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Organic Amendment for the Recovery of Vineyard Soils: Effects of a Single Application on Soil Properties over Two Years

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Abstract: Spent mushroom substrate (SMS) is the organic residue generated during mushroom cultivation, and it is being produced in ever-greater quantities around the world. Different applications for this residue have been proposed for its valorization, but its application as a soil amendment could be one of the most sustainable. SMS improves soil quality by increasing its organic matter (OM), thereby enhancing the sustainability of agricultural systems. The objective of this work was to evaluate the effect of the application of two doses of SMS on the chemical, biochemical, and microbiological characteristics of two degraded vineyard soils in La Rioja (Spain) with different textures, as a new regenerative agricultural practice. The variations in organic carbon (OC), micro- and macronutrients, soil microbial biomass (BIO), respiration (RES), dehydrogenase activity (DHA), and the profile of phospholipid fatty acids (PLFAs) extracted from the soils were evaluated over two years. An initial increase in soil OC content was recorded in both soils, although the content that remained over time differed for each site. In general, SMS enhanced DHA, RES, and BIO in the soils, but the effect varied, possibly being conditioned by the availability of OC for soil microorganisms. In general, changes in the soils' microbial structure after SMS application were not very significant over the two-year experimental period.

Keywords: spent mushroom substrate; vineyard soils; regenerative agriculture

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1. Introduction

Global mushroom production has increased sharply over the last five decades. According to statistics provided by the United Nations Food and Agriculture Organization (FAO), the average annual increase in the cultivation of edible fungi is 5.6% worldwide [1]. This production generates an increasing amount of an organic residue known as spent mushroom substrate (SMS), with roughly 5 kg of SMS being produced per kg of mushrooms [2,3]. Different applications for the valorization of this residue have been proposed, such as energy production, animal feed, pollutant absorption, etc. [1,4,5]. However, its application as a soil amendment after an appropriate re-composting process could be one of the most viable uses for enhancing the sustainability of agricultural production systems [2,6–8]. SMS improves the soil structure by increasing the OM content, water retention capacity, and microbial activity, and by decreasing soil compaction. Accordingly, the use of SMS as an organic amendment is an agronomical practice of interest from an environmental and economic point of view when applied to eroded and degraded soils with a low OM content. The improved properties of SMS-amended soil could be beneficial for crop yield.

However, research is required to establish an application rate and/or to assess the effects of SMS-amendment, depending on the type of soil involved [9].

The production of mushrooms in La Rioja region (N-E Spain) amounts to more than 70 Mt (https://www.larioja.org accessed on 27 September 2021), and this generates significant amounts of SMS. Accordingly, SMS could be applied to the agricultural soils in this area, and especially those dedicated to vine cultivation. Vineyard soils extend over a large area in this region (35.7% of the total cultivated area) (https://www.larioja.org accessed on 28 October 2021), and most of these soils are exposed to processes of erosion and degradation. In general, these soils have an OM content below 1%, and are compacted, unstructured, and unbalanced, thereby diminishing the quality of the grapes produced [10–12]. OM's potential to improve water and nutrient retention and increase soil aggregation has been reported previously [13]. Aggregate stability is a key factor for enhancing the physical fertility of soils, and it can be enhanced by following suitable management practices, utilizing organic amendments to maintain an appropriate soil structure, thereby preventing and even reversing soil degradation [14–16]. Consequently, soil OM added through the use of organic amendments could help to improve soil quality, decrease runoff and erosion, and enhance its reclamation [17].

Within this context, a new methodology for regenerative agriculture has been proposed based on the use of SMS to increase the OM content of soils and thereby enhance the regeneration of vineyard soils in La Rioja. This objective is included in the VITIREG project (Development of regenerative viticulture techniques for improving vineyard soil and grape quality within the DOCa Rioja) conducted by the regenerative agriculture operational group of La Rioja (http://vitireg.org/ accessed on 27 September 2021). The application of SMS as a plant fertilizer has already been reported [18,19], but its application to soils with a greater or lesser degree of degradation has received less attention [20]. However, other organic residues, such as sewage sludge or urban solid wastes, crop residues, livestock waste (manure and slurry), and agro-industrial residues from wine, beer, and olive production, are potential candidates as organic soil amendments [21,22]. These organic residues have usually been proposed for regenerating soils with different degradation issues, being applied in different doses within the context of the circular economy [23]. Previous studies have documented the contribution that organic residues make to reversing the decrease in soil OM content and the disruption of nutrients caused by intensive farming [24,25], although the increase in soil OM will depend on the total OC content in the residues applied.

The objective of this research was to study the effects of applying SMS to two vineyard soils in La Rioja in terms of these soils' chemical, biochemical, and microbial properties, under field conditions. The effect of a single SMS application in two doses to two vineyard soils with different textures was assessed over time. Changes in soil chemical parameters—indicators of soil quality—and biochemical parameters—indicators of soil microbial abundance (biomass—BIO) and activity (respiration—RES and dehydrogenase activity—DHA)—were monitored over a two-year period following the amendment with SMS. The soil microbial structure was also evaluated by analyzing the profile of phospholipid fatty acids (PLFAs) extracted from the soils.

2. Materials and Methods

2.1. Experimental Setup and Soil Sampling

The field assay was conducted over two years (February 2019–March 2021) on experimental plots in two vineyards from "La Rioja Oriental" (N-E Spain) located at ARN1 (42°16′23.6″ N, 2°04′0.058″ W) and ARN2 (42°12′0.029″ N 2°04′13.7″ W). The climate at these sites is dry and warm, with a Mediterranean influence. The maximum and minimum temperatures recorded over the experimental period by in situ weather stations were 38.7 °C and 42.0 °C (maximum) and −5.4 °C and −6.1 °C (minimum) (mean 14.5 °C); the maximum daily precipitation recorded was 43 mm, and the totals for cumulative precipitation were 840 and 1143 mm at the ARN1 and ARN2 sites, respectively.

