., 2021). Intercropping and cover cropping in vineyards have been shown to improve soil quality and reduce erosion, with no negative effects on grape yield and quality (Gattullo et al., 2020; Warren Raffa et al., 2021). On the other hand, such management increases the evapotranspiration of the vineyard system, reducing the available water for grapevine. Therefore, in the Mediterranean climate, a scheduled drip irrigation system is required in most cases (Tarricone et al., 2020).

Another option for efficient and sustainable soil management in Mediterranean vineyards is the use of natural organic and inorganic amendments to improve soil and crop quality (Priori et al., 2020; Wallace and Terry, 2020; Cataldo et al., 2021).

One of the most commonly used amendments is composted organic material, consisting of manure, pruning, and other plant residues. This material has been used in agriculture and horticulture for years, and, more recently, has also found application in viticulture (Gaiotti et al., 2017; Martínez et al., 2018). The results of these studies demonstrated that the use of compost in viticulture increased soil biological activity, as well as plant nutrient availability and root development (Martínez et al., 2018; Wu et al., 2020). The addition of biochar to soil, with or without compost, has also been recently proposed to improve soil water and nutrient retention, water infiltration, and soil aeration, as well as microbiological activities (Schmidt et al., 2014).

In terms of inorganic amendments, pumice has been used in agriculture, and particularly in horticulture, for several purposes. Pumice lightens the soil, making tillage easier, improves soil aeration, increases water retention, and reduces negative effects of crusting and shrink-swelling of clays (Malekian et al., 2012). A recent study (Kong et al., 2021) reported positive effects of the use of pumice in soils as amendment to increase water retention and preventing soil salinity in arid environments.

Studies about the effects of the different organic and inorganic amendments on table grape yield and quality are few (Martínez et al., 2018; Tangolar et al., 2020) and did not test the synergistic effects of different amendments.

The aim of this work was to test various organic and inorganic materials, readily available at low cost, which can be used either individually or in combination to enhance grape yield and quality. This enhancement is assessed based on the presence of sugar, organic acid, polyphenols, antioxidant enzymes, vitamins, and amino acids. The study focuses on improving soils with limited physical (shallow) and chemical (low organic carbon, high $CaCO_3$) properties in southern Türkiye on Mediterranean coast.

2. Materials and methods

2.1. Study area

The study on the effects of various natural mineral and organic amendments on grape yield and quality was conducted over 2 years (2017 and 2018) at the Viticultural research unit of Çukurova University, Adana, Türkiye. The experimental vineyard is located at an altitude of 70 m above sea level, with coordinates 37°01′50″ N and 35°22′46″ E. The soil of the vineyard was classified as Haplic Calcisol (Loamic, Hypercalcic) according to the International classification system (IUSS-WRB Working Group, 2015) (Figure), with clay loamy texture, subalkaline pH (7.83), high calcium carbonate content (53.6%), low organic carbon stock (about 25 t C ha^{-1}, calculated from 1% organic matter at 20 cm with 1.25 $g.cm^{-3}$ bulk density). The climate of the site is classified as hot-summer Mediterranean climate (Csa) subclass, according to the Köppen climate classification system, with a mean annual temperature of 24 °C, mean relative humidity of 64%, and total precipitation of 205 mm during vegetative season (April to October)[4].

2.2. Plant material and treatments

The treatments were applied to a 4-year-old "Prima" (*Vitis vinifera* L.) cultivar grafted on "1103 Paulsen" rootstock. The vines were trained with the Guyot system (0.50 m high) at a density of 6666 plants per hectare, spaced at intervals of 1.0 m × 1.5 m, and oriented from south to north. No bunch and berry thinning, or growth regulator applications, were performed. Instead, 15 buds per vine were left during pruning in order to promote plant homogeneity.

Seven treatments were tested in the field to improve soil quality, as well as grape yield and quality for 2 years. Inorganic amendment, such as pumice (P) (Table 1), was used alone or in combination with organic amendments. Three treatments involved using organic amendments such as commercial dry compost (DC) (Table 2), mixture of grapevine pruning residues and farm cattle manure (PR+FM), mixture of cereal straw and farm manure (S+FM). A control treatment, with no amendments, was also tested (Tables 3 and 4).

Basaltic pumice (P) was obtained from Delihalil quarries located in Osmaniye town, 80 km east of the study site. Basaltic pumice is rich in plant nutrient sources such as Fe, Mg, K, P, and Ca with a bulk density of 0.9 g cm^{-3} and a porosity reaching 50% (Demir et al., 2005) with a diameter from 0.7 to 15 mm. Pruning residues (PR) were obtained from the neighbouring experimental vineyard farm of Çukurova University after pruning in January, along with cereal straw (S) and mature farm cattle manure (FM) from the university's animal husbandry facilities. Compost produced from farm manure, prunings, and straw underwent a six-month composting process each year. The compost was then applied to experimental plots in February 2017 and in January 2018 by incorporating to the 0–20 cm soil by hand-driven rotator. The outcomes of the applications were evaluated using samples collected in 2018.

2.3. Grape yield, cluster, and berry and juice analysis

In June 2018, when the total soluble solids reached 13-15 °Brix, indicating approximately full ripening of the berries, five clusters from each plot were randomly sampled (Winkler et al., 1974) and transported to the laboratory in a refrigerated box. The weight of the clusters (g) and the weight of the berries (g 100 $berry^{-1}$) were measured in the laboratory on the same day. The concentration of total soluble solids (TSS) (°Brix) was determined using a digital refractometer (Atago, Japan). The titratable acidity (TA) (g 100 mL^{-1} grape juice) was expressed as tartaric acid, and the pH was determined using a pH meter. The TSS/TA ratio was used to calculate the ripeness index. Grape yield (g $vine^{-1}$) was calculated by multiplying the number of grape bunches by the average bunch weight.

2.4. Phytochemical analysis

Berry samples weighing approximately 500 g were randomly selected from different plants of treatments as two replicates for phytochemical analysis. Samples were collected and stored at −20 °C until processing. Phytochemical analyses were performed at the Department of Genetic and Bio-Engineering, Faculty of Engineering, Yeditepe University, İstanbul.

2.4.1. Sugar analysis

The high-performance liquid chromatography-evaporative light scattering detection (HPLC-ELSD) method was used to determine soluble sugars (glucose, fructose, and sucrose) in berries (Ma et al., 2014; Zhu et al., 2018). All samples and standards were filtered through 0.45-μm Millex Millipore filters before introducing samples of 10 μL to the HPLC-ELSD. A solvent ratio was adjusted to 85 acetonitrile: 15 water (v/v) with a flow rate of 1 mL min^{-1}. The column and drift tube temperatures were set to 45 °C and 82 °C, respectively, and the nebulizer gas flow was set to 2 mL min^{-1}. Peaks were measured using HPLC grade sugar calibration standards (Sigma-Aldrich, Shanghai, China).

2.4.2. Organic acid analysis

Ten millilitres of deionized water was added to the berry sample (1 g), and the solution was homogenized using an

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[4] MGM (2022). Adana General Climate Data. General Directorate of Meteorology [Online]. Website https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler istatistik.aspx?k=A&m=ADANA [Accessed 24 January 2022].

