



Changes in vineyard soil parameters after repeated application of organic-inorganic amendments based on spent mushroom substrate

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ABSTRACT

The changes of physicochemical and biochemical parameters of a silty loam (S1) and sandy loam (S2) vineyard soils added with spent mushroom substrate (SMS) or SMS composted with ophite (OF) as rock dust (SMS + OF) were studied. Two doses of SMS or SMS + OF (25 and 100 Mg ha⁻¹) were applied for two consecutive years (2020–2021) and changes of soil physicochemical parameters, and dehydrogenase activity (DHA), respiration (RES), microbial biomass (BIO), and the phospholipid fatty acids (PLFAs) profile were assayed on a temporal basis. The results showed an increase in soil organic carbon (OC) content, total and mineralised N, P, and K, especially when the highest SMS dose was applied to soils. Repeated application caused OC content over time up to 2.3 times higher than initial content in the silty loam soil. This increase was not observed in sandy soil, possibly due to a higher bioavailability of OC, as indicated by the evolution of extractable humic acid/fulvic acid pools. In both soils, all biochemical parameters increased after amendment, being favoured both by the OC and by the presence of OF. Significant positive correlations were found between DHA, RES and BIO, and OC content especially in the first part and then levelled off after the second dose application. Total bacterial or fungal PLFAs patterns reflected the variation of BIO by SMS application. The higher growth of fungi vs. bacterial community in amended soils was recorded after the first SMS application, although the opposite effect occurred after the second application, with similar results in both soils. The findings indicate that the application of SMS or SMS + OF in vineyard soils could be an appropriate agronomic management practice for maintaining soil sustainability, although doses and application times of these amendments should first be evaluated depending on soil texture.

1. Introduction

Soil degradation is a serious problem in many parts of the world, threatening agricultural development and human food security (Ray et al., 2019; Zhang et al., 2022). Sustainable agricultural practices are increasingly necessary because long-term intensive agriculture may compromise the soil's physical, chemical and biological quality (Amoah-Antwi et al., 2020; Beig et al., 2022; Swoboda et al., 2022). Different strategies have been proposed as potential management practices to avoid or delay this process (Amami et al., 2021; Ray et al., 2019; <https://ejpsol.eu/soil-research/i-sompe>). One of these strategies involves the application of organic amendments to soil to increase its organic matter (OM) content (Bonanomi et al., 2020; Zhang et al., 2022). This practice improves water and nutrient holding capacity and

increase soil aggregation, as previously reported (Amoah-Antwi et al., 2020; Hernandez et al., 2017). Consequently, wastes of different origin (urban, industrial or agricultural) are frequently used as organic amendments in agricultural management practices, as their potential to improve soil physical structure, stimulate soil microbial activity, and maintain soil fertility and quality have been well documented (El-Naggar et al., 2019; Ibrahim et al., 2022; Zhang et al., 2022). The suitable management of organic amendments is a good way to maintain appropriate soil aggregate stability and structure, as basic factors for enhancing soil physical fertility (Lee et al., 2021; Manfredi et al., 2019) avoiding soil erosion, preventing and reversing soil degradation through the addition of OM to soils (Medina et al., 2012; Unagwu, 2019).

Spent mushroom substrate (SMS) is an organic waste generated by the production of edible mushroom; approximately 5 kg of SMS are

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produced per kg of mushrooms (Grimm et al., 2021). This output is increasing because global mushroom farming has increased in the last five decades at a rapid rate, with an average annual growth of 5.6% worldwide (Kulshreshtha, 2019). The application of this waste as a soil amendment after an appropriate composting process is considered to be a viable process. It could be of interest to enhance the sustainability of agricultural production systems as it is indicated in different reviews on the use and valorisation of SMS (Kit-Leong et al., 2022; Mohd Hanafi et al., 2018; Umor et al., 2021) given their ability to enrich the soil in OM and nutrients (Joniec et al., 2022) similar to other organic residues such as vegetable, fruit or garden waste compost (Arthur et al., 2011).

In addition to OM, other factors such as mineral nutrients are also considered important for improving crop production (Swoboda et al., 2022). Mineral nutrients are extracted from the soil with each harvest, and need to be adequately replaced by fertilisers or other amendments to avoid their total extraction and depletion and improve soil quality. In this context, the application of rock dust to soil after weathering or intensive soil management practices is now considered a natural resource for soil re-mineralisation (Cunha et al., 2022). It provides a large amount of nutrients, and has been recommended for its potential beneficial effects on increasing cation exchange capacity, pH, and mineral content (Swoboda et al., 2022). Some authors (Basak et al., 2021; Cunha et al., 2022) have reported a good performance in the productivity of plants subjected to the use of rock dust, mainly those with a long cycle, compared to plants subjected to conventional fertilisation. The literature is not clear about the best types of rock dust to use, but glacial and primary minerals, such as dolerite or feldspars, have been studied (Cunha et al., 2022). Moreover, the performance of rock dust can be improved by mixing these products with organic wastes, such as manure, sewage sludge and crop residues, to obtain nutrient-enriched materials, although the benefit of this compost will depend on the physical, biological and chemical nature of the mineral and organic materials that are co-composted (Basak et al., 2021; Meena and Biswas, 2014). García-Gómez et al. (2002) have found that a more active microbial population appears during the initial stages of the composting process of SMS with rock dust from glacial silt and quartz dolerite. This is due to changes in the physical properties of the material and the addition of essential nutrients for microbial growth and metabolism.

The application of soil organic amendments to increase OM and improve its structure and sustainability has been extensively studied as previously indicated. However, only a few studies have evaluated the effects of mixed organic-mineral compost on soil system although the benefits when minerals are associated with organic wastes, or not, are known to provide soils with enhanced chemical characteristics and plant yield (Enciso-Garay et al., 2016; Li et al., 2020a; Jones et al., 2009). In this context, a new organic-mineral compost was explored in this work based on SMS as organic material and the sub-volcanic rock ophite (OF) as mineral component has been proposed for application to agricultural soils for increasing their OM content and improving their re-mineralisation.

SMS is generated in significant amounts in La Rioja region (N-E Spain) by mushroom farming, amounting each year to more than 70 Mt (<https://www.larioja.org>). Accordingly, SMS and SMS composted with OF (SMS + OF) could be applied to the agricultural soils in this area in line with the objectives of the circular economy for the application of wastes generated in situ (Breure et al., 2018). The application of SMS and SMS + OF in vineyard soils in this region could be most expedient because these lands have been subjected to traditional soil management practices in viticulture, and they are facing an alarming loss of soil quality that has triggered a process of erosion and degradation (Peregrina et al., 2012). Vineyard soils extend over a large area in La Rioja (35.7% of the total cultivated area) (<https://www.larioja.org>), and most of these soils have OM levels of less than 2% and are compacted, unstructured and unbalanced, which impacts on grape quality and/or production, affecting the sustainability of vineyards in general

(Calleja-Cervantes et al., 2015a, 2015b; Pérez-Álvarez et al., 2013).

Studies on the application of amendments to vineyard soils with different organic wastes have been reported (Bonanomi et al., 2020; Bustamante et al., 2011; Herrero-Hernández et al., 2022; Marín-Martínez et al., 2021; Medina et al., 2015; Sánchez-Monedero et al., 2019). In general, these studies involve single applications of wastes in different dosages in soils in laboratory or field experiments, reporting the part of organic wastes play in reversing the decrease in soil OM content and the disruption of nutrients caused by intensive farming, although the increase in soil OM depends on the total OC content of the wastes applied. However, field-scale trials with repeated application of compost have scarcely been reported in vineyard soils, even though these practices could have a potential effect on carbon sequestration and the enhancement of carbon stocks (Calleja-Cervantes et al., 2015a,b; Morlat and Chaussod, 2008). Some studies have reported results at the end of the continuous application of different organic wastes as amendments for several years or even after each application, but to our knowledge no studies exist on the application of compost based on SMS + OF to vineyard soils.

In order to improve the vineyard soils, we intended to investigate 1) the changes in soil physicochemical and biochemical properties following the application of SMS or SMS + OF to two vineyard soils with different textures for two years under field conditions, and 2) the effects of both amendments applied at two dosages as a potential agronomic management practice for vineyard sustainability. These objectives are framed in the VITIREG Project of the Regenerative Viticulture Operational Group of La Rioja (<http://vitireg.org/>).