Experimental plots measuring 90 m^2 were laid out for each of the two soils (Aridisol, Typic Haplocalcid) [26]. ARN1 has a silty loam texture (27.8% sand, 55.3% silt, and 16.9% clay), and ARN2 has a sandy loam texture (56.7% sand, 27.0% silt, and 16.2% clay). Both soils have an OM content < 2%.

ARN1 and ARN2 plots were amended using SMS in doses of 25 and 100 Mg ha^{-1} (dry weight) (ARN-25 and ARN-100, respectively), which are equivalent to application rates of = 5 and 20 g C kg^{-1} soil, respectively. A randomized experimental plot was designated for each treatment. A single application of SMS was incorporated into the first 25–30 cm of the topsoil layer in February 2019, using a rotavator. Soil samples were taken twice in the first year—one month after SMS application (March 2019) and eight months after application, once the harvest had ended (October 2019)—and subsequently in March 2020 and March 2021 (one and two years after SMS application). Five soil cores were collected in each plot from 0 to 30 cm for physical and chemical analysis, and from 0 to 15 cm for biochemical and microbiological analysis. Composite samples of five cores were transferred to polypropylene bottles. All of the samples were transported to the laboratory in portable refrigerators.

2.2. Spent Mushroom Substrate

Spent mushroom substrate, generated after *Agaricus bisporus* cultivation and production, and aerobically re-composted for three months, was kindly supplied by Sustratos de La Rioja S.L. (Pradejón, La Rioja, Spain), and its characteristics are specified in Table 1.

Table 1. Characteristics of the SMS applied to the soil.

Parameters [1]	SMS
Humidity (%)	48.0
Dry matter (%)	52.0
pH (1/25 p/v)	7.40
Electrical conductivity (dS m^{-1})	7.00
Organic matter (%)	49.0
Organic carbon (%)	28.4
Humic acids (%)	10.1
Fulvic acids (%)	7.40
Total N (%)	2.14
NH_4^+-N (mg kg^{-1})	0.23
NO_3^--N (mg kg^{-1})	1.91
C/N	15.0
Total P (%)	1.86
Total Ca (%)	13.5
Total K (%)	2.48
Total Mg (%)	1.62
Total Na (%)	0.36
Total S (%)	8.58
Total Fe (mg kg^{-1})	4570
Total Cd (mg kg^{-1})	0.24
Total Cu (mg kg^{-1})	58.5
Total Ni (mg kg^{-1})	6.50
Total Pb (mg kg^{-1})	1.38
Total Zn (mg kg^{-1})	310
Total Cr (mg kg^{-1})	9.60

[1] Values for dry SMS residue.

2.3. Physical and Chemical Soil Analysis

The characteristics of the unamended and amended soils were determined in triplicate using previously air-dried and sieved (<2 mm) samples and standard analytical methods [27]. Briefly, soil pH and electrical conductivity (EC) were determined in a soil/water suspension (1/2.5 and 1/5 *w/v* ratio, respectively). Total OC and N were determined using a LECO CN628 (LECO Corporation, Saint Joseph, MI, USA) elemental analyzer. OM was

calculated from the OC results multiplied by 1.724. NH_4^+-N and NO_3^--N were determined via colorimetry using a segmented flow autoanalyzer AA3 (Bran+Luebbe GmbH, Norderstedt, Germany). Assimilable P was determined by the Olsen method, while assimilable macronutrients (Ca, K, and Mg) and micronutrients (Cu, Fe, Mn, and Zn) were extracted by using ammonium acetate at pH 7 and quantified using a Varian model 720-ES inductively coupled plasma-optical emission spectrometer (Varian Instruments, Palo Alto, CA, USA). Soil particle size distribution was determined using the pipette method. Inorganic carbon was determined as $CaCO_3$ by using a Bernard calcimeter. 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). Moreover, alkali soluble and acid insoluble carbon (humic acid, HA) and alkali and acid soluble carbon (fulvic acid, FA) were also determined in soil extracts one and eight months after SMS application, following the traditional method of HA and FA extraction from soil OC using a sodium pyrophosphate solution [28].

2.4. Biochemical and Microbial Analysis

Biochemical and microbial parameters were determined in triplicate in surface soil samples (0–15 cm). Soil respiration (RES) was determined by measuring the pressure drop caused by O_2 consumption 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 CO_2 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 O_2 consumption. The results were expressed as mg O_2 kg^{-1} dry soil.

As a measure of overall microbial activity, soil dehydrogenase activity (DHA) was determined by the Tabatabai method [29]. 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 by using 7 mL of methanol, centrifuged (3000 rpm, 10 min), and then extracted twice more. The three fractions were mixed and diluted to 25 mL using methanol. The absorbance of the supernatant was measured in a spectrophotometer at 485 nm. The results were expressed as mg TPF kg^{-1} dry soil.