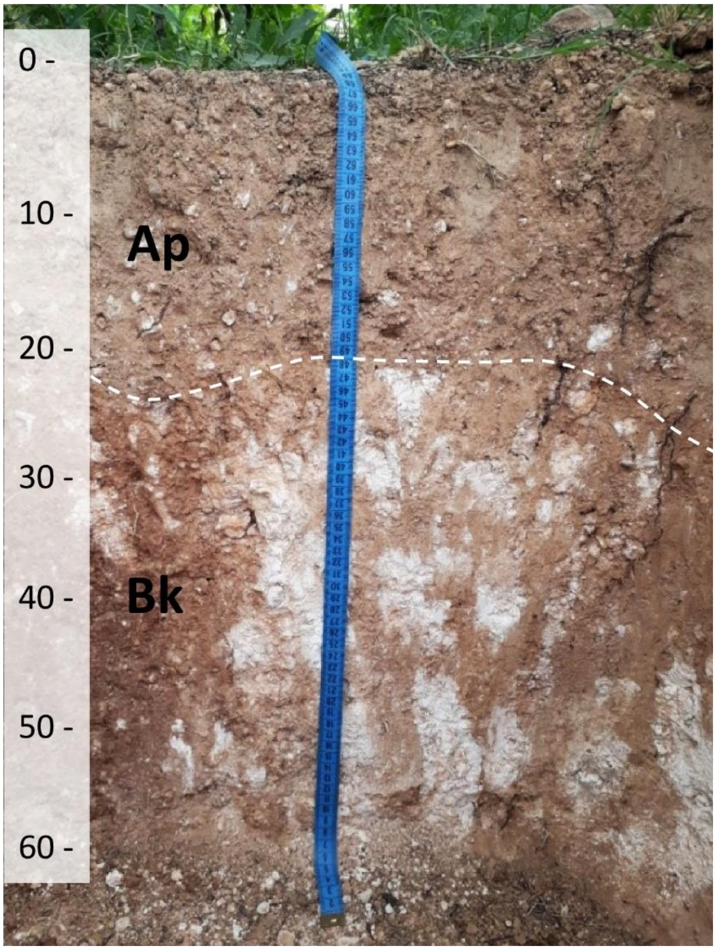

Figure. The soil profile within the experimental vineyard. Ap is the tilled horizon, whereas Bk is the subsurface calcic horizon (Bk), characterized by frequent white soft and hard concentrations of calcium carbonate. The soil is classified as Haplic Calcisol (Loamic, Hypercalcic) according to the FAO system (IUSS-WRB Working group, 2015).

Table 1. The chemical and physical properties of the basaltic pumice.

Properties	Values
SiO_2	46%
Al_2O_3	21%
Fe_2O_3	7%
CaO	11%
MgO	7%
$Na_2O_+ K_2O$	8%
Water retention	19.6%
Porosity	71.3%
pH	7.0–7.4
Bulk density	1–2 g cm^{-3}

Table 2. The chemical and physical properties of the dry compost.

Properties	Values
Total nitrogen	2%
Total P (P_2O_5)	2%
Water-soluble K_2O	2%
EC	0.95 dS m^{-1}
Organic matter	50%
Total humic + fulvic acids	10%
C/N	12.6
pH (1:1)	6.8–8.8

Table 3. Treatments and applied amounts of various amendments in different ratios.

Treatments	Ratio (V:V)	Applied amount (each year)
Control		No application
Pumice (P)	1	50 t ha^{-1}
Dry compost (DC)	1	4 t ha^{-1}
Pruning residue:Farm manure (PR+FM)	1:2	50 t ha^{-1}
Straw:Farm manure (S+FM)	1:2	50 t ha^{-1}
Pumice:Dry compost (P+DC)	1:2	50 t ha^{-1} + 4 t ha^{-1}
P+(S+FM)	-	50 t ha^{-1} + (50 t ha^{-1})
P+(PR+FM)	-	50 t ha^{-1} + (50 t ha^{-1})

Table 4. Macro- and micronutrients, pH, and electrical conductivity (EC) of the materials used in the study.

Materials [x]	Macroelements (mg 100 g^{-1})				Microelements (mg kg^{-1})			pH	EC (dSm^{-1})
	P	K	Ca	Mg	Fe	Mn	Zn		
Control	27.7 c[y]	403 cd	4234 ab	308 bc	7.56 bc	13.42 bcd	2.12 c	7.68	0.361 d
P	30.8 c	354 d	3911 d	202 d	5.50 d	11.34 d	2.26 c	7.72	0.335 d
DC	28.2 c	456 cd	4306 a	303 bc	6.58 cd	13.18cd	2.48 bc	7.76	0.383 cd
PR+FM	52.6 a	776 ab	3740 e	341 abc	8.40 ab	20.62 ab	2.73 ab	7.75	0.511 abc
S+FM	53.5 a	767 ab	3889 d	381 ab	8.19 ab	19.76 abc	2.98 a	7.75	0.638 a
P+DC	33.4 bc	479 c	4170 b	274 cd	5.86 d	13.29 bcd	2.24 c	7.66	0.449 bcd
P+(S+FM)	53.3 a	660 b	3598 f	288 cd	8.11 ab	21.57 a	2.78 ab	7.79	0.510 abc
P+(PR+FM)	43.8 ab	818 a	4044 c	399 a	9.24 a	23.10 a	3.01 a	7.69	0.561 ab
LSD 5%	11.5	114	120	86	1.32	7.33	0.38	NS	0.15
p-value	0.0001	<0.0001	<0.0001	0.0045	0.0002	0.0155	0.0005	0.494	0.0053

[x]: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, [y]: Means (n = 3) followed by different letters on the same column are significantly different according to the LSD test at $p \leq 0.05$. NS: Nonsignificant.

Ultra Turrax (IKA, T-25) for the organic acid analysis. After centrifugation at 1200 rpm for 50 min (Kitir et al., 2019), 1 mL of the centrifuged supernatant was filtered through a 0.45-µm Millex Millipore filter and then injected into HPLC (LC-10A HPLC Series, Shimadzu, Kyoto, Japan). The HPLC, equipped with a pump system and a UV/Vis detector (SPD-20A) monitored at 210 nm, was used for the analysis of organic acids. Identification and quantification of acids were done by injecting 20 µL of stock solution to separate different acids on a Supelcogel TMC-610H column (30 cm × 7.8 mm, i.e. Supelco, Bellefonte, PA, USA) by using 0.1% phosphoric acid as the mobile phase at a flow rate of 0.6 mL min^{-1} in the isocratic mode of the HPLC at room temperature (26 °C).

2.4.3. Amino acids

A fresh sample of 1 g was treated with 0.1N HCl, homogenized with Ultra Turrax (IKA-T 25), and incubated at 4 °C for 12 h. The supernatants were filtered through 0.22-µm filters (Millex Millipore) after centrifugation at 1200 rpm for 50 min. The supernatants were then transferred to a vial, and the amino acids were analysed with HPLC as described by Antoine et al. (1999) and Kitir et al. (2019). Measurements were taken on Zorbax Eclipse-AAA 4.6 × 150 mm, 3.5 µm columns (Agilent 1200 HPLC) were taken at 254 nm, and the amino acids were identified by comparison with standards of O-phthaldialdehyde (OPA), fluorenylmethyl chloroformate (FMOC), and 0.4 N borate. In the mobile phase chromatography system, the following solutions were used: Phase A: 40 mM NaH_2PO_4 (pH: 7.8) and Phase B: acetonitrile/methanol/water (45/45/10, v/v/v) solutions. The mobile phase flow rate through the system was 2 mL min^{-1} and the column temperature was 40 °C. After the 26-min derivation process in HPLC, aspartate, glutamate, arginine, serine, glutamine, histidine, glycine, asparagine, alanine, tyrosine, cysteine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine, and sarcosine were analysed.