2. Materials and methods

2.1. Spent mushroom substrate and ophite

Spent mushroom substrate (SMS) was generated after *Agaricus bisporus* cultivation and production in the eastern Rioja region, and aerobically re-composted for three months. Ophite (OF) is a sub-volcanic igneous rock composed of manganese, iron, zinc and copper, among other metals, with a high concentration of magnesium taranite and plagioclase, and a medium-low concentration of epidote, quartz, and montmorillonite-chlorite. It was provided by Clean-Biotec S.L.L. (Logroño, Spain) and used as a mineral dust to re-mineralise the soil. The SMS was mixed with the OF at 15% and re-composted for a month, and both SMS and SMS + OF were kindly supplied by Sustratos de La Rioja S. L. (Pradejón, La Rioja, Spain). The characteristics of SMS, OF and SMS + OF were determined on a dry weight basis by the usual methods of analysis (Marín-Benito et al., 2009; Sparks, 1996) (Table 1).

2.2. Experimental set-up and soil sampling

A field assay was conducted over 2020 and 2021 in two vineyards: S1 (42°16'23.6"N, 2°04'0.058"W) and S2 (42°12'0.029"N 2°04'13.7"W), in the eastern part of the region "La Rioja Oriental" (N-E Spain). The climate at these sites is dry and warm, with a Mediterranean influence. Rainfall and air temperature were monitored over the whole experiment at an onsite weather station. The experimental layout was based on a randomised complete block with five treatments, and three replicates per treatment (15 plots of 3 m × 3 m). The treatments corresponded to unamended soil, soil amended with SMS at the rates of 25 and 100 Mg ha⁻¹ (S + SMS25 and S + SMS100, respectively), and 25 and 100 Mg ha⁻¹ of SMS + OF at 15% (S + SMS25+OF, S + SMS100+OF) on a dry weight basis. These rates are equivalent to the application of 5 and 20 g C kg⁻¹ soil, respectively. Prior to amendment, the soil was tilled using a field cultivator, and then SMS or SMS + OF was mixed with the topsoil (0–30 cm) in each plot using a rotavator. No additional fertilisation was carried out. The study was conducted in the grape growing season (March–October), and the soil was kept free of vegetation over the experimental period.

Table 1

Characteristics of spent mushroom substrate (SMS), ophite (OF) and unamended soils (S1 and S2) determined on dry weight basis.

Parameters	SMS	OF	SMS + OF	S1	S2
Texture	–	–	–	Silty Loam	Sandy Loam
Sand (%)	–	–	–	27.3 ± 4.99	48.7 ± 4.71
Silt (%)	–	–	–	50.2 ± 2.30	31.6 ± 2.99
Clay (%)	–	–	–	22.5 ± 2.84	19.7 ± 3.67
pH	7.60 ± 0.04	8.37 ± 0.34	7.48 ± 0.01	8.09 ± 0.06	8.50 ± 0.11
Electrical conductivity (dS m ⁻¹)	10.9 ± 0.08	0.25 ± 0.00	10.6 ± 0.27	0.74 ± 0.03	0.16 ± 0.02
CaCO ₃ (%)	14.6 ± 0.27	2.38 ± 0.14	10.7 ± 0.33	17.6 ± 0.78	17.0 ± 1.75
Organic matter (%)	41.9 ± 0.34	0.09 ± 0.04	31.2 ± 0.68	1.42 ± 0.10	0.98 ± 0.19
Organic carbon (%)	24.3 ± 0.20	0.05 ± 0.02	18.1 ± 0.39	0.82 ± 0.06	0.57 ± 0.11
Total N (%)	2.05 ± 0.00	0.02 ± 0.00	1.67 ± 0.03	0.12 ± 0.02	0.10 ± 0.00
C/N	11.9 ± 0.09	3.21 ± 1.55	10.8 ± 0.07	7.22 ± 1.03	6.50 ± 1.04
CEC (cmol _c kg ⁻¹)	42.9 ± 1.01	1.66 ± 0.03	32.9 ± 1.84	6.56 ± 0.12	5.01 ± 1.13
NH ₄ ⁺ -N (mg kg ⁻¹)	298 ± 2.97	5.51 ± 3.25	31.9 ± 5.33	6.43 ± 0.06	0.90 ± 0.00
NO ₃ ⁻ -N (mg kg ⁻¹)	587 ± 8.69	88.8 ± 5.89	673 ± 13.6	214 ± 15.0	46.3 ± 2.20
Available P (g kg ⁻¹)	1.14 ± 0.06	0.00 ± 0.00	1.01 ± 0.16	0.04 ± 0.01	0.02 ± 0.00
Available Ca (g kg ⁻¹)	28.5 ± 0.08	5.97 ± 0.31	31.9 ± 0.99	13.1 ± 0.51	14.3 ± 0.66
Available K (g kg ⁻¹)	18.1 ± 0.08	0.04 ± 0.00	14.4 ± 0.19	0.63 ± 0.01	0.32 ± 0.02
Available Mg (g kg ⁻¹)	3.64 ± 0.01	0.18 ± 0.01	3.17 ± 0.06	0.28 ± 0.04	0.11 ± 0.01
Available Cu (mg kg ⁻¹)	1.43 ± 0.04	0.29 ± 0.00	1.41 ± 0.05	0.24 ± 0.11	0.27 ± 0.05
Available Fe (mg kg ⁻¹)	14.5 ± 1.36	1.81 ± 0.30	17.2 ± 0.33	1.83 ± 0.08	1.38 ± 0.05
Available Mn (mg kg ⁻¹)	28.7 ± 0.76	8.38 ± 0.35	14.7 ± 0.19	10.6 ± 0.56	2.69 ± 0.17
Available Zn (mg kg ⁻¹)	9.61 ± 0.18	0.20 ± 0.00	7.94 ± 0.20	0.06 ± 0.00	0.06 ± 0.00

SMS and SMS + OF wastes were applied twice (March 2020 and 2021), and soil samples were taken one month after each SMS application (April 2020 and 2021), and then seven months later, once the grape harvest had ended (October 2020 and 2021). Five soil cores were collected in each plot from a depth of 0–30 cm for physicochemical analysis, and from 0 to 15 cm for biochemical and microbiological analysis. Composite samples of five cores were placed in polypropylene bottles and transported to the laboratory at IRNASA-CSIC (Salamanca, Spain) in portable refrigerators.

2.3. Soil physicochemical analysis

The S1 and S2 vineyard soils from the experimental plots are both classified as Aridisol, Typic Haplocalcid (Soil Survey Staff, 2010). S1 has a silty loam texture (27.3% sand, 50.2% silt, and 22.5% clay), and S2 has a sandy loam texture (48.7% sand, 31.6% silt, and 19.7% clay). Both soils have an OM content <2%, OM was calculated from the OC results determined using a LECO CN628 (LECO Corporation, Saint Joseph, MI) elemental analyzer and multiplied by 1.724. The characteristics of the unamended and amended soils from triplicate plots were determined using previously air-dried and sieved (<2 mm) samples. Standard analytical methods as indicated in Herrero-Hernández et al. (2022) were

used to determine soil pH, electrical conductivity (EC), total OC and N, total soluble N (NO₃⁻-N and NH₄⁺-N), assimilable P, assimilable macronutrients (Ca, K, and Mg) and micronutrients (Cu, Fe, Mn, and Zn), the cation exchange capacity (CEC), soil particle size distribution, and inorganic carbon (Table 1). In addition, clay minerals (illite and kaolinite) were qualitatively identified in the soil clay fraction via the X-ray diffraction technique using a Philips PW-1710 diffractometer (Eindhoven, the Netherlands). Alkali soluble and acid insoluble carbon (humic acid, HA) and alkali and acid soluble carbon (fulvic acid, FA) were also determined in soil extracts following the traditional method of HA and FA extraction from soil OC using a sodium pyrophosphate solution (Stevenson, 1982).

2.4. Soil biochemical analysis

Biochemical and microbial parameters were determined in surface soil samples (0–15 cm) from triplicate plots. Soil dehydrogenase activity (DHA) was determined by the Tabatabai method (Tabatabai, 1994). Briefly, 6 g of fresh soil was mixed with 60 mg of calcium carbonate and 1 mL 3% 2,3,5-triphenyltetrazolium chloride and 2.5 mL of ultrapure water. The reaction mixture was incubated at 37 °C for 24 h in the dark, and then the 1,3,5-triphenylformazan (TPF) was extracted. The absorbance of the supernatant was measured in a spectrophotometer at 485 nm. The results were expressed as mg TPF kg⁻¹ dry soil.

Soil respiration (RES) was determined by measuring the pressure drop caused by the O₂ consumed by microorganisms in 50 g of fresh soil over four days using OxiTop Control BM6 containers fitted with an OxiTop Control OC 110 measurement system (WTW, Weilheim, Germany). The CO₂ produced by the metabolism of soil microorganisms was trapped in 10 mL of NaOH 1 M. The metabolic activity of microorganisms was measured based on O₂ consumption. The results were expressed as mg O₂ kg⁻¹ dry soil.