The microbial biomass (BIO) and the microbial community structure of the soil samples were determined using phospholipid fatty acid (PLFA) analysis, as described by Frostegård et al. [30]. Lyophilized soil samples (2 g) were extracted using a one-phase chloroform:methanol:phosphate buffer solvent by sonication. Extracts were purified using SPE, and phospholipids were separated from non-polar lipids and transesterified to fatty acid methyl esters using methanol-KOH. Finally, hexane extracts containing the resultant fatty acid methyl esters were analyzed by gas chromatography. Quantification involved an Agilent 7890 gas chromatograph (Agilent Technologies, Wilmington, DE, USA) equipped with a 25 m Ultra 2 (5% phenyl)-methylpolysiloxane column (J&W Scientific, Folsom, CA, USA) and a flame ionization detector. PLFAs were identified using bacterial fatty acid standards and software from the Microbial Identification System (Microbial ID, Inc., Newark, DE, USA). Nonadecanoic acid (19:0) was used as an internal standard for the quantitative determination of PLFAs. The total microbial biomass was estimated based on the total sum of PLFAs, and expressed as nmol g^{-1}. Specific PLFAs [31] were used as biomarkers to quantify the relative abundances of both gram-negative bacteria (monounsaturated fatty acids and cyclopropyl 17:0) and gram-positive bacteria (iso and anteiso saturated branched chain fatty acids), as well as actinobacteria (10-methyl fatty acids), and fungi (18:2ω6 cis and 16:1ω5), which were used to identify larger groups within the soil biomass.

2.5. Statistical Analysis

Normal data distribution was verified using the Shapiro–Wilk test, and Levene's test was used to check the homogeneity of variance. Data underwent two-way analysis

of variance (ANOVA), with the main factors being soil treatment and sampling times. The Tukey post hoc test at $p \leq 0.05$ was used to determine significant differences among means, and to evaluate the effects of the different soil treatment and sampling times on the chemical, biochemical, and microbial parameters. A correlation matrix of bivariate data including biochemical and microbial parameters and soil chemical characteristics was analyzed based on Pearson's coefficients. ANOVA and correlation analyses were carried out using the IBM SPSS Statistics v26 software package (IBM, Armonk, NY, USA).

3. Results and Discussion

3.1. Effect of SMS on Chemical Properties

Tables 2 and 3 show the characteristics of the unamended and SMS-amended ARN1 and ARN2 soils after different time periods. The use of SMS led to a decrease in the pH of the amended soils up to eight months after its application. This effect was significant ($p \leq 0.05$) in the ARN1 soil, with a silty loam texture, when amended with the higher SMS dose and in the ARN2 soil, with a sandy loam texture, when amended with both SMS doses. An increase in organic acids released by the microbial decomposition of SMS or by phosphate solubilizing might play an important role in initial soil acidification [32,33]. Accordingly, a non-significant negative correlation ($p > 0.1$) was observed between OC content and pH for ARN1 at one and eight months following SMS application. However, for ARN2, the correlations between pH and OC or N ($p \leq 0.05$ (0.000)) and between pH and available P, K, or Mg content ($p \leq 0.05$ (0.016–0.000)) were significant when all of the samples from the different time periods were considered jointly. An increase in pH was simultaneously observed over time corresponding to the decrease in soil OC content due to the evolution of SMS, and the pH values recorded in both amended soils were higher than in the unamended ones two years after SMS application (Tables 2 and 3). A greater pH increase was observed in the sandy loam soil (ARN2) two years after SMS application. Some authors have suggested that the buffer capacity of calcareous soils may play a role in the recovery of pH values to initial levels [10,14]. However, different results are reported in the literature after long-term compost application, indicating increases and decreases in the pH of soils depending on their initial pH and the nature of their organic residues [12,32,34]. It is important to consider these changes in pH values, as they could affect the availability of microelements and/or the solubility of certain non-essential metallic elements.

Conversely, EC values shifted in the opposite direction to pH levels (Tables 2 and 3). The application of SMS led to an increase in the EC of amended soils up to eight months after its application. This effect was significant ($p \leq 0.01$) in ARN1 and ARN2 soils amended with the higher SMS dose. A positive and significant correlation between EC and OC, N, NH_4^+-N, NO_3^--N, and available P, K, and Mg contents was found for both soils (p range = 0.024–0.000 for ARN1, and p range = 0.016–0.000 for ARN2) when all of the data from the different sampling times were considered. EC indirectly indicates the concentration of soluble salts and, initially, it increased in the presence of the organic amendment. Slightly higher EC values after the addition of compost and vermicompost to the soils compared to the control soil have been reported in previous studies [35,36]. Nevertheless, EC values decreased over time for both ARN1 and ARN2, and no significant differences were found between different soil treatments two years after SMS application [2].

The OM or OC content of the unamended soils increased after SMS application in both doses in ARN1 ($p \leq 0.05$) and at the higher dose in ARN2 ($p \leq 0.05$) due to the high OM content of the SMS applied (Figure 1). The OM content increased 2.3–2.6 times in the silty loam soil (ARN1) one month after SMS application in both doses, while this content increased only up to 1.8 times in the sandy loam soil (ARN2) when 100 Mg ha^{-1} of SMS was applied. The OM content was significantly reduced by up to 44–60% in the silty loam soil and by up to 30% in the sandy loam soil eight months after SMS application due to the mineralization of the organic residues [12]. However, the OM content of the ARN1-100 soil remained 23–31% higher than in the unamended soil or ARN1-25 soil ($p \leq 0.05$) one and two years after SMS application. The increase in the OM content of the sandy loam soil was

only significant (increasing by 50%) up to one year after SMS application at 100 Mg ha^{-1}. However, the lower dose of SMS application (25 Mg ha^{-1}) did not lead to a significant increase in soil OM content with respect to the unamended soil at any sampling time. In ARN2, neither rate of SMS application significantly increased OM content after two years.

Table 2. Characteristics of unamended soil (ARN1) and of soil amended with SMS at two doses (ARN1-25 and ARN1-100) over the experimental period.