A 500-mg frozen berry sample was crushed using liquid nitrogen, homogenized and extracted with 10 mL of 3-sulfosalicylic acid, which was then filtered through a Whatman filter paper (ø2) for proline measurement. In a test tube, 2 mL of filtrate was reacted with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid at 100 °C for 1 h, and then the reaction was stopped in an ice bath. This mixture was extracted with 4 mL of toluene, mixed vigorously with a test tube stirrer for 15–20 s and the concentrations of proline from the extracts were measured spectrophotometrically at 520 nm (Bates et al., 1973).

2.4.4. Antioxidant enzyme activity

Berries weighing 200 mg were homogenized in 5 mL of 100mM phosphate buffer (pH: 7.0) containing 1 percent (w/v) polyvinyl pyrrolidone (PVP) and all analyses were performed at 4 °C to determine superoxide dismutase (SOD), peroxidase (POD), glutathione S-transferase (GST), glucose-6-phosphate dehydrogenase (G6PD), ascorbate peroxidase (APX), glutathione reductase (GR), and catalase (CAT) activities. The homogenate was centrifuged at 1200 rpm for 15 min, and the supernatant fraction was tested for enzyme activity. The rate of hydrogen peroxide degradation was used to determine the CAT activity, as described by Abedi and Pakniyat (2010). The CAT activity was determined by a decrease in reaction mixture absorbance at 240 nm that was caused by adding H_2O_2. The reaction mixture contained 50 mM phosphate buffer (pH: 7.0), 10 mM H_2O_2 and 100 µL of extract. The activity was calculated using a 39.4 mM cm^{-1} oxidation extinction coefficient for H_2O_2. Peroxidase activity was measured at 436 nm to determine its ability to convert guaiacol into tetraguaiacol (Angelini et al., 1990). Superoxide dismutase activity was determined using Abedi and Pakniyat's (2010) method of measuring inhibition in the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. Total SOD activity was determined by monitoring the prevention of NBT chloride depletion. Under a 40W fluorescent lamp, 200 µL of the reaction mixture (50 mM phosphate buffer (pH: 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 63 mM NBT, 50 mM riboflavin, 13 mM methionine, and 50 µL of plant extract) were placed in a 96-well microplate. After 8 min of exposure to light, the absorbance at 560 nm was measured. A control reaction mixture, without illumination was also prepared and handled in the same manner. One unit of SOD activity was determined to be the amount of enzyme that inhibited the sNBT reduction by 50%.

2.4.5. Phenolic compounds

Phenolic compounds in plant samples were analysed as defined by Li et al. (2017) using an Agilent 1200 Series high-performance liquid chromatography coupled with an Agilent 6410 triple quadrupole mass spectrometer (HPLC-QqQ-MS/MS, Agilent Technologies, Santa Clara, CA, USA). Prior to injection into the HPLC, a 0.1-mL berry sample was filtered through a 0.45 µm polyethersulfone membrane. Phenolic compounds from the samples were separated on a Poroshell 120 EC-C18 column (4.6 × 150 mm, 2.7 µm) with a flow rate of 0.4 mL/min. (A) 0.1 percent v/v formic acid in water and (B) 0.1 percent v/v formic acid in acetonitrile: methanol (1:1, v/v) were used in the mobile phase. The gradient elution protocol was as follows: 10% B to 46% B from 0 to 28 min; 46% B to 10% B from 28 to 29 min; and 10% B isocratic from 29 to 34 min. The column was maintained at 55 °C during the gradient elution program. With a spray voltage of 4 kV, a gas temperature of 350 °C, and a nebulizer pressure of 35 psi, negative electrospray ionization was employed on QqQ-MS/MS. The temperature of the ion source was set to 150 °C. To identify phenolic compounds in the samples, a multiple reaction monitoring mode for the transition of the precursor to production was used. Procyanidin B1, procyanidin B2, procyanidin C1, (+)-catechin, (−)-epicatechin, (−)-epigallocatechin and gallocatechin, myricetin, myricetin-3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-glucuronide, dihydroquercetin, syringetin-3-O-glucoside, gallic acid, protocatechuic acid, caffeic acid, and 4- hydroxycinnamic acid were used as external standards for quantifying flavanols and phenolic acids.

2.4.6. Anthocyanins

Samples were ground into powders (approximately 0.2 g) in a mortar filled with liquid nitrogen. Following that, a slightly modified extraction method based on Butelli et al. (2008) was used. Two millilitres of 100% MeOH was used to extract the powder (Sigma, St. Louis, MI, USA). The powder/solvent mixture was kept at 4 °C for 12 h and shaken every 15 min for the first 2 h to avoid light exposure. After centrifugation at 2800 rpm for 30 min, the supernatant was filtered through a 0.22-µm membrane filter and analysed using an Agilent Technologies 1200 series HPLC. The mobile phase consisted of two solvents: Solvent-A, which was 87% water, 11% acetonitrile (ACN), and 2% acetic acid, and Solvent-B, which was 40% water, 58% ACN, and 2% acetic acid. At a flow rate of 1 mL min^{-1}, the gradient elution was as follows: 0 min 4% B, 20 min 20% B, 35 min 40 percent B, 40 min 60% B, 45 min 90% B, and 55 min 4% B. The detection wavelength was 520 nm, and the column oven temperature was set to 30 °C. Anthocyanin standards (petunidin-3-(*trans*-coumaroyl)-rutinoside-5-glucoside) of Anhui Biothun (Anhui, China) were used for the analyses. Purified anthocyanin extracts were dissolved in 100% MeOH before being analysed by mass spectrometry on a Q-TOF 5600 (Applied Biosystems, CA, USA) system in positive mode with m/z values ranging from 300 to 1000.

2.4.7. Vitamins

Vitamin A

Berry samples were ground for vitamin A (retinol) analysis. Subsequently, berry samples were extracted with a mixture of *n*-hexane and ethanol. Additionally, 1% BHT was added and kept in a dark environment for 1 day. At the end of this period, the samples were centrifuged at 4000 rpm (+4 °C) for 10 min. The obtained supernatant was filtered through a Whatman filter paper, and then 0.5 mL of *n*-hexane was added. Subsequently, drying was performed using nitrogen gas. The residue in the tubes was dissolved in a methanol+tetrahydrofuran mixture. Analyses were carried out in a Thermo Scientific Finnigan Surveyor model high-performance liquid chromatography (HPLC) and in amber glass vials on Tray auto sampler using PDA array detector (Al-Saleh et al., 2006).

