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Carbon and Nitrogen Stocks in Vineyard Soils Amended with Grape Pomace Residues

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Abstract: Fruit crops under soil conservational management might sequester carbon (C) in soils and mitigate greenhouse gases emissions. Using grape pomace residues as soil amendment holds promise for sustainable viticulture. However, its actual capability to increase soil organic carbon (SOC) and nitrogen (N) is unknown, especially in subtropical climates. This research aims to investigate whether grape pomace compost and vermicompost can increase SOC, total N (TN), and C and N stocks in subtropical vineyards. Two vineyards located in Veranópolis, in South Brazil, one cultivated with 'Isabella' and the other with 'Chardonnay' varieties, were annually amended with these residues for three years. We quantified SOC and TN in each condition in different soil layers, as well as C and N content in two different granulometric fractions: mineral-associated organic matter (MAOM) and particulate organic matter (POM). C and N stocks were also calculated. Despite potential benefits, neither treatment enhanced SOC, its fractions, or C stocks. In fact, vermicompost was rapidly mineralized and depleted SOC and its fractions in the 0.0 to 0.05 m layers of the 'Isabella' vineyard. Our findings indicate that the tested grape pomace residues were unable to promote C sequestration in subtropical vineyards after a three-year period.

Keywords: climate change; fruit crops; grapevine; mineral-associated organic carbon; organic residues; particulate organic carbon; soil organic matter

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1. Introduction

Most soils worldwide present low carbon (C) content, especially in cultivated areas, mostly due to improper management practices (such as soil tilling, lack of cover crops, slash-and-burn), and/or tropical and subtropical climates [1]. Consequently, the need to replenish soil organic matter (SOM) becomes paramount for maintaining soil health and fertility [2]. Utilizing organic residues, such as compost or vermicompost, presents a promising approach to enhance soil organic C (SOC) and C stocks, and improve soil health. This strategy is particularly relevant in vineyard soils, where the cultivation of grapevines demands long-term and sustainable soil fertility practices. By adopting practices that promote SOM accumulation, vineyard managers can contribute to mitigating climate change while ensuring adequate plant nutrition and grape yield. Thus, integrating organic amendments into vineyards emerges as an important component of sustainable viticulture in the face of environmental challenges [3].

Studies have reported various benefits in orchards and vineyards resulting from soil conservation management and fertilization with organic nutrient sources [4–7]. Organic nutrient sources vary from fresh residues, compost or vermicompost, made from animal or plant material, such as animal manure or food industry waste, respectively. Their benefits include not only being a source of nutrients—such as N, phosphorus (P), sulfur (S), potassium (K), calcium (Ca), and magnesium (Mg)—but also improving soil health, enhancing soil water retention capacity, increasing total cation exchange capacity [7,8], and promoting C fixation [9]. In addition, the benefits provided by organic fertilization can increase grapevine and cover crops' shoot and root growth, which leads to higher biomass production and, thus, higher C fixation by photosynthesis, besides higher grape yield [6]. These practices have become more common in global viticulture, also coinciding with an increase in the production and commercialization of organic products [10]. Among commonly used organic sources of nutrients, composts and vermicomposts stand out, notably those produced with animal manure or industry residues, which include winemaking residues (grape pomace). This strategy has yet another positive input on sustainability, since it guarantees a safe disposal of industry waste [11,12].

The constant input of organic residues in soils may not only lead to an increase in SOM but also change its nature and persistence [13]. The main divisions, also called fractions, of SOM consider its recalcitrance [13]. While more labile fractions of the SOM may benefit plant growth due to nutrient (especially N) release, increasing recalcitrant fractions of SOM, namely the mineral-associated organic matter (MAOM), is key to elevating and/or maintaining C stocks [13,14]. Interestingly, the different lability in SOM fractions is correlated to SOM granulometric physical fractions [13]. Soils amended with organic fertilizers over time usually have greater SOM and MAOM content [5,15–19], especially via greater plant growth and biomass production [6,17]. Thus, measuring the MAOM and the stable forms of SOM in soils is an accessible way to verify changes in C and N pools in soils amended with organic fertilizers in the long run [20].

In the short term, however, more labile forms of SOM, such as particulate organic matter (POM), might increase in soils amended with organic residues [21,22]. Additionally, the particulate fraction of the organic matter is more sensitive to changes in the cultivation system [21,23]. Thus, evaluating POM is a necessary tool to verify more rapid changes in the C and N pool in soils amended with residues, especially under conservationist (no-tillage) management.

Studies examining the effects of grape pomace residues, such as grape pomace compost and vermicompost, on C and N stocks and their fractions in soils remain insufficient, especially in subtropical vineyards. We believe that conducting field studies in productive organic vineyards could address this knowledge gap. Understanding how this type of residue behaves in subtropical soils is relevant information to quantify the capacity of such agroecosystems to sequester C. Given this, the study aimed to test the effects of different grape pomace fertilizers on SOC and TN in subtropical organic vineyards. Additionally, we examined C and N content in MAOM and POM, as well as C and N stocks. We believe that vineyard soils amended with grape pomace residues might have higher C and N contents, especially in their particulate fractions, and C and N stocks after three growing seasons.