Parameter	ARN1				ARN1-25				ARN1-100			
	1 Month	8 Months	1 Year	2 Years	1 Month	8 Months	1 Year	2 Years	1 Month	8 Months	1 Year	2 Years
Texture	Silty loam											
Sand (%)	28.30				27.05				30.69			
Silt (%)	49.97				50.24				48.48			
Clay (%)	21.73				22.71				20.83			
pH (1/2.5 p/v)	7.96	8.28	7.91	7.98	7.89	8.26	8.12	8.44	7.88	7.96	7.88	8.15
Electrical conductivity (dS m^{-1})	1.45	0.57	0.40	0.51	1.55	0.52	0.32	0.33	1.87	0.97	0.40	0.56
$CaCO_3$ (%)	16.8	15.8	16.9	13.8	16.2	16.4	16.9	15.9	15.2	15.3	16.4	17.5
Organic matter (%)	1.39	1.53	1.33	2.13	3.25	1.28	1.43	1.89	3.73	2.08	1.74	2.63
Organic carbon (%)	0.81	0.89	0.77	1.24	1.88	0.74	0.83	1.09	2.17	1.21	1.01	1.53
Total N (%)	0.19	0.13	0.13	0.13	0.21	0.12	0.13	0.13	0.24	0.15	0.14	0.18
NH_4^+-N (mg kg^{-1})	10.5	2.67	0.00	26.2	8.64	3.77	0.45	19.3	7.29	1.66	1.45	23.7
NO_3^--N (mg kg^{-1})	581	172	57.5	118	716	254	59.5	76.6	836	398	76.3	65.2
C/N	7.4	7.1	5.8	9.5	9.0	6.3	6.2	8.3	9.2	8.2	7.1	8.3
Available P (mg kg^{-1})	57.1	22.0	<5	25.6	61.0	16.0	<5	34.9	79.2	43.0	<5	77.3
Available Ca (g kg^{-1})	15.0	14.1	13.1	12.9	15.0	14.2	13.9	13.4	15.0	14.4	13.4	13.6
Available K (g kg^{-1})	0.99	0.44	0.48	0.44	1.11	0.68	0.48	0.67	1.58	0.64	0.47	0.95
Available Mg (g kg^{-1})	0.47	0.32	0.28	0.30	0.48	0.37	0.27	0.27	0.55	0.37	0.27	0.32
Exchangeable Na cmol$_{(+)}$ kg^{-1}	0.91	0.44	0.20	0.39	0.82	0.36	0.14	0.14	0.99	0.50	0.11	0.12
Exchangeable Ca cmol$_{(+)}$ kg^{-1}	38.0	41.3	30.7	34.3	37.9	41.2	34.1	32.5	38.5	42.3	32.9	35.3
Exchangeable K cmol$_{(+)}$ kg^{-1}	2.88	1.24	1.26	0.74	3.14	1.23	1.27	1.57	4.16	1.84	1.24	2.19
Exchangeable Mg cmol$_{(+)}$ kg^{-1}	3.73	2.42	2.02	2.11	3.71	2.31	1.82	1.95	3.93	2.92	1.96	2.48
Exchangeable NH_4^+ cmol$_{(+)}$ kg^{-1}	7.57	7.03	6.56	3.36	7.15	6.03	6.05	4.73	6.62	6.56	6.92	6.32
Available Cu (mg kg^{-1})	0.65	0.58	0.24	0.75	0.63	0.48	0.65	0.48	0.74	0.55	0.09	0.65
Available Fe (mg kg^{-1})	13.9	1.98	1.82	3.28	57.1	14.4	3.83	2.82	2.37	4.58	1.82	8.81
Available Mn (mg kg^{-1})	14.0	7.92	10.6	12.3	15.9	8.42	11.7	11.7	15.7	9.09	10.5	11.0
Available Zn (mg kg^{-1})	0.45	0.30	< 0.06	0.39	0.52	0.39	<0.06	0.31	0.67	0.39	<0.06	0.66

Table 3. Characteristics of unamended soil (ARN2) and of soil amended with SMS at two doses (ARN2-25 and ARN2-100) over the experimental period.