The microbial biomass (BIO) and the microbial community structure of the soil samples were determined in lyophilised soil samples (2 g) using phospholipid fatty acid (PLFA) analysis, as described by Froste-gård et al. (1993). Total microbial BIO was estimated based on the total sum of PLFAs, and expressed as nmol g⁻¹, and it was determined as indicated in García-Delgado et al. (2018). Specific PLFAs were used as biomarkers to quantify the relative abundance of both Gram-negative and Gram-positive bacteria, as well as of Actinobacteria and fungi (Herrero-Hernández et al., 2022; Zelles, 1999).

2.5. Statistical analysis

Samples from triplicate plots were analysed, and the results presented as a mean of three repetitions. A Pearson's correlation matrix (CM) of the physicochemical characteristics, biochemical and microbial parameters was performed. A two-way analysis of variance (ANOVA) was carried out, with the main factors being soil treatment and sampling times after verifying normal data distribution using the Shapiro–Wilk test, and checking the homogeneity of variance using Levene's test. The Tukey post hoc test at p ≤ 0.05 was used to determine significant differences across means, and to evaluate the effects of the different soil treatment and sampling times on the physicochemical, biochemical, and microbial parameters. CM and ANOVA were carried out using the IBM SPSS Statistics v26 software package (IBM, Armonk, NY, USA). A principal component analysis (PCA) was carried out to analyse the relationships between the samples and the soil properties, and to identify similar groups in terms of physicochemical and biochemical properties at different sampling times by PAST v3.15 software (Hammer et al., 2001).

3. Results and discussion

3.1. Weather conditions

Weather conditions were recorded throughout the experiment (March 2020–October 2021, 210 days per experimental period) (Fig. 1). The mean maximum and minimum temperatures recorded over this period were 38.1 °C and −2.1 °C in S1, and 38 °C and −0.8 °C in S2, with ≈17 °C being the average temperature in both sites. The rainfall registered differed between the two experimental periods (2020 > 2021) and between the two sites (S2 > S1). The cumulative precipitation was 451 mm and 289 mm in S1 during the seven months in 2020 and 2021, respectively, while in S2 these values were 777 mm and 301 mm, respectively. The maximum daily precipitation was recorded over the first 16 days (92.7 mm in S1 and 204 mm in S2) in both soils in 2020 (Fig. 1), followed by light rainfall. In 2021, the highest rainfall was recorded after 185 days (38 mm and 45 mm in S1 and S2, respectively).

3.2. Effect of SMS on soil physicochemical properties

3.2.1. Changes in soil pH and electrical conductivity

Soil pH values were 8.09 (S1) and 8.50 (S2), respectively, before the first application of amendments (Table 1), and the use of SMS (SMS and SMS + OF) generally decreased the pH in both soils over time (Figs. S1A and B in Supplementary Material). The pH decreased in S1 (a silty loam texture) after the application of the high dose of SMS, while in S2 (a sandy soil texture) a significant decrease was observed after the application of both doses of SMS ($p \leq 0.05$). The pH values recovered seven months after the first SMS application in step with the evolution of SMS, although in S2 the high SMS dose partially inhibited this recovery. The changes in pH were similar after the second application of amendments, although pH recovery over time occurred in S2 but not in S1, especially in the presence of SMS + OF. A significant negative correlation between pH and OC was observed in S1 after one and seven months of both SMS applications ($r = -0.98$ – -0.86 , $p = 0.007$ – 0.05). However, significant correlations were only observed in S2 between pH and OC ($r = -0.91$ – -0.97 , $p = 0.008$ – 0.03) after seven months of SMS application in

both years, indicating that pH changes depended more on the SMS dose applied in S1 than in S2. Soil pH fluctuations were reported over time, depending on the initial pH, the nature and dose of the organic wastes, and/or soil buffering capacity (Arthur et al., 2011; Bustamante et al., 2011; Unagwu, 2019; Marín-Martínez et al., 2021). The microbial decomposition of SMS could contribute to these changes (Trivedi et al., 2017), which might occur after repeated application, although it was different for the two soils. Angelova et al. (2013) reported that the production of organic acids during the mineralisation of organic materials could decrease soil pH.

Initially, soil EC values were 0.74 and 0.16 in S1 and S2 unamended soils, respectively (Table 1), and they increased after the application of a high dose of SMS (Figs. S1C and D). EC values were lower after the second SMS application than after the first one, and only increased in S2 after applying a high dose of SMS or SMS + OF. The results changed contrary to pH values, and according to the significant negative correlation observed with pH values in S1 for the two periods following the first application of amendments ($r = -0.95$ – -0.94 , $p \leq 0.02$) and in S2 ($r = -0.96$, $p \leq 0.01$). EC indirectly indicates the concentration of soluble salts that the amendments could provide (Arthur et al., 2011). In fact, EC values in both soils were significantly related to OC content ($r = 0.95$ – 0.88 , $p \leq 0.05$ for S1 and $r = 0.82$ – 0.92 , $p \leq 0.03$ for S2) and to $\text{NO}_3\text{-N}$, and the P and K available ($p \leq 0.01$), although only after the first application of wastes. This effect was not observed after the second SMS application. EC increases after the soil application of compost and vermicompost compared to the control soil (Angelova et al., 2013; Galán-Pérez and Peña, 2019). EC is expected to increase with OF according to the results reported by Li et al. (2020b) on the higher amounts of soluble salts in a compost fortified with rock dust of a different nature compared to compost alone.

3.2.2. Changes in OC content

The OC content of the unamended soils was 0.82% (S1) and 0.57% (S2) (Table 1), and increased to 1.15%–3.89% (S1) and 1.55%–3.92% (S2) one month after the first SMS application at both doses (SMS25 and SMS100) ($p \leq 0.05$), due to the high OC content of the SMS applied (Fig. 2A and B). These values were lower when SMS + OF was applied

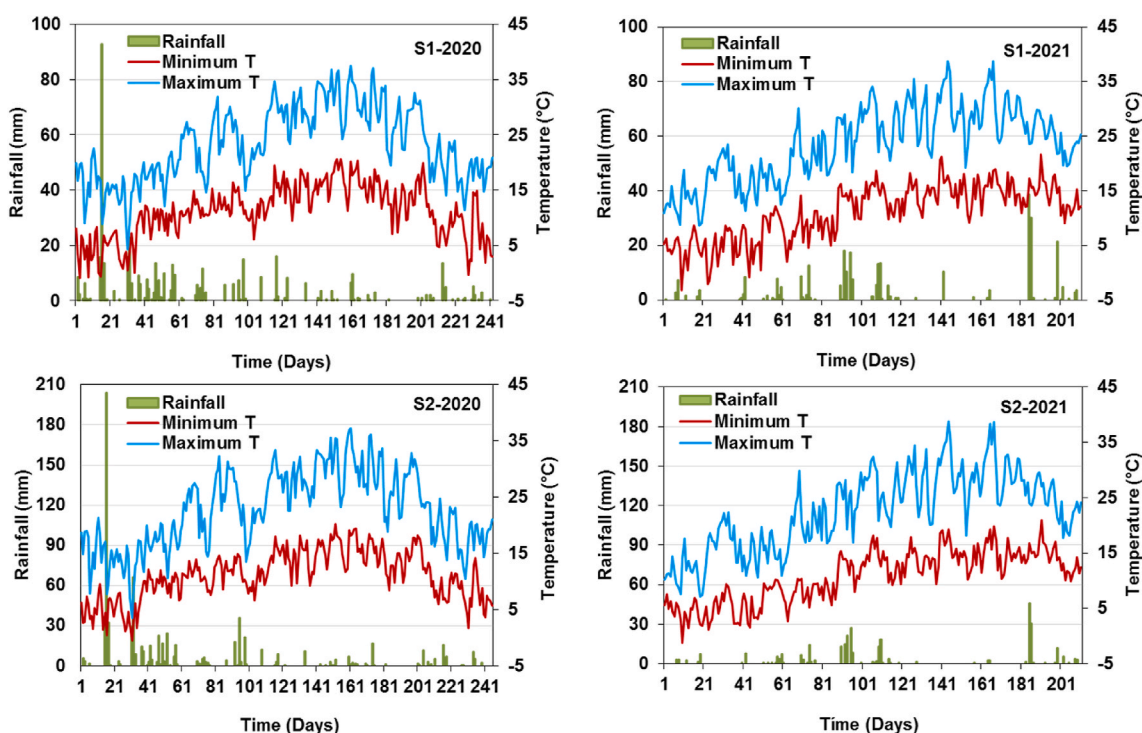


Fig. 1. Rainfall, maximum and minimum air temperatures recorded in soils S1 and S2 over experimental period (March 2020–October 2021).