2. Materials and Methods

2.1. Experimental Site and Design

Two experiments (henceforth called Vineyard 1 and Vineyard 2) were carried out over three growing seasons in commercial organic vineyards situated in Veranópolis (28°47′06″ S; 51°30′32″ W), Rio Grande do Sul, southern Brazil. The region features a humid subtropical climate with hot summers (Cfa). Spontaneous cover crops were consistently maintained in both rows and interrows throughout the year, being mowed during the grapevines' growing season. Vineyard 1, employing a pergola system, featured 'Isabella' (*Vitis labrusca* × *Vitis vinifera*) grapevines grafted onto Paulsen 1103 rootstocks, with spacing of 2.5 m between rows and 1.5 m between plants (2666 plants per hectare). Vineyard 2 featured 'Chardonnay' (*V. vinifera*) grapevines, grafted onto Paulsen 1103 rootstocks, with spacing of 2.8 m between rows and 1.5 m between plants (2380 plants per hectare), also in pergola system, under polyethylene translucid coverage. The soil profiles in both vineyards were classified as Leptic Cambisol [24]. In Vineyard 1 and in Vineyard 2, grapevines were 10 to 12 and 3 years old (since seedling transplantation), respectively. In both areas, soil had been previously limed before vineyard establishment.

Vineyards 1 and 2 were divided into three rows, and, in each row, three fertilization treatments were applied: grape pomace compost (CO), grape pomace vermicompost (VC), and a negative control (C), with no fertilization. The setup was arranged in randomized blocks, with six replicates (therefore, two replicates per row). Each replicate consisted of five grapevines, totaling 7.5 m in length.

The organic fertilizers (CO and VC) were applied in amounts equivalent to 40 kg N ha^{-1}, based on previous studies [8] with grapevines in the same region. The actual CO and VC dose was calculated after analyzing their total N content, which is displayed in Table 3, and humidity at the time of application. Organic fertilizers were applied annually, in each growing season's bud burst, concentrated in rows and under grapevines' canopy projection, as suggested by Brunetto et al. [5]. CO was obtained in an industrial organic composter (Biosolos, Veranópolis, RS, Brazil), which composted raw winery residues (grape pomace) in open air. The manufacturer stated that the compost was made with winery residues (mainly grape skin, seeds, and stalks) and that the residues are composted for 60 days.

VC was made in a closed worm composter, located at the Federal Institute of Education, Science and Technology, Restinga campus, in Porto Alegre (RS), Brazil. Each year, winery residues (grape pomace) were composted for 60 days (thermophilic phase), and then vermicomposted with Eisenia sp. worms for another 120 days (humification phase), with constant addition of water. Grape pomace mainly comprised grape skin, seeds, and stalks. Both CO and VC were stored at room temperature after stabilization.

The monthly rainfall and mean temperatures were collected from the experiment intallment up until February 2023 (end of last growing cycle). These data were obtained at an automatic meteorological station (National Institute of Meteorology conventional station) located 150 m from the vineyards. Results are shown in Figure 1.

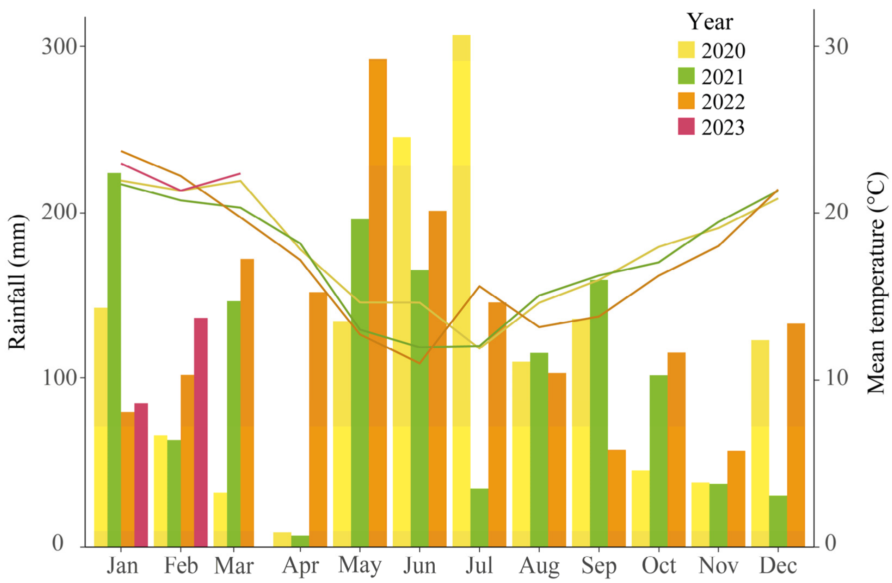

Figure 1. Accumulated monthly rainfall (mm), shown in bars, and monthly mean temperatures (°C), shown in lines, from January 2020 to February 2023, obtained from an automatic meteorological station (National Institute of Meteorology conventional station) located 150 m from the vineyards.

2.2. Soil Characterization and Physical and Physicochemical Attribrutes

Prior to beginning the experiment in 2020, soil samples from the 0–0.2 m layer were collected from Vineyards 1 and 2 for the following physicochemical soil analyses: exchangeable Ca and Mg (1:20 1 mol L^{-1} KCl), exchangeable K (1:10 Melich-1 extractor), available P (1:10 Melich-1 extractor), pH (1:10 distilled water), SOM, through dry combustion (FlashEA 1112 instrument from Thermo Electron Corp, Milan, Italy), and soil texture [25]. The soil samples comprised 15 subsamples, taken randomly throughout each vineyard, within the limits of the experiment. Subsequently, after three growing seasons (2023), soil samples were collected from each experimental unit, and the aforementioned variables were reanalyzed, with the exception of soil texture. Soil sampling was performed using a tubular soil probe. These results are shown in Table 1.