Parameter	ARN2				ARN2-25				ARN2-100			
	1 Month	8 Months	1 Year	2 Years	1 Month	8 Months	1 Year	2 Years	1 Month	8 Months	1 Year	2 Years
Texture	Sandy loam											
Sand (%)	52.54				45.12				47.99			
Silt (%)	25.93				29.58				28.99			
Clay (%)	21.53				25.29				23.01			
pH (1/2.5 p/v)	8.61	8.51	8.50	8.44	8.46	8.41	8.60	8.62	8.05	8.01	8.48	8.53
Electrical conductivity (dS m^{-1})	0.21	0.19	0.16	0.18	0.24	0.32	0.32	0.16	0.77	1.29	0.22	0.20
$CaCO_3$ (%)	12.7	18.4	16.9	15.1	17.0	18.4	18.5	16.7	17.9	17.4	18.5	20.3
Organic matter (%)	1.31	0.80	0.99	1.46	1.35	0.94	1.02	1.36	2.38	2.76	1.49	1.33
Organic carbon (%)	0.76	0.46	0.57	0.85	0.79	0.55	0.59	0.79	1.38	1.60	0.86	0.77
Total N (%)	0.09	0.09	0.10	0.08	0.08	0.08	0.09	0.07	0.15	0.17	0.11	0.05
NH_4^+-N (mg kg^{-1})	2.76	4.55	0.92	6.29	4.29	5.82	0.00	4.31	11.7	12.5	0.81	7.78
NO_3^--N (mg kg^{-1})	56.2	83.6	26.3	35.7	45.7	60.9	8.00	16.7	341	223	26.9	4.96
C/N	8.6	5.2	5.8	10.6	7.1	7.0	6.2	10.8	9.0	9.4	7.1	15.1
Available P (mg kg^{-1})	33.0	52.1	20.0	52.5	37.1	46.0	38.1	44.9	53.1	116	77.2	23.6
Available Ca (g kg^{-1})	14.8	14.6	14.3	13.6	15.0	14.9	13.9	14.0	15.0	15.1	13.9	14.4
Available K (g kg^{-1})	0.27	0.28	0.32	0.31	0.29	0.34	0.24	0.37	0.73	1.09	0.45	0.39
Available Mg (g kg^{-1})	0.21	0.13	0.11	0.11	0.14	0.16	0.10	0.13	0.20	0.34	0.14	0.12
Exchangeable Na $cmol_{(+)}$ kg^{-1}	0.05	0.07	0.01	0.04	0.09	0.15	0.01	0.04	0.43	0.42	0.03	0.04
Exchangeable Ca $cmol_{(+)}$ kg^{-1}	34.0	40.3	33.1	30.3	33.2	40.9	30.3	32.5	36.2	40.5	32.6	35.2
Exchangeable K $cmol_{(+)}$ kg^{-1}	0.75	0.73	0.84	0.50	0.82	0.85	0.59	0.81	2.32	3.38	1.16	0.82
Exchangeable Mg $cmol_{(+)}$ kg^{-1}	1.68	1.09	0.92	0.81	1.18	1.31	0.76	1.02	2.05	3.51	1.12	0.88
Exchangeable NH_4^+ $cmol_{(+)}$ kg^{-1}	5.57	5.56	5.85	4.24	4.57	4.87	3.80	4.09	6.03	7.79	5.05	3.46
Available Cu (mg kg^{-1})	0.50	0.55	0.27	0.54	0.46	0.52	0.178	0.74	0.60	0.75	0.201	0.63
Available Fe (mg kg^{-1})	4.45	4.38	1.38	2.14	4.42	6.93	4.115	2.47	5.83	3.24	< 0.055	6.59
Available Mn (mg kg^{-1})	3.38	2.89	2.69	1.72	3.47	1.33	1.72	2.22	4.97	2.85	2.043	1.39
Available Zn (mg kg^{-1})	0.26	0.44	<0.06	0.40	0.32	0.29	<0.06	0.38	0.49	0.71	< 0.06	0.19

Herrero-Hernández et al. [37] have reported a 3.5–6.5 times increase in the OM content in the upper horizon of a sandy loam soil amended with SMS at doses of 40 and 100 Mg ha^{-1}, but also found that OM content decreased by up to 1.4–1.8 times 355 days after SMS application to a vineyard soil. In addition, the application of poultry manure or waste compost to a sandy loam soil led to an increase >38% when applied at doses of 10–30 Mg ha^{-1} [14], while the application of sheep/goat manure or distillery organic waste compost at doses of 4–5 Mg ha^{-1} in a soil with similar texture produced an initial increase > 100% compared to the unamended soil's OC content [12]. A significant increase in the OC content in the 0–10 cm and 10–20 cm layers has been recorded by Marín-Benito et al. [38] in green-compost-amended soils versus non-amended soils seven months after application, indicating that the application of this organic amendment improved soil properties over the long term.

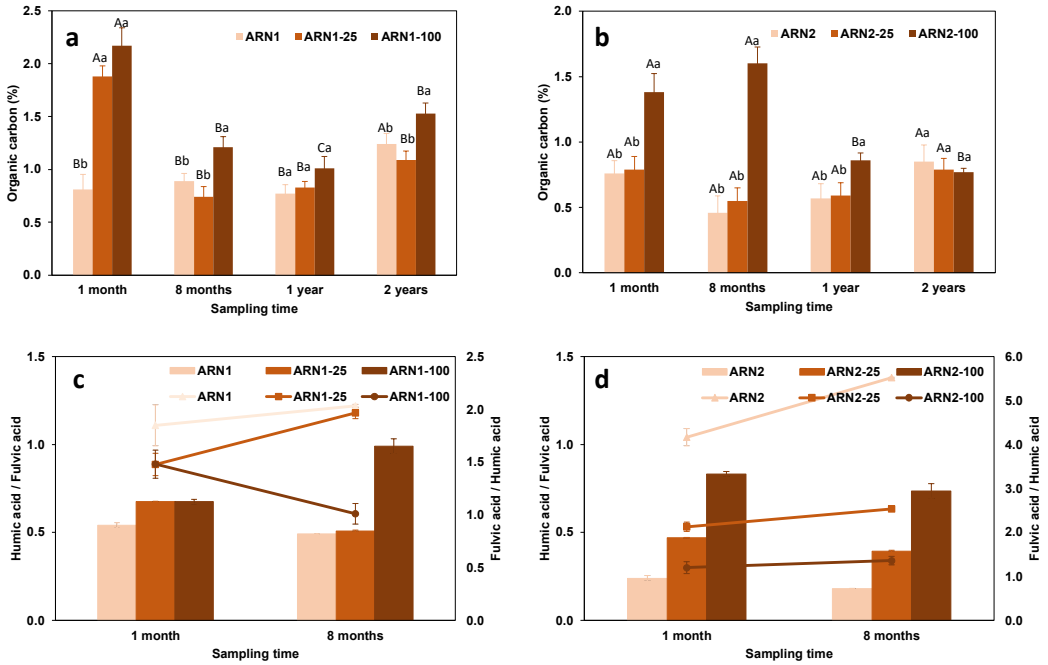

Figure 1. Changes in the percentages of OC in ARN1 (**a**) and ARN2 (**b**), and HA/FA (bars) and FA/HA (lines) ratios for ARN1 (**c**) and ARN2 (**d**) soils, unamended and SMS-amended at two rates at different sampling times after SMS application. Error bars represent the standard deviation of the mean. Different lower-case letters above the bars denote significant differences ($p \leq 0.05$) between samples at each sampling time, and different upper-case letters above the bars denote significant differences ($p \leq 0.05$) for each sample at different sampling times.