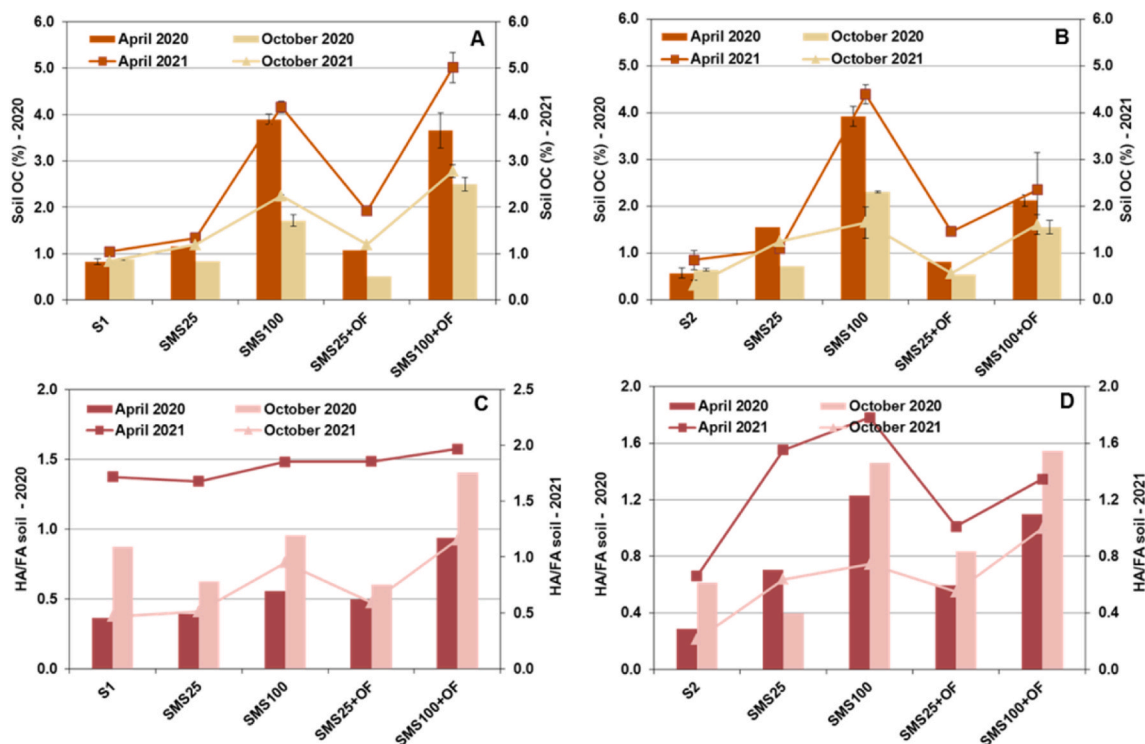


Fig. 2. Changes in organic carbon (OC) content and HA/FA ratios in the vineyard soils S1 (A,C) and S2 (B,D) unamended and amended at two doses of SMS and SMS + OF (SMS25, SMS100 and SMS25 + OF, SMS100 + OF) after one month (dark colours) and seven months (light colours) of the application in the year 2020 (bars) and 2021 (lines).

than with SMS alone due to the latter's lower OC content (Table 1). The OC content was significantly reduced by 28.0%–56.1% in S1 and 26.8%–53.4% in S2 seven months after the first SMS application due to the mineralisation of the organic wastes over time (Herrero-Hernández et al., 2022; Marín-Martínez et al., 2021). Despite this decrease, the OC content of soils amended at 100 Mg ha⁻¹ remained 1.9–2.8 times (S1) and 2.4–3.5 times (S2) higher than in the corresponding unamended soil ($p \leq 0.05$), but no increases were observed following the application of a low dose of SMS (25 Mg ha⁻¹) seven months after the first application. An initial increase in OC content followed by a decrease over time in S1 and S2 was also observed after the second application of wastes (Fig. 2A and B). However, the residual OC content seven months after the second application always increased with respect to the initial OC content of the unamended soils (up to 3.4 times in S1, and up to 2.8 times in S2) following the application of the high SMS dose (Fig. 2A and B).

It is also noteworthy that the OC residual content after two SMS applications (October 2021) increased between 1.1 and 2.3 times in the silty loam soil amended at different dosages compared to those of the corresponding soils in the same period (October 2020). This retention or capture of OC was not generally observed in the sandy soil (Fig. 2A and B), indicating the influence of soil texture in the possible sequestration of OC provided by SMS and its evolution over time. The OC content in S2 + SMS100 were higher than in the unamended soil, but the residual OC content did not increase over time (i.e., no sequestration of OC after the second application of SMS compared to the first one). The continuous application of wastes in S2 would therefore be necessary to maintain the OC content, while a continuous increase in OC content could be obtained in S1 after repeated SMS application. The amount of sequestered carbon depends on the balance between organic inputs and mineralisation processes (Morlat and Chaussod, 2008). In this context, Herrero-Hernández et al. (2011, 2022) report that the OC in a sandy loam soil such as S2 could be more bioaccessible to microorganisms for facilitating OC mineralisation, making OC storage more difficult than in S1.

The application of different organic wastes such as poultry manure,

waste compost (Unagwu, 2019), sheep/goat manure, distillery organic waste compost (Marín-Martínez et al., 2021), and green compost (Marín-Benito et al., 2018) increases the amount of OC in sandy or silty loam soils, but the experiments did not last long time (between 3 and 9 months) to evaluate possible retention of OC in soil over time and its effects on the reclaiming degraded soils. In a long-term experiment, Morlat and Chaussod (2008) found increased OC amounts up to 2.1 times the level of unamended sandy vineyard soil following the yearly application of composted cattle manure and SMS for 28 years successively. The increase in soil OC could reach a saturation value if the losses through mineralisation are approximately equal to the C inputs from the organic treatments. Calleja-Cervantes et al. (2015a,b) reported the need to apply different amounts of organic amendments over a 12-year period to increase OM content by 0.1% in a loamy-clay vineyard soil. The different maturity states of the OM in each amendment could explain the soil's C sequestering potential being favoured by the most complex and recalcitrant molecules (mature and stable) (Kubat and Lipavsky, 2006).

Some authors have used the relationship between the OC associated with the humic acid (HA) and fulvic acid (FA) fractions extractable by NaOH from the soil to ascertain the changes in the maturity and stability of OC and as an indicator of its future evolution in unamended and amended soils (Li et al., 2020a; Marín-Benito et al., 2012). These changes in the HA/FA ratio are influenced by the addition of OM from organic wastes (Angelova et al., 2013), although they are low due to the amounts of OC associated with humic- and fulvic-like substances extractable using NaOH, which are small compared to the large amount of OM in the organic amendments.

The HA/FA ratio was also determined for unamended S1 and S2 and for amended soils one and seven months after each SMS application (Fig. 2C and D). In general, an increase in this ratio was found for amended soils compared to unamended ones. These changes were significant in S2 for both SMS doses applied, but only in S1 when the high rate was applied ($p \leq 0.05$). Relative increases in the HA/FA ratio through the application of SMS to soils could occur if the FA fraction

decreases due to the effect of microorganisms during the experimental timeframe. A different OC evolution mechanism involving changes in this ratio might occur in S1 and S2 due to their different texture. The silt + clay fraction content in S1 is twice that in S2, and the content of the fine fraction, as well as the type and amount of clay minerals, has a direct influence on the OC stabilisation provided by organic amendments in the soil (Aguilera-Huertas et al., 2022; Medina et al., 2015; Sarkar et al., 2018). This process could explain the higher OC capture after two SMS applications in the silty loam soil (S1) compared to the sandy soil (S2).

3.2.3. Changes in macronutrients, micronutrients and exchange cation capacity

As expected, available P, K, Ca and Mg increased in the amended soils due to the nutrient provided by the organic amendments (Paula et al., 2017). P and K content (Fig. S2) initially increased with the high dose of SMS (S1) or with both doses of SMS (S2), but then decreased over time ($p \leq 0.05$). Changes in Ca and Mg content were non-significant after amendment application (data not shown). The P content was 40.4 mg kg^{-1} and 20.1 mg kg^{-1} in unamended S1 and S2, respectively (Table 1), and increased by 3.7–8.8 times (S1) and 13.2–4.4 times (S2) following the first and second applications of the amendments, respectively (Figs. S2A and B). In general, the highest increases were observed for both soils amended with the high dose of SMS + OF. P content decreased in the amended soils by 50% and 49% (S1) or by 73% and 57% (S2) seven months after amendment application for both experimental years. However, a real increase in the P content of both amended soils was recorded after the second application (October 2021) compared to the first application (October 2020) (Figs. S2A and B), with this cumulative effect being between 12% and 98% in S1 and 23%–190% in S2.