Vitamin B

A total of 10 g of the samples prepared by crushing the frozen berries were weighed and homogenized. The samples were then transferred to a conical flask containing 25 mL of extraction solution. The solution was sonicated using a shaking water bath at an ambient temperature of 70 °C was used for 40 min. Following sonication, the sample was cooled and filtered to make a volume of 50 mL with extraction solution. The extraction solution was again filtered with filter trips (0.45 μm) and 20 μL aliquot solutions were injected into the HPLC by using an autosampler. A reversed-phase C-18 analytical column (STR ODS-M, 150 mm 4.6 mm ID, 5 m, Shimadzu Corporation, Japan) was used to separate the B complex vitamins. At 40 °C, the mobile phase consists of a 9:1 (v/v) combination of 100 mM sodium phosphate buffer (pH: 2.2) containing 0.8 mM sodium-1-octanesulfonate and acetonitrile. The flow rate was kept constant at 0.8 mL min^{-1} using a PDA detector with a 270 nm absorption rate. The peak area of the corresponding chromatogram was used to calculate B vitamins using the following equation:

B vitamins (mg 100 g^{-1}) = Concentration of standard × (Area of sample/Area of standard) × Dilution factor (Mozumder et al., 2019).

Vitamin C

The extraction solution was combined with 2.5 mL of frozen, crushed berry material (3% MPA and 8% acetic acid for MPA-acetic acid extraction and 0.1% oxalic acid for oxalic acid extraction). The mixture was titrated with indophenol solution (25% DCIP and 21% $NaHCO_3$ in water) until a light but distinct rose-pink colour appeared and persisted for more than 5 s (AOAC, 1990).

2.5. Statistical analyses

The experiment was designed to include three replications of two vines per experimental unit. Utilizing the JMP statistical program based on SAS, one-way analysis of variance (ANOVA) with randomized complete block design was performed. ANOVA with three replications was used for yield, cluster, berry, and juice characteristics, and ANOVA with two replications was used for phytochemical analysis. The least significant difference (LSD) test was used on the data to compare the means at a 5% significance level ($p \leq 0.05$).

3. Results and discussions

3.1. Effect of treatments on yield, cluster, berry, and juice properties

The use of organic and inorganic amendments had effects on grape yield and quality (Table 5). Yield was significantly higher than that of the control in PR+FM (+0.8 kg per plant), in P and P+S+FM (both about +2 kg per plant), and in P+PR+FM (+3 kg per plant). The P+PR+FM combination had the best results also in terms of bunch and berry weight. On the other hand, this treatment did not seem to have any influence on the juice properties in terms of TSS, acidity, pH, and ripening index, which were not statistically different ($p > 0.05$) from those of the control. The S+FM mixture exhibited the highest performance in TSS, pH, and ripening index, with 15.37%, 3.74, and 26.18, respectively. In general, all the treatments including farm manure (FM) showed positive effects on yield, bunch weight, berry weight, and TSS. Organic-based compost, farm manure, and vermicompost are known to improve productivity in poor soils (Popović et al., 2020). On the contrary, the mixture of pumice and dry compost (P+DC) resulted in slightly lower yield, bunch, and berry weight than the control, and the low nitrogen content in the mixture inhibited the organic material decomposition (Fog, 1988; Vivanco and Austin, 2011; Schuster, 2016; Dong et al., 2019; Hou et al., 2021). This may be attributed to the decomposition of cellulosic high organic matter content of the DC soil, where microorganisms deplete soil nitrogen before plants can uptake it. This may also be due to the low maturity of commercial compost used.

Table 5. Two-year cumulative effects of the various treatments on “Prima” grape yields, cluster, berry, and juice properties.

Treatments[x]	Yield (g vine^{-1})[y]	Bunch weight (g)	Berry weight (g 100 berry^{-1})	Total soluble solids (%)	Acidity (g 100 mL juice^{-1})	pH	Ripening index (TSS/Acidity)
Control	5875de	345.6de	433.9bc	12.63 c	0.692ab	3.41e	18.39cd
P	7719b	454.0b	458.7ab	13.57bc	0.647abcd	3.54cd	21.11bc
DC	6340cd	372.9cd	485.9ab	14.37ab	0.679abc	3.49de	21.21bc
P+DC	5180e	304.7e	372.9 d	12.50c	0.640bcde	3.60bc	19.52cd
S+FM	6354cd	373.8cd	438.3abc	15.37a	0.587de	3.74a	26.18a
P+S+FM	7864b	462.6b	432.6bc	14.13b	0.610cde	3.62bc	23.15ab
PR+FM	6674c	392.6c	394.0cd	13.97b	0.568e	3.69ab	24.76a
P+PR+FM	8860a	521.2a	490.9a	12.60c	0.722a	3.49de	17.53d
LSD 5%	752	44.26	53.51	1.193	0.078	0.085	3.385
p-value	<0.0001	<0.0001	0.004	0.001	0.010	<0.0001	0.0008

[x]: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, [y]: Means (n = 3) followed by different letters on the same column are significantly different according to the LSD test at $p \leq 0.05$.

In addition, due to the high water retention of pumice (Table 2), nutrients are initially retained by it. As a result, mixtures with farm manure tend to have naturally higher nitrogen levels than those with dry compost, resulting in higher grape yield and bunch weight.

The possible reasons for the positive effects of pumice, P+S+FM, and P+PR+FM on yield, bunch weight, and berry weight could be that pumice, straw, and pruning residues provide aeration and water retention together with the nutrient content of farm manure. Treatments containing farm manure always showed positive effects on yield, bunch and berry weight, and TSS. This showed that farm manure nutrients are readily available, such as nitrogen at 2%, and, as stated by Oldfield et al. (2019), high yield occurs in soils with less than 2% organic matter, which is also the case for the studied soils.

3.2. Effects of treatments on grapes’ phytochemical properties

The organoleptic characteristics of the grapes, which affect their taste, are crucial elements that enhance their marketability (Baccichet et al., 2021). Sugars and organic acids are among the most important characteristics to determine the quality of the grapes. The amount of anthocyanins increases with the increasing amount of sugar in the grapes towards ripening (Winkler et al., 1974). Anthocyanins produce the distinctive colours of the fruit and thus contribute to the quality of the fruit. Thus, anthocyanins are also ripening indicators because most fruits accumulate these compounds mainly during the ripening process (Jaakola, 2013).

The difference between sugars and organic acids other than sucrose sugar and citric acid and their total values was not statistically significant ($p > 0.05$) (Table 6). The values ranged between 192.24 (S+FM) and 233.53 (Control) g kg^{-1} fresh weight for total sugars and between 14.21 (PR+FM) and 19.48 (P) g kg^{-1} fresh weight for total organic acids. It was observed that the obtained values were generally in agreement with the values obtained for different grapes reported in the literature (Kelebek, 2009; Tangolar et al., 2015, 2016). Thus, it was evaluated that experimental applications did not have a significant effect ($p > 0.05$) on the taste at the level of total sugars and organic acids. The total sugar content in the control appeared to be relatively higher than in all other treatments, likely due to the concentration effect, i.e. the relatively low weight of the bunches of the control resulted in high sugar content per unit weight (Table 5).

Total organic acids and total sugars were not significantly different ($p > 0.05$) between treatments and control, although organic acids were generally slightly higher in all treatments with pumice. The lowest acidity was found in PR+FM with 14.21 g kg^{-1} fresh weight, showing that nutrition capacity of the farm manure has a positive effect on the taste of the grape. Although the total sugar content of PR+FM was lower than that of the Control, P, and P+DC treatments, the lower total organic acid content stands out, showing that the application has a positive influence on the fruit’s taste.