The present study found that the type and/or texture of the soil has an effect on the OC retention provided by the SMS and on its evolution over time. An indication of the maturity and stability of OM in organic soils can be determined by assessing the relationship between the OC associated with the HA and FA fractions extractable from the soil using NaOH [28]. This ratio was obtained here as an indicator of the evolution of OM [39]. This HA/FA ratio was <1 for all of the unamended and amended soils one and eight months after SMS application (Figure 1), indicating that most of the OC occurred as FA rather than HA [32]. Furthermore, one and eight months after SMS application, this indicator was higher in amended soils than in unamended ones (Figure 1). The changes in this indicator were significant in ARN2 at one and eight months after SMS application, but a significant change was recorded only in the ARN1-100 soil eight months after SMS application. These results are consistent with the observed decrease in the FA/HA ratio for all of the amended soils compared to the unamended ones (Figure 1); which is the opposite of the HA/FA ratio and, in general, its decrease indicates an increase in HA.

The application of SMS to soils could change the FA/HA ratio, enhancing HA due to the effect of microorganisms over the experimental time period [39]. The decrease in this FA/HA ratio was greater in the amended ARN2 sandy loam soil eight months after SMS application. In this sandy loam soil, the OC may be more bioaccessible to microorganisms, therefore facilitating this OC evolution [37]. The changes in these indicators were lower for ARN1 than for ARN2, possibly due to a different OC evolution mechanism. The silt+clay fraction content in ARN1 is twice that of ARN2, and it has been reported that this fraction, as well as the type and amount of clay minerals, have a direct influence on the OC stabilization provided by organic amendments in the soil [40]. This evolution of the OM humic fraction in the silty loam soil could explain the lower evolution of OM in ARN1 recorded two years after SMS application. Angelova et al. [32] have indicated that changes in the HA/FA ratio in favor of HA could be heavily influenced by the addition of OM from organic residues, although this depends on the quality of the compost used. These

authors have found significant changes in the HA/FA ratio after adding 5 and 10 g kg^{-1} of vermicompost or compost, and indicate that the increase was small relative to the large amount of OM applied because of the small amount of OC associated with humic- and fulvic-like substances extractable using NaOH [32].

The quantities of N, P, K, and Mg also increased in the amended soils, as was expected due to the organic amendment's role in increasing the nutrient availability for plants in the soil [18]. A significant correlation was found between soil OC content and N, P, K, and Mg (p range 0.042–0.001 in ARN1, and p range 0.009–0.000 in ARN2). The increase in organic N and its evolution over time followed a similar pattern to the increase in OC following the application of SMS to both soils. The significant correlation we observed between OC and N content in both soils (r = 0.82–0.84, p = 0.001) has been previously reported by other authors [41]. As with the OC levels, there was only a significant increase in N for the ARN1 soil amended with the higher rate of SMS at the end of the experiment, while the N content decreased two years after SMS application to the ARN2 soil.

In general, the role of organic amendments in facilitating microbial mineralization of organic N within soil is limited in short-term experiments [42]. NH_4^+-N and NO_3^--N concentrations (Tables 2 and 3) performed differently in the two soils. NH_4^+-N decreased up to 1.2–1.4 times in SMS-amended ARN1, while NO_3^--N increased up to 1.4 times ($p \leq 0.05$) one month after SMS application. However, both concentrations decreased over time, with no major differences recorded at the end of the experiment between the SMS-amended soils. However, NH_4^+-N and NO_3^--N increased up to 4.2 and 6.0 times, respectively, in the SMS-amended ARN2 soil ($p \leq 0.01$), with a significant increase in these concentrations eight months after SMS application. An increase in N mineralization in vineyard soils due to the effects of rain and temperature over time has been reported in a previous study [10]. The concentration of NH_4^+-N was lower than other values reported for amended soils, and some authors have found a relationship between N mineralization and the degree of stabilization of the organic residues applied [12]. It should be noted that an increase in N mineralization was observed in the soils at both sites at the end of the experiment. Moreover, NH_4^+-N concentration had increased more than 100% two years following treatment in both the unamended and amended ARN1 soil. This result could be explained if conventional tillage had been carried out before the sampling period, as is indicated in a Mediterranean vineyard agroecosystem [43]. Sodhi et al. [44] have reported that this nutrient could be physically protected within macro aggregates. Accordingly, NH_4^+-N concentrations were lower than those of NO_3^--N, as is frequently found in other amended vineyard soils [12], indicating that the soluble mineral N pool is dominated by NO_3^--N.

Analysis of the SMS-amended soils showed a significantly higher availability ($p \leq 0.05$) of P, K, and Mg concentrations in the soil amended with the higher SMS rate compared to the unamended soil. Initially, available P and K concentrations increased 1.6 and 2.6 times, respectively, in the ARN1-100 soil, and 1.4 and 1.6 times, respectively, in the ARN2-100 soil compared to unamended soil. The trend was similar in both soils, with P and K concentrations increasing up to eight months following the application of SMS, and then gradually decreasing to the values recorded for unamended soils. As previously cited, significant correlations between available P, K, and Mg, and soil OC and N contents indicate that the organic amendment has the potential to improve the K and P concentrations of a degraded soil, as has been reported for soils amended with other organic residues, such as poultry manure, although the effect of this amendment would not be permanent over time [14,45]. Courtney and Mullen [6] have compared the effects of SMS and mineral "NPK" fertilizer, and found that an SMS application of 100 Mg ha^{-1} had the strongest positive effect on barley (Hordeum vulgare) grain yield (a 59% increase compared to a no-fertilizer control). The authors report that SMS can be beneficial for crop yield and soil properties, but that it is important to consider the rate of SMS application.