K content was 629 mg kg^{-1} and 322 mg kg^{-1} in unamended S1 and S2, respectively (Table 1), and increased by 2.7–5.9 times (S1) and by 3.8–5.8 times (S2) in SMS soils after the first and second SMS applications, respectively (Figs. S2C and D). In general, the highest increases were observed for SMS100 and SMS100 + OF amended soils. K content then decreased after the grape harvest by 58% and 37% (S1) and by 58% and 51% (S2) for both years. However, similarly to P content, there was an increase in K content of between 24% and 260% in S1 and 90%–217% in S2 after the second application (October 2021) compared to K content after the first application (October 2020) in the corresponding amended soils (Figs. S2C and D).

Similarly, P and K content increases after continuous application of organic amendments in vineyard soils, but different results have been reported depending on the original OM applied (Calleja-Cervantes et al., 2015a, 2015b). Significant correlations were found between soil OC content and P, K, Ca or Mg content ($r = 0.89\text{--}0.99$, p range = $0.050\text{--}0.000$) in S1, and $r = 0.84\text{--}0.99$, p range = $0.050\text{--}0.000$ in S2) after both years of amendment application, indicating that SMS and SMS + OF could be potential sources of P and K in soils. The organic amendments' potential for improving the available nutrient content of a degraded soil has been reported following the application of other organic wastes in general (Amoah-Antwi et al., 2020; Bonanomi et al., 2020) or these wastes combined with rock dust (Basak et al., 2021; Cunha et al., 2022; Swoboda et al., 2022). In turn, the significant correlation found between the nutrients P and K for the two experimental periods and for both soils ($p = 0.000\text{--}0.05$) points to a similar origin. This confirms the supply of these nutrients to the soils by SMS and SMS + OF, although the maintenance of their adequate level in soils depends on the dosage applied or the successive waste applications (Calleja-Cervantes et al., 2015a, 2015b).

The carbon exchange capacity (CEC) was 6.56 (S1) and 5.01 (S2) $\text{cmol}_+ \text{ kg}^{-1}$ (Table 1), and changes in this parameter could be expected in amended soils because CEC is provided by the organic or mineral soil components (Arthur et al., 2011). However, only significant increases in CEC were observed after the second application of SMS100 or SMS100

+ OF in S1 (up to four times) and in S2 (up to 2.7 times) (data not shown). Morlat and Chaussod (2008) also reported increases in CEC through the application of a high dose of SMS for 28 years. Increases in CEC through SMS + OF application would be expected, as reported for the application of other rock dust amendments (Dias et al., 2018). However, the CEC of is very low (Table 1), and its contribution to soil CEC was not observed during the experimental period.

The amount of other nutrients such as Mn, Zn, Cu, and Fe could also be provided by organic and inorganic amendments, constituting an additional advantage of inorganic NPK fertilisation (Swoboda et al., 2022). However, no significant differences were found following the application of composted organic and mineral materials (data not shown), contrary to other authors (Basak et al., 2021; Karaca, 2004).

3.2.4. Changes in the mineralisation of organic N

Fig. S3 features organic N, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations determined in the two soils over time. Organic N increased after SMS application in both soils, mainly in those amended with the highest dose, and decreased over time (Figs. S3A and B). Changes in N content were similar to OC content, and a significant correlation was found between OC and N content in both soils ($r = 0.98\text{--}0.99$, $p = 0.0014\text{--}0.000$), as previously reported by other authors (Peregrina et al., 2012; Cebadero-Cayetano et al., 2020). Over time, organic N could be mineralised by soil microbial activity, producing different forms of mineral N, such as $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ (Lou et al., 2017; Peregrina et al., 2012) (Fig. S3).

Soil $\text{NH}_4^+\text{-N}$ content increased up to 1.2 times in S1 and up to five times in S2 after the first amendment application (Figs. S3C and D). The $\text{NH}_4^+\text{-N}$ content was higher in amended S1 than in S2 one month after the first and second applications of the amendments, with the opposite being observed seven months after both applications. These contents increased up to 3.0–2.7 times in amended S1 and S2, respectively, after the second application, but a real increase was only observed in S2 + SMS100 over time. $\text{NO}_3^-\text{-N}$ always increased in both soils after amendment application, up to 4.7–4.3 times (S1) and up to 11–17 times (S2) one month after the first and the second SMS applications, respectively, although it decreased in both soils over time. A significant correlation was found between N and $\text{NO}_3^-\text{-N}$ content in both soils and for both periods of soil treatment with SMS ($r = 0.91\text{--}0.93$, $p = 0.03\text{--}0.02$) in S1 and ($r = 0.90\text{--}0.87$, $p = 0.05\text{--}0.04$) in S2. N mineralisation depends on the degree of stabilisation of the applied organic wastes (Marín-Martínez et al., 2021). An increase in this mineralisation over time due to the effects of rainfall and temperature has been reported elsewhere, albeit not observed here (Fig. S3) (Bustamante et al., 2011).

$\text{NH}_4^+\text{-N}$ concentrations were lower than those of $\text{NO}_3^-\text{-N}$, as often found in other amended vineyard soils (Marín-Martínez et al., 2021), indicating that the soluble mineral N pool is dominated by $\text{NO}_3^-\text{-N}$ (Bustamante et al., 2011). In this sense, an accumulation of $\text{NO}_3^-\text{-N}$ content after the second application compared to the first one was observed in general in S1, but not in S2 (Figs. S3E and F). More $\text{NO}_3^-\text{-N}$ could leach in a sandy soil (S2) than in a loamy soil (S1) in response to the higher aggregate rainfall recorded in S2 (Fig. 1).

3.2.5. Overall impact of SMS amendments on soil chemical properties

Fig. 3 presents the PCA of the chemical properties for the soils S1 and S2. The combination of both analyses shows how some variables are related to each other. For S1, as reflected in the PCA (Fig. 3A), OC was the main variable contributing to component 1 (PC1), explaining 89.47% of the data variability, while EC associated mainly to component 2 (PC2), explaining 8.99%. These two variables determined the biggest differences between the soil treatments. The pH variable explained a much lower percentage of the data distribution in the PCA, while all the other variables barely contributed to this variability.

For S1, significant positive and negative correlations were found between OC – EC ($r = 0.503$, $p = 0.024$), and between OC – pH ($r = -0.814$, $p = 0.000$), respectively, considering all the sampling data jointly. Furthermore, negative but non-significant correlations were