Table 1. Soil physical and physicochemical analyses in the 0.0–0.20 cm soil layer, in Vineyard 1 and Vineyard 2, before beginning the experiment (August 2020) and after three years of application (April 2023) of the following treatments: control (C—no organic fertilizers), grape pomace vermicompost (VC), and grape pomace compost (CO).

	Clay (%)	pH	Exchang. K (mg dm^{-3})	Available P (mg dm^{-3})	Exchang. Ca ($cmol_c$ dm^{-3})	Exchang. Mg ($cmol_c$ dm^{-3})
2020						
Vineyard 1	33	6.7	229	26.29	10.85	1.87
Vineyard 2	30	5.3	137	4.48	4.74	1.35
2023						
Vineyard 1						
C		6.04	87.84	20.52	8.88	1.81 b [2]
VC	*NA*	6.19	93.90	31.93	7.63	1.67 ab
CO		5.93	90.53	19.59	8.40	2.16 a
p-value [1]		0.46	0.43	0.32	0.40	0.04
Vineyard 2						
C		4.71	101.59	10.66	5.91	2.50
VC	*NA*	5.52	115.59	20.03	4.76	1.92
CO		5.35	100.02	15.74	4.95	1.86
p-value [1]		0.82	0.78	0.07	0.83	0.71

[1] *p*-values of ANOVA at α = 5%, [2] different lowercase letters indicate different means among organic fertilizer applications (Tukey test, α = 5%). *NA = not assessed.*

Table 1 shows that there was no difference among soil fertilization managements after three years in Vineyards 1 and 2, with the exception of soil exchangeable Mg. However, in Vineyard 1, soil exchangeable K in the 0.0 to 0.20 m layer severely decreased (Table 1), mainly due to percolation from the topsoil. This probably happened because of the high exchangeable K content at the beginning of the experiment (August 2020), above the soil capacity to retain it. Also, in Vineyard 2, the same phenomenon might have been prevented due to the vineyard's polyethylene coverage, which prevents rainfall in the cultivation rows. Due to their higher adsorption energy, that did not happen to P, Ca, and Mg. Notably, available P content increased in Vineyard 2 soil without any fertilization (C) (Table 1). This probably happened due to the adoption of a full-year coverage of cover crops from the beginning of the experiment. These species could have solubilized P in the plants' rhizosphere, via organic acid exudation [18,22], absorbed and assimilated it, and then kept cycling this nutrient.

2.3. Grape Pomace Residue Composition

After maturation, samples of VC and CO were dried in a forced-air-circulation oven (MA035/2, Marconi, Piracicaba, Brazil) at ±65 °C until constant mass in order to calculate their humidity (%). The dried materials were then characterized regarding their chemical composition and properties. Samples of the air-dried CO and VC were digested in H_2SO_4 and H_2O_2(2:1) [25] and HNO_3 and $HClO_4$ (3:1) [25]. The sulfuric–peroxide digestion extract was distilled in a micro-Kjeldahl N distiller (Tecnal, TE-0363, São Paulo, Brazil) and titrated in standardized ±0.025 mol L^{-1} of H_2SO_4 to quantify total N content [25]. The nitric–perchloric extract was diluted and the concentration of P was quantified using the molybdate-blue technique, in a UV–visible spectrophotometer (V-5000, Shanghai Metash Instruments Co, Shanghai, China), at 882 nm [26]; K concentration was quantified in a flame photometer (910, Analyser, Brazil); and Ca, Mg, copper (Cu), and zinc (Zn) concentrations were quantified by atomic absorption spectrophotometry (AAnalyst 200, Perkin-Elmer, Shelton, CT, USA). Total C content was quantified in finely ground CO and VC via dry combustion followed by gas chromatography, using an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, Milan, Italy), in triplicates.

CO P and K contents were 2.64 and 2.76 times higher than VC's (Table 2). Ca content was 5.61 times higher in VC than in CO (Table 2). The Cu content in compost, however, was 2.41 times higher in CO than in VC (Table 2). Also, VC had a mean C:N ratio of 15.79, which is notably lower than the 23.92 ratio found in CO (Table 3).

Table 2. Mean elementary composition of grape pomace vermicompost (VC) and grape pomace compost (CO) during the three years of treatment application.

Element	CO	VC
P (g kg^{-1})	2.59	6.84
K (g kg^{-1})	8.61	23.73
Ca (g kg^{-1})	1.74	0.31
Mg (g kg^{-1})	1.59	1.29
Cu (mg kg^{-1})	49.88	120.35
Zn (mg kg^{-1})	47.78	46.70

Table 3. Organic Fertilizer N Content, Doses, and C Input.