A further advantage of the application of organic amendments to soil is the possible input of other nutrients such as Mn, Zn, Cu, and Fe, which are not provided by inorganic

NPK fertilization. Increases in these micronutrients over a two-year period after consecutive applications of organic amendments have been reported [45]. However, they were not observed in the SMS-amended soils in this research, possibly because of the single SMS application over the experimental time period.

3.2. Effect of SMS on Soil Biochemical Properties and PLFAs Analysis

The application of SMS increased RES, DHA, and BIO values in the ARN1 and ARN2 soils after SMS application at certain sampling times. In general, RES in ARN1 was higher than in ARN2 for the treatment rates and sampling times studied ($p \leq 0.01$ and $p \leq 0.05$, respectively) (Figure 2). Initially, RES increased in both soils after SMS application, although this change was not significant in all cases (p range 0.08–0.02); RES only increased in ARN1 after the application of SMS at the higher rate. It has previously been reported that organic amendments are associated with a higher impact on soil RES compared to inorganic fertilizers [45]. In our study, the effect of the SMS on RES remained over time when applied at the higher dose, but there were no significant differences between the RES values recorded for the unamended soils and those amended with the lower SMS dose. RES decreased after one year in ARN1, and after eight months in ARN2. In ARN1, the lowest RES values were recorded two years after treatment, while in ARN2, the lowest RES values were observed one year after SMS application. RES has previously been related to soil OC content [35], although our study found a significant correlation only between RES and OC for the ARN2 soil (r = 0.62, p = 0.031), and a non-significant correlation between RES and OC in ARN1, despite ARN1 exhibiting a higher OC content over time compared to ARN2 (Table 2). This could be explained as a result of the increased availability of labile C at ARN2 versus ARN1, as any increase in microbial activity and RES stimulation depends on the labile C pool, together with other variables, such as soil humidity [33].

Soil DHA also increased in the presence of SMS (p range 0.000–0.002) in both soils when the organic residue was applied at the higher dose (100 Mg ha^{-1}) (Figure 2). Initially, DHA was higher in ARN1 than in ARN2 when the higher dose of SMS was applied, and DHA was higher in the ARN1-100 than in ARN1 and ARN1-25. However, DHA was higher in ARN2-100 than in ARN1-100 at other sampling times. The increase in DHA is due to the stimulation of soil microorganisms following the introduction of OC [46]. The DHA values decreased over time, and a significant correlation with OC content was found only in ARN1 soil (r = 0.765, p = 0.004). The highest DHA values for ARN2 were detected in ARN2-100, with a lower change recorded in ARN2 and ARN2-25. The higher level of enzyme activity in ARN2-100 is related to the FA/HA ratio; the ARN2-100 condition is the one in which FA/HA decreased the most compared to the unamended soil, as previously indicated. ARN1 recorded a steady decline in activity, probably due to the reduction in readily available OC during the early stages of decomposition, resulting in more recalcitrant residues that were more difficult to degrade, and thus slowing the rate of microbial activity [47]. However, the DHA values in amended soils remained higher than in the unamended soils two years after SMS application.

Soil BIO followed a similar pattern to DHA in both soils (Figure 2). Initially, higher BIO values were found in ARN1-100 and ARN2-25 (p range 0.009–0.039). The BIO in the SMS-amended soils was significantly higher than that observed in the unamended ones (Figure 2). This was attributed to the high OM content of the SMS applied, which enriched the soil OC, a known energy source for soil microbes. After eight months, the BIO increased in both soils compared to the corresponding unamended ones [46], although there were non-significant differences between soils amended with low and high SMS doses. After two years, there were non-significant differences between the BIO values of the unamended and amended soils (Figure 2).

Soil BIO is affected by several factors, such as temperature, nutrient sources, water content, and the type of OM applied [14]. However, our analysis revealed a non-significant correlation between the BIO values and OC content or the other chemical parameters of the soils studied. Similarly, Carlson et al. [47] did not find a significant correlation between

these factors for other soils amended with different organic residues. Some authors have suggested that the OC supplied by organic amendments is readily metabolized and may therefore have an effect on the microbial community by providing an available energy source to be degraded by soil microorganisms [9,14]; these authors also report significant positive correlations between OC content and BIO in amended soils.