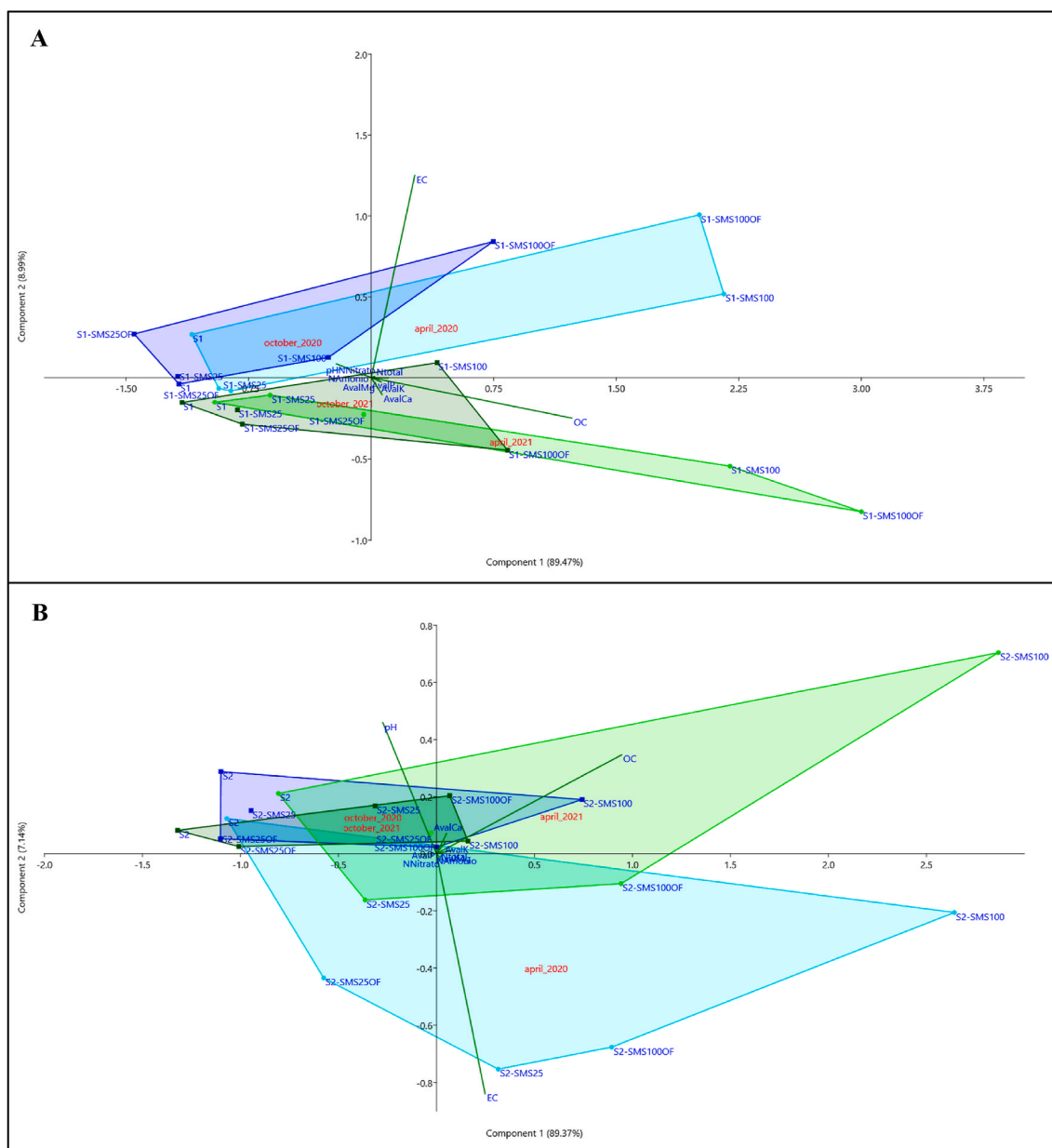


Fig. 3. Principal component analysis (PCA) for the chemical parameters (OC, EC, pH, N, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, available P, K, Ca and Mg) and the four sampling times of vineyard soils S1 (A) and S2 (B) unamended and amended at two doses of SMS and SMS + OF showing loading scores in the two principal components. Unamended and SMS-amended soils taken one month (April) and seven months (October) after the application were denoted by light and dark blue colours in 2020 and by light and dark green colours in 2021, respectively. Percent variability explained by each principal component is shown in parentheses after each axis legend ($n = 3$).

found between pH and EC ($r = -0.382$, $p = 0.097$). The results indicate that the initial effect of the amendment (April 2020 and 2021) favoured an increase in OC content and its accumulation in the soil over time (October 2020 and 2021), especially in the samples amended with the highest dose of SMS (S1 + SMS100, S1 + SMS100+OF). However, OC levels decreased over time due to the mineralisation of the organic waste, as previously indicated. The chemical parameters were scarcely associated with the unamended and amended samples at the lowest dosages (S1 + SMS25, S1 + SMS25+OF). The concentrations in these samples varied little throughout the field trial, and they were located on the left of the PCA, as they had low loadings for PC1 and PC2. In addition, although the application of wastes increased the nutrient content that persisted over time, it hardly influenced the distribution of the samples in the PCA.

The behavior was similar for S2 (Fig. 3B). OC was the main contributing variable to PC1 that explained 89.37% of the data

variability, while the EC and pH associated mainly with PC2 explained 7.14%. These three variables determined the greatest differences between the soil treatments, while the other variables barely contributed, as indicated for S1, although OC and EC had a lower weight in the components of S2, and pH had a greater influence on it. Considering all the sampling data together, a significant correlation was found between OC – EC ($r = 0.635$, $p = 0.003$) and OC – pH ($r = -0.762$, $p = 0.000$, and EC – pH ($r = -0.736$, $p = 0.000$). At the initial application of the wastes (April 2020), the samples were mainly associated with the variables EC and OC (located in the lower part of the PCA), while samples corresponding to the other periods were particularly associated with OC and pH (located in the upper part of the PCA). A correlation of the samples with a high OC content (S2 + SMS100 and S2 + SMS100+OF) was initially recorded (April 2020 and 2021), although it decreased seven months after the application of the wastes. It confirmed the influence of soil texture on the capture of OC, as already indicated. It is also worth

noting the relationship between the samples corresponding to April (2020), and EC as a parameter initially indicating the concentration of soluble salts (Dominguez-Gutierrez, 2022; Guo et al., 2001), and the unamended and amended soils at the lowest dose of SMS with the pH as in S1 (Herrero-Hernández et al., 2022).

3.3. Effect of SMS on soil biochemical properties and PLFAs analysis

3.3.1. Changes in microbial biomass and activities

Soil DHA, RES and BIO values generally increased in S1 and S2 due to the additional nutrients delivered by SMS application (Joniec et al., 2022) (Fig. 4).

Initially, soil DHA values increased up to 1.9 times in S1 + SMS100 compared to the unamended soil, but a non-significant increase was observed in S1 + SMS25. Soil DHA was higher in S2 than in S1, and increased up to 3.8 times in S2 + SMS100+OF compared to the unamended soil, and increased following the application of both wastes. However, the increases in DHA were greater in S1 than in S2 over time (Fig. 4). Soil DHA values followed a similar pattern after the second SMS application, but DHA increased more in S2 than in S1 initially and with the SMS applied over time. The influence of OC content and/or its bioavailability for stimulating microbial activity has been widely reported (Pose-Juan et al., 2017). This is shown by the significant correlations found between soil DHA and OC content determined initially and over time in S1 ($r = 0.92\text{--}0.97$, $p \leq 0.02$) and in S2 ($r = 0.88\text{--}0.95$, $p \leq 0.05$), respectively, after the application of wastes for both years. Soil DHA also significantly correlated with other chemical parameters (pH, EC, total N, and available P, K and Mg) (p range = 0.000–0.05), which correlated with the OC of the SMS applied. Changes in DHA through the application of organic wastes in soils have been reported and increases in this enzymatic activity are usually found, although lower or similar values have also been reported after the application of SMS (Herrero-Hernández et al., 2022), green compost (García-Delgado et al., 2018), and sewage sludge (Galán-Pérez and Peña, 2019), indicating the influence of different factors for controlling this activity. There is a significant correlation between DHA and total bacterial PLFAs in S1 ($r = 0.85\text{--}0.90$, $p \leq 0.06$) and S2 ($r = 0.91$, $p \leq 0.03$), as recorded at certain sampling times, which could explain the results forthcoming. However, the relationship with individual PLFAs, specifically the PLFAs of Gram-negative and Gram-positive bacteria or Actinobacteria and fungi, were not obtained for the two amended soils (data not shown).

Herrero-Hernández et al. (2022) observed also increases of the relative abundance of PLFAs, specifically diagnostics of Gram-negative bacteria and fungi for soils after initial amendment with SMS, although this population decreased over time.

The soil RES values obtained followed a similar pattern to those of soil DHA (Fig. 4). Soil RES was generally higher in S1 than in S2 for the different treatments and samplings after the first application ($p \leq 0.01$ and $p \leq 0.05$, respectively) (Fig. 4). RES increased in both soils with the SMS dose applied, up to 1.9 times in S1 and within the 2.2–3.2 range in S2 compared to the unamended soils, but the differences between the RES values for unamended and amended soils at the lower SMS dose were not always significant. After the second SMS application, the opposite trend was observed (Fig. 4) because RES in S2 was greater than in S1, and RES values increased with respect to the first application, except for the SMS + OF amendment. Soil RES values in S1 were not significantly different following the second application for any period or dose applied, and the values were lower than those following the first application.

In general, RES was enhanced by the organic amendments (Galán-Pérez and Peña, 2019) in agreement with the correlation found in S2 between RES and OC content ($r = 0.96\text{--}0.88$, $p = 0.009\text{--}0.049$) and other soil parameters (pH, EC, total N, and available P, K and Mg) ($r = 0.87\text{--}0.96$, $p = 0.009\text{--}0.043$) related with soil OC. However, a significant correlation was only observed in S1 between RES values and OC content ($r = 0.88$, $p = 0.05$) over time, following the first application. Although the effect of OC content was significant, the results suggest a greater stimulation of the microorganisms in S2 due to the higher bioavailability of labile C pool in this sandy soil, as indicated above, which will become more readily available through amendments over time (Galán-Pérez and Peña, 2019). The correlation between RES and total bacterial PLFAs in S1 ($r = 0.89$, $p = 0.04$) and in S2 ($r = 0.94$, $p = 0.02$) after the first SMS application could explain this microbial activity. This relation was supported by significant correlations obtained with the specifically diagnostics PLFAs of Gram-negative and Gram-positive bacteria in S1 ($r = 0.88\text{--}0.90$, $p = 0.03\text{--}0.05$) (data not shown), and Gram-negative and Gram-positive bacteria and fungi in S2 ($r = 0.91\text{--}0.95$, $p = 0.01\text{--}0.03$) at certain sampling times (data not shown). In addition to chemical parameters, other variables, such as soil moisture content and temperature, are also crucial for this soil microbial parameter (Trivedi et al., 2017).