Organic Fertilizers		2020/21	2021/22	2022/23
VC	N content [1] (dry base)	0.57%	1.59%	1.48%
	Fertilizer dose (dry weight) (Mg ha^{-1})	7.02	2.52	2.70
	C input (Mg ha^{-1})	1.22	0.58	0.62
	C:N [1] ratio	17.38	14.47	15.51
CO	N content [1] (dry base)	1.02%	1.08%	0.92%
	Fertilizer dose (dry weight) (Mg ha^{-1})	3.92	3.70	4.35
	C input (Mg ha^{-1})	0.94	0.89	1.04
	C:N [1] ratio	23.51	22.27	25.99

[1] Total C and N quantification is detailed in Section 2.3.

2.4. Soil Sampling and Granulometric Fractionation of SOM

After three years of treatment application (April 2023), soil samples were collected from each replicate of Vineyards 1 and 2. The samples were collected from the 0–0.05, 0.05–0.10, 0.10–0.20, and 0.20–0.40 m soil layers in the vineyards' rows (under grapevine canopies) using a tubular soil probe. Each sample comprised three subsamples. Briefly after sampling, soil samples were left to air-dry until they reached a constant mass. After that, they were sieved through a 2 mm mesh to remove the stone fractions and root debris. Finally, they were stored in closed pots.

All the soil samples were fractionated based on their granulometric properties, similar to a methodology proposed by Poeplau et al. [27]. First, 20 g of the air-dried 2 mm sieved soil samples was dispersed in 10 mL of NaOH 1 mol L^{-1} and 40 mL of deionized water with two 5 mm diameter nylon beads for 4 h on a shaker at 150 rpm. The soil suspension was then sieved through a 53 µm mesh. The retained fraction (>53 µm) was re-suspended in distilled water to separate light (floating material) and dense fractions. However, no material was recovered in the light fraction in this study. Therefore, all the >53 µm material comprised the sand fraction (>53 µm). The material that passed through the sieve comprised the silt + clay fraction (≤53 µm). Both fractions were oven-dried at

50 °C in a forced-air-circulation oven (MA035/2, Marconi, Piracicaba, Brazil) and ground to the consistency of flour in a ball mill. The concentration of C and N was then measured in each fraction via dry combustion followed by gas chromatography in an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, Milan, Italy). Organic C present in the sand fraction was named particulate organic carbon (POC), and organic C present in the silt + clay fraction was named mineral-associated organic C (MAOC). Similarly, N content of the sand fraction was named particulate nitrogen (PN), and the N present in the silt + clay fraction was named mineral-associated nitrogen (MAN).

Additionally, SOC and TN were quantified via dry combustion followed by gas chromatography in an elemental analyzer (Flash EA 1112, Thermo Electron Corporation, Milan, Italy), in the air-dried soil samples, after 2 mm sieving and grinding to a flour consistency in a ball mill.

2.5. Soil C and N Stocks

After three years of treatment application (April 2023), soil bulk density was measured in Vineyards 1 and 2 in the same layers where samples were collected for C and N analyses (0–0.05 m; 0.05–0.10 m; 0.1–0.2 m; and 0.2–0.4 m), with methodology of Teixeira et al. [28]. Additionally, each sample was dispersed in a mixture of distilled water and 1 mol L^{-1} NaOH and then sieved through a 2 mm screen. The retained mineral material was called the stone fraction and weighed. Soil stoniness (SS) was calculated by the ratio (m:m) of the stone fraction to the total bulk soil sample. SS was quantified because the C and N content was quantified in fine soil; C and N stocks, however, concern the bulk soil. Multiplying the C or N content by the total mass of bulk soil would overestimate C and N stocks, since the stone fraction possesses virtually no C or N. These results are shown in Table 4.

Table 4. Soil bulk density (Ds) and soil stoniness (SS) in Vineyard 1 and Vineyard 2 after three years of application (April 2023) of the following treatments: control (C—no organic fertilizers), grape pomace vermicompost (VC), and grape pomace compost (CO).

Treatments	Soil Layers							
	0.0–0.05 m		0.05–0.10 m		0.10–0.20 m		0.20–0.40 m	
	Ds ($Mg\ m^{-3}$)	SS ($g\ g^{-1}$)	Ds ($Mg\ m^{-3}$)	SS ($g\ g^{-1}$)	Ds ($Mg\ m^{-3}$)	SS ($g\ g^{-1}$)	Ds ($Mg\ m^{-3}$)	SS ($g\ g^{-1}$)
	Vineyard 1							
C	1.15	0.08	1.38	0.11	1.51	0.07	1.51	0.04
VC	1.01	0.07	1.37	0.09	1.50	0.07	1.47	0.03
CO	1.18	0.05	1.41	0.09	1.48	0.07	1.48	0.03
	Vineyard 2							
C	0.85	0.17	0.97	0.16	1.02	0.13	1.08	0.12
VC	0.82	0.18	0.98	0.17	1.04	0.17	1.07	0.14
CO	0.80	0.17	0.99	0.17	1.05	0.14	1.09	0.14

Soil C and N stocks, as well as their fractions' stocks, were then calculated as follows:

$$\mathrm{Stock}(\mathrm{Mg\ ha^{-1}}) = \sum_{i=1}^{4} X_i \cdot V_i \cdot Ds_i \cdot (1 - SS_i) \qquad (1)$$

where:

X: C or N fraction (SOC, POC, MAOC, TN, PN, or MAN) ($g\ kg^{-1}$);
V: soil volume (m^3), which is each layer thickness multiplied by 1 ha;
Ds: soil bulk density ($kg\ m^{-3}$);
SS: soil stoniness ($g\ g^{-1}$);
1–4: each of the four analyzed soil layers: 0–0.05, 0.05–0.1, 0.1–0.2, and 0.2–0.4 m.