Among the treatments DC, P+DC, and P+S+FM showed the highest total anthocyanin values with 261.53, 261.28, and 249.81 mg kg^{-1} fresh weight respectively (Table 7). This may be due to the relatively early ripening of the grapes in the treatments. The total anthocyanin content varied between 199.20 mg kg^{-1} (P) and 261.53 mg kg^{-1} (DC) in our study (Table 7). These were close to the values reported by Eshghi et al. (2014), who reported that total content of anthocyanin in berries of 35 grape cultivars varied from 42.74 mg kg^{-1} to 619.04 mg kg^{-1}, based on fresh weight. The results of the present study were in line with Tangolar et al. (2016). Cantos et al. (2002) also indicated that anthocyanins were the main phenolic

Table 6. Effects of different treatments on sugar and organic acid contents (g kg^{-1} fresh weight) of "Prima" berries.

Treatments^x	Glucose^y	Fructose	Sucrose	Total sugars	Total sugar per plant (g plant^{-1})	Malic acid	Tartaric acid	Citric acid	Total organic acids
Control	74.94	153.18	5.41b	233.53	1372 ab	6.84	9.02	0.17c	16.03
P	94.82	126.18	4.41bc	225.41	1740 ab	5.91	13.40	0.17c	19.48
DC	86.55	112.00	5.43b	203.98	1293 ab	5.84	12.44	0.21bc	18.49
P+DC	94.24	120.64	5.16b	220.03	1140 b	7.44	12.82	0.34b	20.60
S+FM	76.67	112.82	2.75c	192.24	1222 b	7.13	10.10	0.25bc	17.48
P+S+FM	91.00	104.72	8.24a	203.97	1604 ab	4.96	10.70	0.66a	16.33
PR+FM	88.69	113.45	4.07bc	206.20	1376 ab	5.37	8.68	0.16 c	14.21
P+PR+FM	78.00	131.27	5.12b	214.39	1900 a	5.90	11.92	0.17 c	17.99
LSD 5%	NS	NS	1.81	NS	638	NS	NS	0.14	NS
p-value	0.955	0.685	0.006	0.957	0.0201	0.368	0.476	0.0009	0.494

^{x}: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, ^{y}: Means (n = 3) followed by different letters on the same column are significantly different according to the LSD test at $p \leq 0.05$. NS: Nonsignificant.

Table 7. Effects of different treatments on anthocyanin contents (mg kg^{-1} fresh weight) of "Prima" berries.

Treatments^x	Malvidin -3-glu^y	Peonidin -3-glu	Petunidin -3-glu	Delphinidin-3-glu	Cyanidin -3-glu	Total
Control	79.48	52.91 bc	46.29 c	29.94 c	7.13 ab	215.76 bc
P	69.48	46.59 c	46.24 c	29.51 c	7.38 ab	199.20 c
DC	77.35	67.31 a	58.08 ab	55.51 a	*3.28 c*	261.53 a
P+DC	84.40	63.01 ab	59.19 a	50.71 ab	3.97 bc	261.28 a
S+FM	75.07	49.31 c	40.95 c	27.34 c	7.83 a	200.51 c
P+S+FM	77.90	64.86 a	68.12 a	34.42 bc	4.51 abc	249.81 a
PR+FM	72.07	48.63 c	48.42 bc	28.79 c	7.62 a	205.53 bc
P+PR+FM	72.01	57.89 abc	57.79 ab	40.62 abc	6.07 abc	234.39 ab
LSD 5%	NS	11.55	10.62	16.96	3.64	31.70
p-value	0.407	0.020	0.007	0.032	0.102	0.007

^{x}: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, glu: glucoside, ^{y}: Means (n = 3) followed by different letters on the same column are significantly different according to the LSD test at $p \leq 0.05$. NS: Nonsignificant.

compounds in red grapes ranging from 69 "(Crimson Seedless)" to 151 "(Flame Seedless)" mg kg^{-1} fresh weight of grapes. However, Galet (1993) and Kelebek (2009) reported that the total amount of anthocyanins in 23 different grape cultivars range between 42 and 4893 mg kg^{-1}, while Mazza and Francis (1995) reported that the total amount of anthocyanins in the berry of some grape cultivars ranged between 300 and 7500 mg kg^{-1}. Rusjan and Korošec-Koruza (2007) also determined higher amount of total anthocyanins (2179–2219 mg kg^{-1}) in red grapes. Researchers noted that comparing results to the literature is challenging due to the various kinds studied, ecological settings, ripening stage, and analytical methodologies. For example, while Ozer (2003) suggested that the treatments with FM generally yielded lower anthocyanin, which may be attributed to higher N contents retarding maturation of crops. Parrado et al. (2007) found that the application of organic matter from red grape bioethanol production waste induced an increase in anthocyanin levels. The difference between applications for malvidin-3 glucoside was not significant ($p > 0.05$), but for other anthocyanins, significant differences ($p \leq 0.05$) were found between applications. DC and P+S+FM for peonidine-3-glucoside; P+DC and P+S+FM for petunidin-3-glucoside; DC for delphinidin-3-glucoside; S+FM and PR+FM applications for cyanidin-3-glucoside yielded higher values. The fact that there was no difference in terms of malvidin-3 glucoside, which contains the highest rate amount of the anthocyanins in coloured grapes (Winkler et al., 1974; Kelebek, 2009), was evaluated as an indication that the applications did not create enough statistical difference on the berry colour during the trial period.

In the present study, four flavonols and two flavanols (Table 8) were quantified. All of these compounds, except for quercetin-3-glucoside, were affected by the treatments.

In particular, myricetin-3-glucoside and isorhamnetin-3-glucoside showed significantly higher levels in P, S+FM, and PR+FM treatments, whereas catechin levels were higher in DC, P+DC, and PR+FM. Total flavonols showed significantly higher levels only in S+FM, whereas total flavanol levels were higher in PR+FM, S+FM, and DC. Therefore, in general, S+FM and PR+FM applications were the most prominent in terms of the flavonol and flavanol compounds, reported in Table 8.

The S+FM and P+S+FM applications were statistically more prominent ($p \leq 0.05$) in terms of total phenolic acids, in particular for gallic and *p*-coumaric acids (Table 9). These values are in accordance with results from Tangolar et al. (2016), reporting total phenolic acids in the "Prima" cultivar between 403.29 and 535.6 mg kg^{-1} fresh weight. Additionally, Cantos et al. (2002) found that total phenolic compounds ranged from 115 to 361 mg kg^{-1} fresh weight. Total phenolic compound levels, as reported by Lachman et al. (2009), varied among berry skins from different regions, ranging from 370.1 mg L^{-1} to 238.7 mg L^{-1}. Our results are also in agreement with the values found by Kelebek (2009) and Eshghi et al. (2014). In the present study, the accumulation of total phenolic acids, especially flavanols, was enhanced by FM addition, in comparison to the control treatment. This suggests that supplementing FM to PR and SM mixture has a positive effect on the accumulation and biosynthesis of phenolic compounds.

Several studies suggest that phenolic acids can act as antioxidants by scavenging hydroxyl radicals (Chandrasekara, 2018). Thus, they are crucial not only for plant health but also for human health. In this experiment, the highest total phenolic acid value was obtained in the S+FM mixture, followed by P+S+FM, which is also rich in organic material rather than minerals. Here, we can argue that organic material induces phenolic acid development more than mineral material, such as pumice alone.