Initially, the effect of SMS on soil BIO values was not observed in the

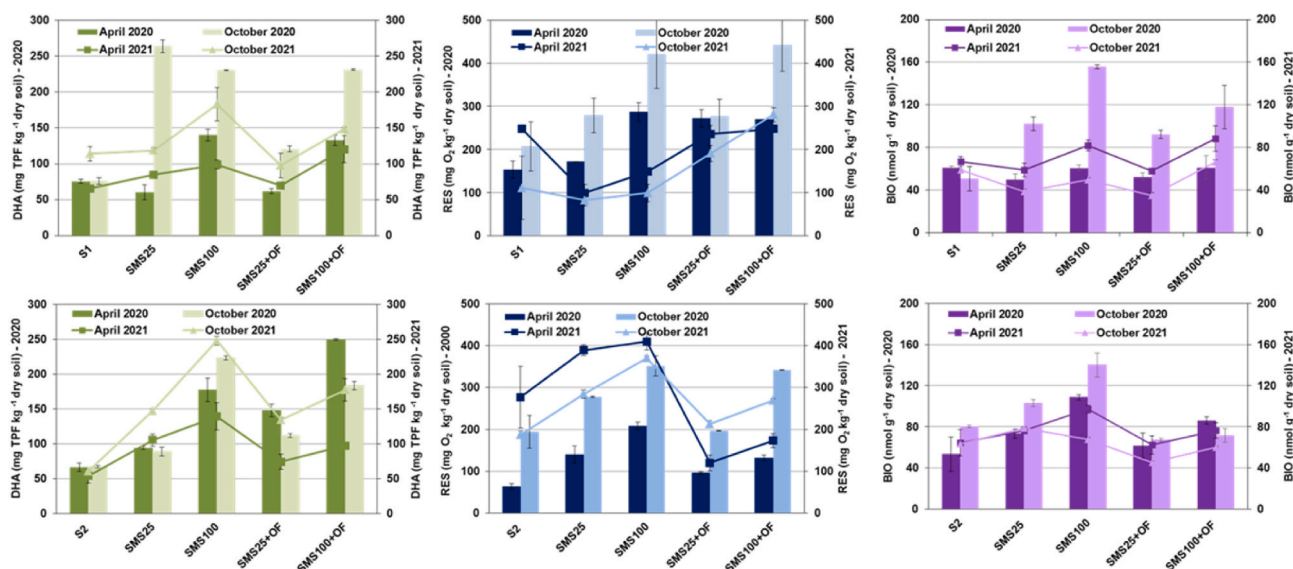


Fig. 4. Mean values of DHA, RES and BIO in the vineyard soils S1 and S2 unamended and amended at two doses of SMS and SMS + OF (SMS25, SMS100 and SMS25 + OF, SMS100 + OF) after one month (dark colours) and seven months (light colours) of the application in the year 2020 (bars) and 2021 (lines).

amended S1, and it was only observed in S2 ($p = 0.001$), where the BIO value increased up to two times in S2 + SMS100 compared to the unamended soil (Fig. 4). As indicated for other biochemical parameters, microbial activity was higher in the sandy loam soil (S2) than in its silty counterpart (S1), due to the higher availability of OC, despite the lower residual OC content in S2 than in S1 (Herrero-Hernández et al., 2022). Labile OC has been singled out as a readily available energy source to be degraded by soil microbes compared to less labile or non-labile (recalcitrant) OC (Unagwu, 2019). A significant correlation between soil BIO and the OC content was obtained in S2 after the first application ($r = 0.99, p = 0.001$).

An increase in soil BIO following the application of organic wastes has been widely reported in laboratory and field assays (García-Delgado et al., 2018; Pose-Juan et al., 2017; Singh et al., 2016). BIO increased for both soils over time after the first SMS application (up to 1.8–3 times in S1, and up to 1.75 in S2 + SMS100) (Fig. 4), as observed for DHA and

RES values. Significant correlations were found at different sampling periods between the biochemical parameters BIO and RES ($r = 0.87, p \leq 0.05$) and DHA ($r = 0.85, p \leq 0.05$) in S1, and BIO and RES ($r = 0.96, p \leq 0.01$) and DHA ($r = 0.95, p \leq 0.014$) in S2. The waste did not have a similar effect on the soils after the second application: BIO initially increased for both soils, but only in S + SMS100 and S + SMS100 + OF and to a lesser extent (up to 1.5 times), and it decreased for both soils over time with no significant differences between treatments (Fig. 4).

Total bacterial or fungal PLFAs reflected the changes found in BIO for the two soils at different times, and significant correlations were determined between BIO and the bacteria or fungi in S1 ($r = 0.89–0.99, p = 0.002–0.04$) and in S2 ($r = 0.98–0.99, p = 0.002–0.000$). These results are supported by the significant correlations obtained with individual PLFAs specifically diagnostics of Gram-positive ($p \leq 0.018$) and Gram-negative ($p \leq 0.018$) or fungi ($p \leq 0.02$) in S1 and Gram-positive ($p \leq 0.033$) and Gram-negative ($p \leq 0.007$) or fungi ($p \leq 0.010$) in S2

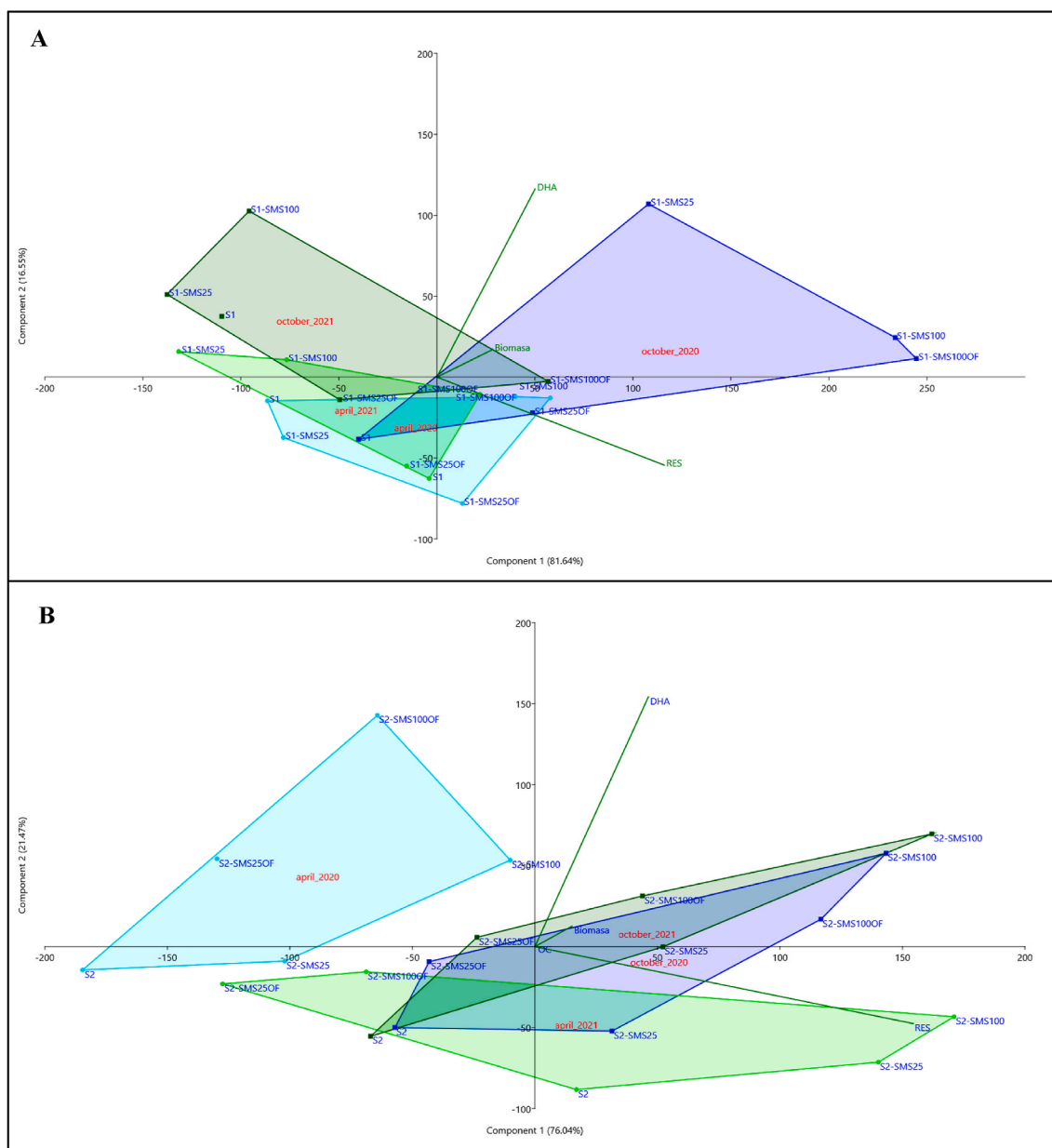


Fig. 5. Principal component analysis (PCA) for the biochemical parameters (DHA, RES and BIO) and the four sampling times of vineyard soils S1 (A) and S2 (B) unamended and amended at two doses of SMS and SMS + OF showing loading scores in the two principal components. Unamended and SMS-amended soils taken one month (April) and seven months (October) after the application were denoted by light and dark blue colours in 2020 and by light and dark green colours in 2021, respectively. Percent variability explained by each principal component is shown in parentheses after each axis legend ($n = 3$).