As shown in Table 4, Ds in Vineyard 2 was particularly low (Ds < 1.0 Mg m^{-3}) in the first 0.10 m of the soil profile. This attribute is result of a combination of high SOM (Table 6)—although not high enough to be characterized as a folic horizon [24]—and high porosity, due to previous soil plowing at vineyard installment (2017).

2.6. Statistical Analyses

The obtained experimental data were tested for homogeneity with the Shapiro–Wilk test. The effect of the organic fertilizers upon each response variable was tested with analysis of variance (ANOVA). Whenever the null hypothesis (equal means) was rejected with alpha equal to 0.05, means were compared via the Tukey test. The "ExpDes" package [29] in R statistical software (version 4.4.0) [30] was utilized for these analyses. Data visualization figures were created using the "ggplot2" package [31] in R statistical software [30].

3. Results and Discussion

After three years, SOC stocks did not differ among treatments in the layers below 0.05 m in both Vineyards 1 and 2, with the exception of the 0.2–0.4 m layer in Vineyard 1 (Figure 2). In the 0–0.05 m layer of Vineyard 1, however, the C stock was lower in soils amended with VC (Figure 2a). On the other hand, in Vineyard 2 the mean SOC stocks were not different among any treatments (Figure 2b). We highlight that SOC stocks are higher in the 0.20–0.40 m layer due to its thickness, not SOC content (Tables 5 and 6). SOC and SOC stock were lower in this layer in Vineyard 1 (Figure 2a) when amended with VC. Notably, the lack of increase in SOC in any soil layers in Vineyards 1 and 2 might be related to the high rainfall and high summer temperature, typical of this study site's climate. These climatic conditions might intensify residue mineralization to higher rates and prevent C from being sequestered in more stable forms [17,20,21,32].

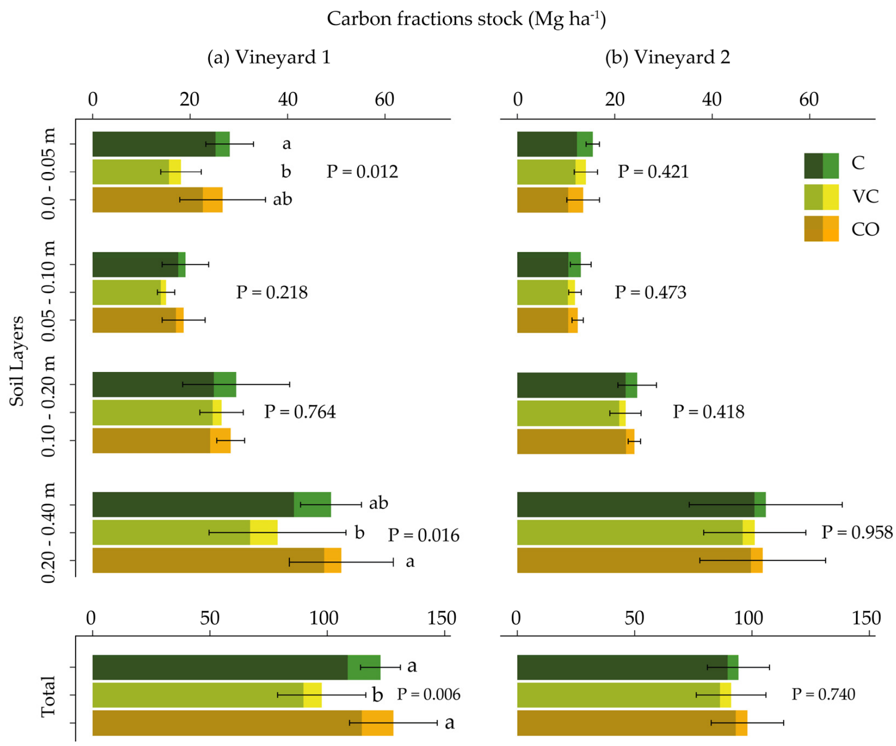

Figure 2. C stocks on 0.0–0.05 m, 0.05–0.10 m, 0.10–0.20 m, and 0.20–0.40 m soil layers, in Vineyard 1 ('Isabella') (**a**) and Vineyard 2 ('Chardonnay') (**b**), after three years of the following treatment applications: C—control (no organic fertilization), VC—fertilization with grape pomace vermicompost, and CO—fertilization with grape pomace compost. Darker colors indicate the MAOC fraction and the lighter colors indicate the POC fraction. *p*-values of ANOVA test are shown and different letters indicate different means among treatments (Tukey test, α = 5%).

Table 5. Soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC) content in different layers (0.0–0.05 m, 0.05–0.1 m, 0.1–0.2 m, and 0.2–0.4 m) in Vineyard 1 ('Isabella') after three years of grape pomace vermicompost (VC) and compost (CO); C stands for control (no organic fertilization).