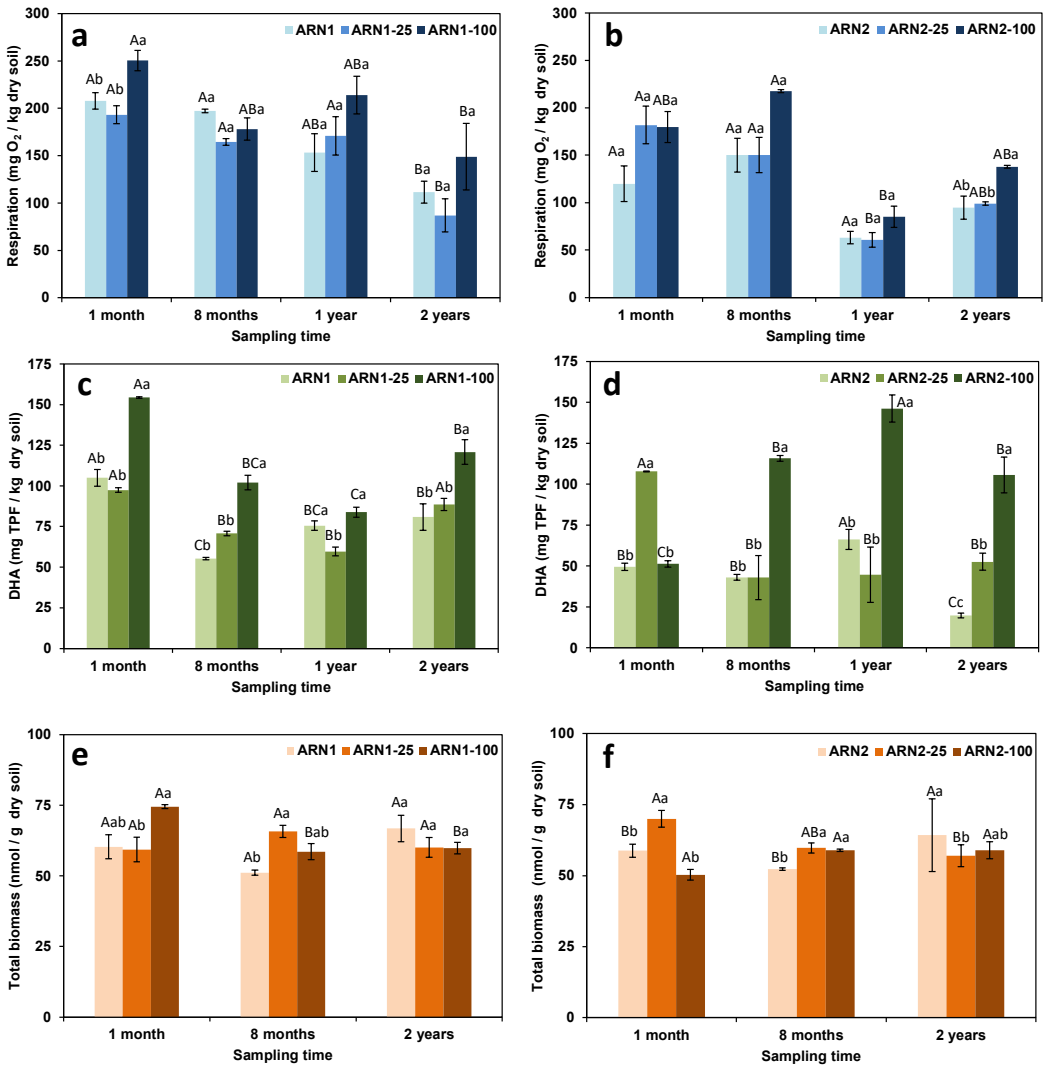

Figure 2. Soil microbial respiration measured by O_2 consumption (**a**,**b**), dehydrogenase activity—DHA (**c**,**d**), and total biomass (**e**,**f**) for ARN1 (**a**,**c**,**e**) and ARN2 (**b**,**d**,**f**) soils, unamended and SMS-amended at two rates at different sampling times after SMS application. Error bars represent the standard deviation of the mean. The lower-case letters above the bars denote significant differences ($p \leq 0.05$) between samples at each sampling time, and the upper-case letters above the bars denote significant differences ($p \leq 0.05$) for each sample at different sampling times.

The present research found correlations between microbial activity levels and OC content (DHA for ARN1 and RES for ARN2). These relationships did not lead to changes in the soils' overall BIO populations, but they did manifest themselves in the increased relative abundance of PLFAs. Specifically, our diagnostics revealed a greater abundance of gram-negative bacteria within both soils amended with SMS at the higher rate, and a greater abundance of fungi within all amended soils. However, this latter population decreased over time (data not shown). Significant correlations were found in the ARN2 soil between fungi and available Ca (r = 0.718, p = 0.029), and between gram-negative bacteria and DHA (r = 0.865, p = 0.003). It is well known that microorganisms play a key role in

OM decomposition, nutrient cycling, and other chemical transformations within soil [45]. Moreover, fungi are also capable of degrading more recalcitrant organic material, such as lignin and cellulose [47]. Our data revealed that these changes were higher in amended soils than in unamended ones, indicating that the application of organic residues increased the soils' overall fertility and quality, as fungi are important for forming soil aggregates, which in turn improve porosity and soil structure [47].

4. Conclusions

In general, the application of SMS to vineyard soils prompted an initial increase in all chemical parameters with the exception of pH. The application rates of SMS and the soil textures played a key role in the extent of these changes. Initially, soil OC increased 2.3–2.6 times in the silty loam soil, but only 1.8 times in the sandy loam soil. However, this increase in OC content persisted only in the soils treated with the highest SMS rate, remaining present after two years in the silty loam soil and after one year in the sandy loam soil. The silty loam soil has a silt+clay content twice that of the sandy loam, and a different OC evolution may have occurred as a result of the direct influence of this clay fraction on OC stabilization. In turn, the effect of the SMS application on the different biochemical and microbial properties of the soils was variable, and may have been conditioned by the availability of OC for soil microorganisms. The effect of OC was observed in DHA and RES, but BIO was not affected by OC content or other soil properties. Changes in the soils' microbial structure after SMS application, indicated by the relative abundance of PLFAs, were not very significant. The results of this study have contributed to our understanding of the long-term effects of an organic amendment and its doses of application for regenerating two degraded vineyards soils with different textures, findings which could be extrapolated to other eroded and degraded vineyard soils in La Rioja region.

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