(data not shown), although this was not observed for all the sampling times after SMS application. In addition, changes in the microbial community structure following SMS application were observed over time after both SMS applications due to the variation of the ratios between bacterial (Gram-positive and Gram-negative, or total) and fungi PLFAs. In general, a decrease in total bacteria/fungi ratio was observed in the amended soils compared to the unamended ones, indicating a lower growth of the bacterial community in amended soils (corresponding mainly to Gram-negative bacteria vs. fungi). An exception was the SMS100 + OF amended soil, where this bacteria/fungi ratio generally increased (data not shown) possibly due to OF stimulates microbial activity as it is widely indicated in section 3.3.2 (García-Gómez et al., 2002; Li et al., 2020a). Fungi are capable of degrading more recalcitrant organic materials, such as lignin and cellulose, and they are important for increasing the soil's overall fertility and quality. However, different effects of SMS application were recorded after the second application, leading to a greater increase in total bacteria along with a decrease in fungi (data not shown) (Moreno et al., 2013). These results could be due to the variability in SMS organic compounds after composting, as the effect of the soils on the microbial structure was not significant, despite their different texture.

3.3.2. Overall impact of SMS amendments on soil biochemical properties

Fig. 5 presents the PCA of soil biochemical parameters for S1 and S2. The combination of both analyses shows how some variables are related to each other. For S1 (Fig. 5A), RES was the main contributing variable to PC1, which explained 81.64% of the data variability, while the DHA associated mainly with PC2 explained 16.55%. These two variables explained the biggest differences between the soil treatments. The BIO variable was more closely associated with PC1; nonetheless, it explained a lower percentage of the data distribution in the PCA compared to the other two parameters. Significant positive correlations were found between the three biochemical parameters when considering all the sampling data jointly ($r = 0.528-0.740$, $p = 0.017-0.000$). The scores for each treatment and sampling time in the PCA showed the different evolution of the treatments over time. In general, the amended soils were positively related to the biochemical parameters after the first SMS application (2020), and an upward trend was observed in the values of RES, DHA and BIO over time, as shown in the PCA with the samples from October located on the right. This relationship was slightly weaker after the second application of the wastes (2021), with most of the samples located on the left of the PCA, along with all the unamended soils at the four sampling times. This tallies with the decrease in the biochemical values recorded for these samples. The unamended soils were the ones less correlated with microbial BIO and activity compared to the amended soils.

At the initial application of the wastes in April in both years, RES was positively related to S1 + SMS100 and S1 + SMS100+OF in 2020, and S1 + SMS100+OF in 2021. These results reflect the initial stimulation of the overall activity of the SMS microorganisms applied at the highest dose (100 Mg ha^{-1}). Furthermore, differences were observed between S1 + SMS25 (negative relationship with PC1) and S1 + SMS25+OF (negative relationship with PC2) in 2020, and between S1 + SMS100 (negative relationship with PC1) and S1 + SMS100+OF (positive relationship with PC1) in 2021. A highlight is the strong relationship between SMS + OF samples and RES and BIO. The SMS is a carrier of new microbial populations that could stimulate microbial activity (García-Delgado et al., 2015; Pose-Juan et al., 2015). Moreover, the rock dust has the ability to improve soil structure, fix oxygen, and increase soil aeration due to its characteristics (particle size and specific surface), as well as provide nutrients (e.g., Zn, Fe, and Cu) and physical microsites, thereby enhancing microbial activity (Li et al., 2020a; Swoboda et al., 2022).

Seven months after the first application of wastes (2020), DHA was particularly related to S1 + SMS25, while S1 + SMS25+OF, S1 + SMS100, and S1 + SMS100+OF were associated with RES and BIO. This

increase in microbial activity could be due to the soil moisture conditions prompting a rapid response by microorganisms (Yan et al., 2016). Seven months after the second application (2021), S1 + SMS100+OF was positively related to the biochemical parameters, suggesting again that the combination of a high dose of SMS with OF stimulates microbial activity (García-Gómez et al., 2002; Li et al., 2020a). The other amended samples, nevertheless, had the opposite relationship with RES. A lower availability of OC in the silty loam soil after the second application, with a final increase in the OC detected with respect to the content initially determined in the soil could explain these results. Moreover, the soil microbial community does not respond in a similar way to environmental conditions (Blume et al., 2002; Grayston et al., 2004) due to changes in the availability of resources that favour the growth of different microbial groups, each one with a different activity-BIO relationship (Papatheodorou et al., 2004). The minimum temperature recorded between March and October 2021 was $-3.3 \text{ }^\circ\text{C}$, and the cumulative precipitation was 289 mm (three and 1.6 times lower than the figure for 2020, respectively), which reduced the activity of soil microorganisms and possibly explains the weaker relationship with the biochemical parameters.

For S2, as reflected in the PCA (Fig. 5B), RES was the main contributing variable to PC1, which explained 76.04% of the data variability, while DHA associated mainly to PC2 explained 21.47%. These two variables explained the greatest differences between the soil treatments. The BIO variable explained a low percentage of the data distribution in the PCA, as observed in S1. Positive but non-significant correlations ($p \geq 0.1$) were found between the three biochemical parameters when considering all the sampling data jointly. In general, the samples from October (2020) and April–October (2021) were positively related to the biochemical parameters, while the samples from April (2020) were barely related to these variables, located on the left of the PCA. The PCA also showed that the application of wastes caused a clear change in the BIO and global activity of the microbial community, as the unamended soils were located in the lower left area. However, most of the amended soils were located on the right of the PCA, with a positive relationship with DHA, BIO and RES. This could be explained by the greater bioavailability of OC in the sandy loam soil (S2) than in the silty loam soil (S1), despite its lower residual content, as previously indicated (Fig. 2). Soil structure is a key feature for controlling OM degradation processes mediated by soil microbiota (Van Veen and Kuikman, 1990), and the positive impacts of microbial activity in sandy loam soils are more significant than in soils with other textures, such as clay soils (Ameloot et al., 2013; Cheng et al., 2017).

At the initial application of wastes (2020), DHA was related to S1 + SMS100 and S1 + SMS100+OF, while the other samples had a weak relationship with the biochemical parameters. A fluctuation in soil moisture content at the beginning of the experiment, as a result of the precipitation of 204 mm initially recorded, could have distorted the composition of the amended soils, as reflected in the low OC levels in these samples. At the second application (2021), RES was closely related to S + SMS25 and S + SMS100 (positive relationship with PC1), although SMS + OF samples were not (negative relationship with PC1). This behaviour was the opposite to that observed for S1, where these samples stimulated microbial RES. Seven months after the application (2020 and 2021), amended samples were positively related to RES. Higher levels of microbial RES were recorded in S2 compared to S1, due to its sandy texture, larger pore size, and greater aeration. Furthermore, BIO and OC were related in a way that decreased with the time.

4. Conclusions

The new organic-mineral compost based on SMS as organic material and the sub-volcanic rock OF as mineral component explored in this work by first time improved the soil quality. Effect of SMS and SMS + OF wastes applied in the vineyard soils significantly increased their OC content. OC was maintained over time after the second application in

the silty loam soil (S1), but it is not occurred for the sandy soil (S2), due to the increased availability of OC in this soil. The increase in soil nutrient content (total N and P or K available) persisted over time, especially in soils amended with SMS and SMS + OF at the highest dosage. Accordingly, applying SMS enhanced the fertility of the vineyard soils tested, as soil nutrient content is an indicator of soil quality and its subsequent yield. In addition, the results reflect an increase in BIO and microbial activity after SMS application. The readily metabolised OC from the SMS was the main factor for explaining the changes in the biochemical parameters DHA, RES and BIO. These changes were enhanced by the presence of in the organic amendment, and they were related to the soil characteristics and the time elapsed after SMS application. The positive effect of SMS on the biochemical properties, mainly RES and BIO, was weakened after the second application. Seasonal changes in weather conditions, such as temperature or moisture in the surface soil, could help to explain this evolution. These factors also influenced the dynamics of the structure of microbial communities. Fungi content prevailed over total bacteria in both soils, although the opposite effect was observed after the repeated application of wastes. The findings found in this work were relevant for stablishing of new protocols based on the SMS or SMS + OF application in vineyard soils in La Rioja and to plan future strategies of management practices in widely cultivated soils. In addition, future work should emphasize on the relevance of the different soil textures to maintain adequate levels of OC and/or nutrients, and prevent their degradation and on the optimisation of dosages and/or successive applications could help to uphold their future sustainability.

Credit author statement

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Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2023.115339>.

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