		SOC (g kg^{-1})	MAOC (g kg^{-1})	POC (g kg^{-1})
0.0–0.05 m	C	53.5 ± 0.54 a	42.3 ± 0.63 a	11.2 ± 0.51 a
	VC	37.2 ± 0.89 b	31.7 ± 0.76 b	5.6 ± 0.14 b
	CO	48.9 ± 0.88 ab	37.7 ± 0.70 ab	11.2 ± 0.40 a
	p-value	0.01	0.04	0.03
0.05–0.1 m	C	30.2 ± 0.73	23.5 ± 0.24	0.67 ± 0.58
	VC	24.3 ± 0.30	21.3 ± 0.25	0.30 ± 0.09
	CO	29.1 ± 0.48	24.5 ± 0.41	0.47 ± 0.21
	p-value	0.18	0.22	0.27
0.1–0.2 m	C	21.0 ± 0.54	18.9 ± 0.52	2.10 ± 0.07
	VC	19.0 ± 0.18	17.6 ± 0.15	1.40 ± 0.05
	CO	20.8 ± 0.20	19.3 ± 0.22	1.50 ± 0.03
	p-value	0.61	0.14	0.94
0.2–0.4 m	C	17.7 ± 0.17 a	16.9 ± 0.18 a	0.80 ± 0.04
	VC	13.4 ± 0.43 b	12.7 ± 0.40 b	0.70 ± 0.05
	CO	17.2 ± 0.12 ab	16.3 ± 0.10 ab	0.80 ± 0.06
	p-value	0.04	0.03	0.89

p-values of ANOVA test are shown and different letters indicate different means among organic fertilizers (Tukey test, α = 5%).

The mean SOC in different soil fractions was different in the 0.0–0.05 m layer among different treatments in Vineyard 1 (Table 5). The highest values of SOC, MAOC, and POC were found in soils without organic fertilizers added, although not being different than soils amended with CO (Table 5). Notably, soils amended with VC had lower SOC, MAOC, and POC content than control in the 0–0.05 m layer (Table 5). Interestingly, in this layer, around 15% of C was in the POC fraction, which is lower when compared to 21 and 23% in C and CO, respectively (Table 5). POC represents the dynamic fraction of C in soils and can be mineralized faster than the MAOC, which is protected in organic-matter–mineral physico-chemical interactions [32–34]. This difference in the C fraction content in the soil layers was also observed for C stocks in its fractions (Figure 2).

In Vineyard 2, there was no difference in mean SOC, MAOC, or POC content in the soil among treatments (Table 6). Although not statiscally different, in Vineyard 2, the soil without any organic fertilizer amendment (C) had higher values for SOC (Table 6), similarly to Vineyard 1 (Table 5).

Table 6. Soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC) content in different layers (0.0–0.05 m, 0.05–0.1 m, 0.1–0.2 m, and 0.2–0.4 m) in Vineyard 2 ('Chardonnay') after three years of grape pomace vermicompost (VC) and compost (CO); C stands for control (no organic fertilization).

		SOC (g kg^{-1})	MAOC (g kg^{-1})	POC (g kg^{-1})
0.0–0.05 m	C	41.1 ± 0.49	36.8 ± 0.43	4.2 ± 0.16
	VC	37.5 ± 0.84	31.6 ± 0.65	5.9 ± 0.32
	CO	38.3 ± 0.52	33.6 ± 0.51	4.8 ± 0.10
	p-value	0.62	0.28	0.46
0.05–0.1 m	C	29.9 ± 0.31	27.2 ± 0.24	2.7 ± 0.16
	VC	28.0 ± 0.29	25.4 ± 0.25	2.6 ± 0.14
	CO	27.0 ± 0.35	25.0 ± 0.30	1.9 ± 0.07
	p-value	0.36	0.38	0.59

Table 6. *Cont.*

		SOC (g kg^{-1})	MAOC (g kg^{-1})	POC (g kg^{-1})
0.1–0.2 m	C	26.7 ± 0.29	25.7 ± 0.28	0.9 ± 0.02
	VC	25.5 ± 0.11	24.6 ± 0.10	0.9 ± 0.02
	CO	23.7 ± 0.29	22.7 ± 0.27	0.9 ± 0.03
	p-value	0.16	0.14	0.94
0.2–0.4 m	C	25.9 ± 0.65	21.7 ± 0.55	0.42 ± 0.23 a
	VC	25.6 ± 0.51	23.8 ± 0.48	0.18 ± 0.09 b
	CO	25.0 ± 0.41	21.2 ± 0.34	0.38 ± 0.25 ab
	p-value	0.96	0.64	0.04

p-values of ANOVA test are shown and different letters indicate different means among organic fertilizers (Tukey test, α = 5%).

Soil TN, MAN, and PN showed a very similar pattern among layers and treatments as SOC, MAOC, and POC, respectively, in both Vineyards 1 and 2 (Table 7; Table 8). TN was the lowest in the 0.0 to 0.05 m layer of soils fertilized with VC in Vineyard 1 (Table 7), as it was the lowest in SOC (Table 5). Although not different according to ANOVA, soils fertilized with VC usually showed lower values of TN and MAN than the other conditions in Vineyard 1 (Table 5). Similarly to SOC, in Vineyard 2 there were no effect of the different residues in TN, MAN, or PN (Table 8). We observed, however, that POC and PN have a higher decrease with soil depth than the more stable fractions (MAOC and MAN). Nitrogen stocks also showed a similar pattern among layers and treatments to C stocks, in both Vineyards 1 and 2 (Figure 3). In Vineyard 1, total N stocks were lower in soils amended with VC (Figure 3a), which indicates that the mineralization of SOM also depletes N stocks. In Vineyard 2, as it was for C stocks, there were no differences among treatments (Figure 3b).