The activities of some of the enzymes analysed from the berries are presented in Table 10. With the exception of GST, the activities of all analysed enzymes were influenced by the treatments. The highest activities were found in S+FM for GR, in S+FM and P for G6PD, in P+S+FM for CAT, in S+FM and P+PR+FM for POD, in P+S+FM for SOD, and in P+PR+FM for APX. The enzyme activities were higher in the pumice, the straw, and the farm manure mixtures than the others. As with phenolic acid development, mixtures dominated by organic material generally showed higher values of enzyme activities than mineral mixtures (Table 10). Antioxidant enzymes not only increase plant resistance, especially for abiotic stresses such as salinity, water and drought stress (Movludi et al., 2014; Pandey et al., 2017), but also increase the nutritional value of grapes and their positive effects on human health (Krishnamurthy and Wadhwani, 2012). Thus, adding organic matter to the soil provides significant benefits in this regard. Antioxidant enzymes (e.g., SOD, glutathione peroxidase (GPX) and glutathione reductase (GR), CAT, etc.) are able to stabilize, or deactivate free radicals before they attack cellular components (Krishnamurthy and Wadhwani, 2012). To minimize the damage caused by free radicals, these substances reduce their energy and interfere with the oxidative chain reaction. Researchers have indicated that antioxidant enzymes are a substantial link between free radicals and more than sixty different health conditions, including the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks, and atherosclerosis (Jideani et al., 2021). For these reasons, by reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, our body's potential to reduce the risk of free-radical-related health problems becomes more palpable. Zhang et al. (2014) suggested that CAT, POD, and SOD are also important antioxidant enzymes that play a crucial role in the elimination of superoxide and

Table 8. Effects of different treatments on flavonol and flavanol contents (mg kg^{-1} fresh weight) of "Prima" berries.

Treatments ^{x}	Rutin^y	Myricetin-3-glucoside	Isorhamnetin-3-glucoside	Quercetin-3-glucoside	Total flavonol	Catechin	Epicatechin	Total flavanol
Control	29.57 bc	4.67 c	9.88 d	17.98	62.10 bc	107.02 b	160.72 c	267.74 c
P	28.92 bc	6.24 a	15.71 b	19.33	70.20 ab	143.22 ab	186.91 bc	330.13 bc
DC	28.11 bc	4.21 d	10.60 cd	18.11	61.03 bc	190.27 a	186.93 bc	377.20 ab
P+DC	31.00 ab	4.18 d	9.88 d	18.20	63.26 bc	185.33 a	167.93 c	353.27 abc
S+FM	35.77 a	5.28 b	17.39 a	19.90	78.34 a	155.34 ab	225.61 ab	380.96 ab
P+S+FM	31.93 ab	3.99 d	11.64 c	16.22	63.78 bc	155.63 ab	202.59 abc	358.22 abc
PR+FM	24.04 c	5.54 b	18.03 a	21.51	69.12 bc	183.29 a	255.04 a	438.33 a
P+PR+FM	29.54 bc	3.93 d	8.04 d	18.68	60.18 c	105.50 b	170.52 c	276.02 c
LSD 5%	5.836	0.436	1.654	NS	9.218	56.98	53.23	92.70
p-value	0.053	<0.0001	<0.0001	0.204	0.025	0.048	0.042	0.041

^{x}: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, ^{y}: Means (n = 3) followed by different letters on the same column are significantly different according to the LSD test at $p \leq 0.05$. NS: Nonsignificant.

Table 9. Effects of different treatments on phenolic acid contents (mg kg^{-1} fresh weight) of "Prima" berries.

Treatments[x]	Gallic acid[y]	Protocatechic acid	Trans-caftaric acid	Trans-coutaric acid	*p*-coumaric acid	Ferulic acid	Total phenolic acids
Control	24.78 cd	24.30 ab	29.60 abc	9.24 bcd	13.30 d	30.72 ab	131.94 c
P	23.21 d	22.62 bc	28.92 bc	13.13 a	16.37 bcd	30.14 b	134.39 c
DC	25.42 cd	24.66 ab	27.39 bc	8.12 d	14.99 bcd	30.15 b	130.73 c
P+DC	28.41 bc	25.24 ab	31.00 ab	8.62 cd	13.96 cd	27.15 b	134.38 c
S+FM	35.96 a	25.07 ab	35.77 a	10.22 bc	25.58 a	28.40 b	161.00 a
P+S+FM	31.48 ab	27.19 a	31.93 ab	8.95 bcd	20.77 ab	28.64 b	148.96 b
PR+FM	27.61 bcd	16.90 d	24.04 c	10.67 b	19.91 abc	35.36 a	134.49 c
P+PR+FM	27.66 bcd	20.75 c	29.90 abc	8.06 d	16.46 bcd	27.97 b	130.82 c
LSD 5%	4.535	3.034	6.244	1.939	6.075	4.756	9.575
p-value	0.0048	0.002	0.067	0.006	0.022	0.072	0.001

[x]: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, [y]: Means (n = 3) followed by different letters on the same column are significantly different according to the LSD test at $p \leq 0.05$.

Table 10. Effects of different treatments on antioxidant enzyme activities (unit 100 g^{-1} fresh weight) of "Prima" berries.

Treatments[x]	GR[y]	GST	G6PD	CAT	POD	SOD	APX
Control	8.92 bc	139.44	40.32 c	7.81 ab	22.25 cd	8.22 d	8.06 d
P	8.30 cd	168.01	52.43 a	7.42 abc	21.84 d	9.53 bc	8.34 cd
DC	7.09 d	157.77	41.67 bc	5.98 c	23.45 bc	10.09 b	8.78 bc
P+DC	7.11 d	151.08	46.04 bc	6.36 bc	24.05 b	10.16 b	8.58 cd
S+FM	12.29 a	143.07	53.26 a	5.99 c	25.56 a	9.13 c	9.41 ab
P+S+FM	10.12 b	144.52	47.70 ab	8.37 a	16.80 e	13.48 a	6.73 e
PR+FM	8.21 cd	178.16	47.72 ab	6.51 bc	22.56 cd	9.14 c	8.32 cd
P+PR+FM	7.15 d	161.84	42.56 bc	7.17 abc	25.97 a	9.80 bc	9.84 a
LSD 5%	1.53	NS	6.274	1.78	1.26	0.68	0.67
p-value	0.0009	0.114	0.012	0.104	<0.0001	<0.0001	0.0003

[x]: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, [y]: Means (n = 3) followed by different letters on the same column are significantly different according to the LSD test at $p \leq 0.05$. NS: Nonsignificant. GR: Glutathione reductase, GST: Glutathione S-transferase, G6PD: Glucose-6-phosphate dehydrogenase, CAT: Catalase (CAT-EC: 1.11.1.6), POD: Peroxidase (POD - EC: 1.11.1.7); SOD, Superoxide dismutase (SOD – EC: 1.15.1.1); APX, Ascorbate peroxidase.

hydrogen peroxide in plants, thus mitigating the harmful effects of reactive oxygen species.

In Troiani et al.'s study (2003), SOD activity values were determined as 53.00 and 7.67 units 100 g^{-1} for peel and pulp in the "Rubi" cultivar, 11.50 and 141.11 units 100 g^{-1} in the "Borbon" cultivar, and 327 and 44 units 100 g^{-1} in the "Benitaka" cultivar. These values were found to be close to the values we determined in our study as 16.80–25.97 units 100 g^{-1} SOD activity in Prima berries.