Table 7. Soil total nitrogen (TN), mineral-associated nitrogen (MAN), and particulate nitrogen (PN) content in different layers (0.0–0.05 m, 0.05–0.1 m, 0.1–0.2 m, and 0.2–0.4 m) in Vineyard 1 ('Isabella') after three years of grape pomace vermicompost (VC) and compost (CO); C stands for control (no organic fertilization).

		TN (g kg^{-1})	MAN (g kg^{-1})	PN (g kg^{-1})
0.0–0.05 m	C	4.65 ± 0.52 a	3.62 ± 0.40	1.03 ± 0.20
	VC	3.22 ± 0.80 b	2.29 ± 0.56	0.93 ± 0.20
	CO	4.11 ± 0.74 ab	2.97 ± 0.44	1.15 ± 0.31
	p-value	0.01	0.08	0.26
0.05–0.1 m	C	2.63 ± 0.55	2.05 ± 0.44	0.58 ± 0.09
	VC	2.08 ± 0.25	1.48 ± 0.18	0.60 ± 0.05
	CO	2.46 ± 0.42	1.77 ± 0.25	0.68 ± 0.05
	p-value	0.18	0.49	0.82
0.1–0.2 m	C	1.77 ± 0.43	1.38 ± 0.25	0.39 ± 0.07
	VC	1.64 ± 0.16	1.17 ± 0.12	0.47 ± 0.06
	CO	1.76 ± 0.17	1.27 ± 0.20	0.49 ± 0.05
	p-value	0.29	0.25	0.08
0.2–0.4 m	C	1.44 ± 0.19	1.12 ± 0.15	0.32 ± 0.04
	VC	1.15 ± 0.32	0.82 ± 0.09	0.33 ± 0.02
	CO	1.47 ± 0.17	1.06 ± 0.11	0.41 ± 0.05
	p-value	0.07	0.43	0.14

p-values of ANOVA test are shown and different letters indicate different means among organic fertilizers (Tukey test, α = 5%).

Table 8. Soil total nitrogen (TN), mineral-associated nitrogen (MAN), and particulate nitrogen (PN) content in different layers (0.0–0.05 m, 0.05–0.1 m, 0.1–0.2 m, and 0.2–0.4 m) in Vineyard 2 ('Chardonnay') after three years of grape pomace vermicompost (VC) and compost (CO); C stands for control (no organic fertilization).

		TN (g kg^{-1})	MAN (g kg^{-1})	PN (g kg^{-1})
0.0–0.05 m	C	3.41 ± 0.53	3.04 ± 0.42	0.37 ± 0.06
	VC	3.19 ± 0.75	2.69 ± 0.56	0.50 ± 0.13
	CO	3.19 ± 0.49	2.80 ± 0.39	0.39 ± 0.08
	p-value	0.77	0.61	0.36
0.05–0.1 m	C	2.38 ± 0.28	2.17 ± 0.11	0.22 ± 0.05
	VC	2.22 ± 0.24	2.02 ± 0.19	0.21 ± 0.02
	CO	2.15 ± 0.29	1.99 ± 0.24	0.16 ± 0.02
	p-value	0.36	0.53	0.17
0.1–0.2 m	C	2.10 ± 0.23	2.03 ± 0.25	0.07 ± 0.04
	VC	1.91 ± 0.22	1.84 ± 0.19	0.07 ± 0.02
	CO	2.03 ± 0.05	1.95 ± 0.08	0.08 ± 0.02
	p-value	0.29	0.60	0.05
0.2–0.4 m	C	2.26 ± 0.52	2.23 ± 0.41	0.02 ± 0.02
	VC	2.26 ± 0.43	2.24 ± 0.37	0.03 ± 0.01
	CO	2.23 ± 0.31	2.19 ± 0.27	0.04 ± 0.01
	p-value	0.99	0.67	0.12

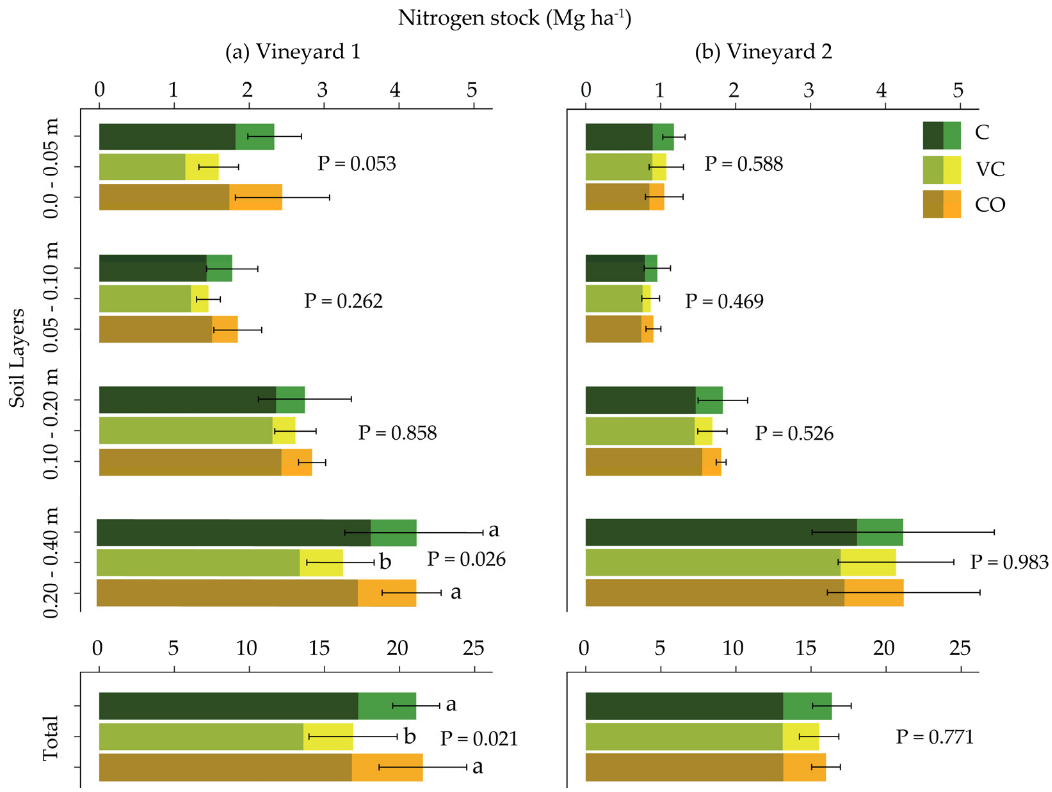

Figure 3. N stocks on 0.0–0.05 m, 0.05–0.10 m, 0.10–0.20 m, and 0.20–0.40 m soil layers, in Vineyard 1 ('Isabella') (**a**) and Vineyard 2 ('Chardonnay') (**b**), after three years of the following treatment applications: C—control (no organic fertilization), VC—fertilization with grape pomace vermicompost, and CO—fertilization with grape pomace compost. Darker colors indicate the MAN fraction and the lighter colors indicate the PN fraction. *p*-values of ANOVA test are shown and different letters indicate different means among treatments (Tukey test, α = 5%).

The annual addition of VC to the soil in vineyard agroecosystems failed to increase SOC content and SOC stocks after a 3-year period (Table 5 and Figure 2). Conversely, amending soil with VC resulted in a lower SOC, MAOC, and POC in the topsoil (0.0–0.05 m), being equivalent to around 30%, 26%, and 50% compared to control (soil without fertilizations) in Vineyard 1 (Table 5). Vermicomposts tend to have more labile forms of C due to the vermicomposting phase and the transformation that occurs both inside and outside earthworms' digestive systems [11,12]. Also, the C:N ratio was lower in every year of application, which means they can provide sufficient N for the soil microbial biomass to grow substantially. Probably due to these characteristics, when added to the soil, VC was mineralized and consumed faster by soil microbiota when compared to CO, which is in accordance with previous studies [11,35]. The lower POC content in Vineyard 1 soil corroborates that explanation, since this fraction also comprehends sand-size particles of the residues. The labile C added via VC can foster soil microbiological activity, which speeds up its own mineralization. Also, studies have shown that vermicomposts have chemical compounds which can enhance microbiological growth and activity in soils [36–38]. After undergoing mineralization, the increased presence of soil microbiota, together with VC-mineralized N (NO_x^-) availability, facilitates the mineralization of SOM and leads to a reduction of SOC and TN contents (and stocks), thereby exemplifying the soil priming effect [39]. However, no microbiological analyses were performed in our study. Nonetheless, these phenomena could possibly explain the results observed, for instance, in Vineyard 1 (Table 5).

However, in Vineyard 2, there was no difference in mean SOC, MAOC, POC, TN, MAN, or PN content in the soil among treatments (Table 6; Table 8). So, the phenomenon observed for VC in Vineyard 1 was not observed in Vineyard 2.

On the other hand, CO did not deplete, nor increase, SOC and the fractions MAOC and POC in both vineyards (Table 5; Table 6), as well as their stocks (Figure 2). The same thing is valid for TN, MAN, PN (Table 7; Table 8), and N stock (Figure 3). Since CO has a higher mean value of the C ratio (23.92), it is significantly less likely to induce priming effects on soils and further mineralize SOM [39]. Also, CO does not undergo the vermicomposting process, and thus has less labile forms of C [11,12].

4. Conclusions

Soil amendment with grape pomace residues (grape pomace compost and vermicompost) was unable to increase C or N stocks, SOC, or TN, nor its mineral-associated and particulate fractions, at the tested amounts, in subtropical vineyards after three years of treatment application. Grape pomace vermicompost, due to its low C:N ratio, acted as a primer and further mineralized SOM, reducing C and N stocks in one of the vineyards. Short-term results prevent stating that the tested residues are an appropriate strategy for increasing C or N stocks in subtropical vineyard soils. However, we highlight that the results of this study are time-limited and that the effects of the residues on C stock and fractions can be distinct in medium- or long-term experiments. The results from these studies allowed initial understanding of the yet understudied field of organic fertilization and organic viticulture in subtropical regions. However, some insights are still impossible to achieve in a three-year experiment. Thus, we highlight and recommend future studies to test higher doses of grape pomace residues, in order to increase SOC, as well as test the results after longer-term experiments.

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