Vitamins are essential components of a healthy diet and serve as indicators of a well-balanced diet. They are also beneficial by acting as antioxidants in different ways (Tardy et al., 2020). For example, vitamins C and A act as neutralizing antioxidants and free radical chain breakers for hydroxyl and superoxide radicals and hydrogen peroxide. Uğur et al. (2020) stated that vitamin C protects against numerous diseases by decreasing free radical damage in the body via its significant antioxidant activity. The P+S+FM treatment showed significant higher contents of A, B1, and B6 vitamins, whereas several treatments (P, DC, P+DC, PR+FM) showed only significant increase of B1 vitamins, when compared with control (Table 11). From Table 11, we can conclude that, grapes contain less vitamin C than pepper, cabbage, broccoli, strawberry, kiwi, orange, and cauliflower, which have vitamin C levels ranging from 150.7 to 21.2 mg 100 g^{-1} according to Uğur et al. (2020).

Table 11. Effects of different treatments on vitamin contents (mg 100 g^{-1} fresh weight) of "Prima" berries.

Treatments[x]	A (Retinol)[y]	B1 (Thiamine)	B2 (Riboflavin)	B6 (Pyridoxine)	C (Ascorbic acid)
Control	32.55e	41.35b	141.95a	73.05b	9.90
P	34.45cde	47.85a	143.35a	89.90ab	10.45
DC	33.85de	51.20a	144.45a	89.80ab	9.70
P+DC	35.30bcd	49.40a	148.05a	86.30b	9.95
S+FM	37.60b	41.10b	159.25a	79.60b	9.95
P+S+FM	41.6a	49.90a	123.35b	109.45a	11.60
PR+FM	34.85cde	50.35a	144.45a	91.00ab	9.60
P+PR+FM	36.85bc	41.50 b	154.35 a	81.70 b	10.25
LSD 5%	2.60	5.85	17.45	20.15	NS
p-value	0.002	0.013	0.041	0.074	0.190

[x]: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, [y]: Means (n = 3) followed by different letters on the same column are significantly different according to the LSD test at p ≤ 0.05. NS: Nonsignificant.

The daily recommended value for vitamin C is 60–110 mg[5],[6] (Uğur et al., 2020). Uğur et al. (2020) reported that the amount of vitamin C required to prevent or treat early deficiency symptoms is between 6.5 and 10 mg day^{-1}. The results of this study align with those reported by other researchers regarding the content of milligrams of vitamin C per 100 grams of fresh weight. For example, Pinheiro et al. (2009) found 10.8 mg of vitamin C in the edible part of in grapes, while Abdrabba and Hussein (2015) reported an average of 14.61 mg of vitamin C in the pulp-seed-peel combination. Additionally, Souza et al. (2012) noted a range of 4.9 to 12.2 mg of ascorbic acid in grape skin (*Vitis vinifera* L.). Despite insignificant differences between trial applications, grapes can generally contribute to meeting daily vitamin C requirements.

B vitamins are required by the body for a variety of functions; for example, thiamine helps the body produce adenosine triphosphate (ATP), a molecule required for energy transportation within cells, riboflavin assists in the metabolism of the food you eat, turning it into energy, and similar to riboflavin, pyridoxine is important for energy production[7]. Tzin and Galili (2010) defined that these amino acids are not only essential components of protein synthesis in plants, but they are also found in various growth hormones and secondary metabolites with a wide range of biological functions and health-promoting properties, such as resistance to abiotic and biotic stresses. Their production in plants is therefore essential for the quality and quantity of production. In addition, the importance of amino acids, which are the building blocks of proteins, in human nutrition, cannot be overestimated (Key et al., 2019; Rose, 2019). There was no significant difference between the treatments for histidine, glycine, asparagine, alanine, isoleucine, leucine, and lysine among the 20 amino acids, evaluated in our study (Table 12). The highest values for arginine were obtained in the S+FM, P+S+FM, and P+PR+FM applications (47,051, 47,360, and 49,187 µg kg^{-1}, respectively); the highest values for proline were obtained in the P and S+FM applications (53,721 and 53,700 µg kg^{-1}, respectively).

Pumice treatment yielded the maximum values for three amino acids (Table 12), namely tryptophan, phenylalanine, and proline. Cystine, tryptophan, glutamate, and glutamine were the most abundant amino acids in dry compost. The P+DC application showed higher levels of glutamate, while S+FM showed higher levels of arginine, serine, tyrosine, sarcosine, and proline values. During the P+S+FM treatment, valine, methionine, tryptophan, and arginine performed better than the other amino acids studied. Glutamine and tryptophan were the most abundant amino acids in PR+FM, while arginine, cysteine, sarcosine, and aspartate were the most abundant amino acids in P+PR+FM. For all amino acid values, the P+PR+FM application produced the maximum value of 289,947 µg kg^{-1}, while the control application produced the lowest value of 257,138 µg kg^{-1}.

Among the mixtures, S+FM, P+S+FM, and P+PR+FM showed relatively higher acid components than others (Tables 12a and 12b). In addition, higher values were measured in terms of amino acids, with the total value

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Table 12a. Effects of different treatments on amino acid contents (μg kg^{-1} fresh weight) of "Prima" berries.

Treatments^{x}	Aspartate^{y}	Glutamate	Arginine	Serine	Glutamine	Histidine	Glycine	Asparagine	Alanine	Tyrosine
Control	11125 de	4111 c	40308 c	16279 c	12409 d	7148	7303	22626	21611	2288 e
P	10921 e	4763 ab	41701 bc	17225 bc	14361 abc	7167	8989	27140	20348	2497 d
DC	11723 cd	5044 a	43897 b	16935 c	15359 a	7223	8982	27058	21243	2586 cd
P+DC	12027 bc	5079 a	42916 bc	17644 abc	14816 ab	7402	8628	26123	22287	2686 bc
S+FM	12780 ab	4565 b	47051 a	18795 a	12328 d	7964	7958	23713	25380	2864 a
P+S+FM	12026 bc	4863 ab	47360 a	17465 abc	13154 bcd	6796	10342	26089	22366	2573 cd
PR+FM	11282 cde	4568 b	41592 bc	17429 abc	15099 a	7224	9098	27483	20431	2553 cd
P+PR+FM	12984 a	4901 ab	49187 a	18426 ab	12560 cd	7719	8171	23458	26047	2830 ab
LSD 5%	784.5	426.1	2992	1381	1808	NS	NS	NS	NS	176.3
p-value	0.003	0.014	0.002	0.052	0.018	0.205	0.116	0.168	0.107	0.002

^{x}: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, ^{y}: Means (n = 2) followed by different letters on the same column are significantly different according to the LSD test at p ≤ 0.05. NS: Nonsignificant.

Table 12b. Effects of different treatments on amino acid contents (μg kg^{-1} fresh weight) of "Prima" berries.

Treatments^{x}	Cysteine	Valine	Met	Try	Phe	IsIso	Leusine	Lysine	Sarcosine	Proline	Total amino acids
Control	2791 b	1864 d	3899 c	4889 bc	4508 d	5231	5247	7953	15709 c	46648 c	257138 e
P	2865 ab	2586 ab	4547 b	6239 a	5288 a	4927	5842	8823	16431 bc	53721 a	280365 cd
DC	3076 a	1913 cd	4229 bc	6165 a	4623 cd	5787	5025	9536	16911 b	52619 ab	282622 bcd
P+DC	2998 ab	1907 cd	4454 b	5923 ab	4777 bcd	5493	5321	8963	17180 b	52147 ab	281712 bcd
S+FM	2894 ab	2198 cd	4459 b	4723 c	4725 bcd	5387	5425	8113	18886 a	53700 a	287865 ab
P+S+FM	2809 b	2861 a	5072 a	6200 a	4979 ab	4885	5969	8966	17379 b	49354 bc	286333 abc
PR+FM	2821 b	2254 bc	4372 bc	6193 a	4770 bcd	5504	5312	9150	16820 b	52620 ab	279574 d
P+PR+FM	3092 a	2102 cd	4551 b	5064 bc	4904 bc	5337	5148	8523	18914 a	52004 ab	289947 a
LSD 5%	71.77	116.08	152.42	309.40	94.05	NS	NS	NS	328.80	1298.06	1841.91
p-value	0.0957	0.0043	0.0292	0.0315	0.0125	0.1861	0.154	0.1489	0.0022	0.0611	<0.0001

^{x}: P: Pumice, S: Straw, DC: Dry compost, PR: Pruning residue, FM: Farm manure, Met: Methionine, Try: Tryptophan, Phe: Phenylalanine, Iso: Isoleusine,

^{y}: Means (n = 2) followed by different letters on the same column are significantly different according to the LSD test at p ≤ 0.05. NS: Nonsignificant.

of all treatments compared to the control. As a result, it was recognized that natural inputs, particularly organic materials, might be used to increase the amino acid content of grapes. The advantages of increasing amino acids in grape by agronomic treatments are considered particularly significant in terms of human nutrition and plant health. Although some amino acid amounts varied among applications, it can be argued that the overall rising trend in amino acids will improve the studied cultivar and ecology.

Huang and Ough (1991), Canoura et al. (2018), Bouzas-Cid et al. (2015, 2018a, 2018b, 2018c), Sánchez-Gómez et al. (2016), Gutiérrez-Gamboa et al. (2017, 2018a, 2018b, 2020), Fernández-Novales et al. (2019) and Wu et al. (2019) reported that the amino acid content of grape berries are affected by different cultivars, rootstocks, and locations, as well as fertilization and other viticultural practices. For example, in the study of Gutiérrez-Gamboa et al. (2020), the effect of the foliar application of a seaweed extract on the "Tempranillo blanco" cultivar was determined for must and wine amino acids and ammonium content. The results suggested that "Tempranillo blanco" behaved as an arginine accumulator cultivar. Biostimulation after seaweed applications at a high dosage to the grapevines increased the concentration of several amino acids in the 2017 season, while it scarcely affected their content in 2018. In another research by Gutiérrez-Gamboa et al. (2018b), results showed that of some elicitors and nitrogen foliar applications to "Garnacha" and "Tempranillo" grapevines decreased the must amino acid concentration. The treatments applied to "Graciano" grapevines affected the grape amino acid content. Based on the percentage of variance attributable, the cultivar had a greater effect on the essential amino acid composition than the treatments and their interaction. Amino acid values obtained in the present study varied from 1864 μg kg^{-1} (in control for valine) to 49187 μg kg^{-1} (in P+PR+FM for arginine). These values align with the valine content (1.07 mg L^{-1}) reported by Fernández-Novales et al. (2019) for "Grenache", as well as the arginine content (38.44 to 89.60 mg L^{-1}) reported by Valdés et al. (2019) for "Tempranillo" berries. In addition, in the review study by Winkler et al. (1974), the amino acid values in the must varied between 6 (for glysine) and 3490 mg L^{-1} (for proline) in the 5 wine grape cultivars. They varied from 0.0 (for tyrosine) to 327 mg L^{-1} (for arginine) in "Merlot" and "Cabernet Sauvignon", and it ranged from <1.0 (for methionine) to 905 mg L^{-1} (for arginine) in 18 grape cultivars.

Arginine and proline amino acids were recorded as the most abundant amino acids in all treatments used in our experiment; valine, cysteine, and tyrosine were determined as the amino acids with the lowest values. These results are in agreement with reports of Winkler et al. (1974), Fernández-Novales et al. (2019), and Valdés et al. (2019), who reported that arginine and proline were the most abundant amino acids in their studies. The results of this study clearly showed that the addition of organic and inorganic materials to the soil, alone or in mixture, had a positive effect on the amino acid content of the grapes.

4. Conclusions

Mediterranean soils have probably been in agricultural use for the longest time in the world, and disappointingly, the quality of these soils is deteriorating due to overproduction but inadequate maintenance. In order to ensure high yield and quality of table grape in Mediterranean area, together with a sustainable use, both economically and environmentally, of external resources, a balanced use of fertilizers and amendments is thus mandatory particularly in soils with physical (depth) and chemical (organic matter, high $CaCO_3$, low micronutrients) limitations. The recycling of bio-waste and/or low-cost natural inorganic materials can contribute to reduce the dependence from synthetic fertilizers, increasing the soil and yield quality.

In the present study, volcanic pumice (P) as a mineral source, straw (S), cattle farm manure (FM), and dry compost (DC) as organic matter sources, single or mixed (with or without pruning residues, PR), were used to improve the quality of shallow Calcisol with low organic matter and nutrients in a table grape vineyard in southern Türkiye. The results of the study demonstrate that inorganic and organic soil amendments, alone or in various combinations, positively affected several characteristics of the grapes. With the information obtained, a decision can be made regarding which amendment to apply, alone or in mixture, when a specific grape characteristic is to be targeted. In this study, P+PR+FM showed the best performance in sugar accumulation per plant (1900 g per plant), and the best accumulation of total amino acids in the berries, in particular aspartate, arginine, and cysteine, while P+S+FM showed the highest anthocyanin content together with DC and P+DC. However, it should be noted that the application doses of the tested combinations and the organic matter material to inorganic material mixture ratios utilized in this study may not be applicable to all soils. Nevertheless, the positive response of the grapevine to the orgonamineral mixture added to the soil in this two-year study demonstrates that the soils studied are significantly deteriorated and require immediate remediation. Aside from that, global environmental issues such as desertification, climate change, and biodiversity loss jeopardize the condition of healthy soils, let alone degraded soils. In conclusion, using every type of inorganic and organic materials that are not detrimental to human or environmental health in agricultural areas should be stressed on both a local and global scale.

As a result, although we achieved positive results both in chemistry and quantity of calcareous Mediterranean soil, additional studies are needed to determine the detailed effects of such treatments on soil quality, such as soil carbon sequestration, biological activities, and soil water dynamics, in order to achieve optimal yields while preserving and even enhancing soil ecosystem services. In addition, the benefits of such soil conditioners should be described to farmers as soon as possible, and the use of natural conditioners should be encouraged so that the treatments can be disseminated over vast areas and food security can be ensured.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Conceptualization, supervision, methodology, investigation, project administration, S.T.; writing, review, editing, discussion, interpretation, and visualization, S.T. and S.P.; investigation, resources, pomological analysis, formal analysis, data curation, S.T. and S.T.; resources, phytochemical analysis, M.T.; applications, visualization, writing the original draft, E.A.

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