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2 **SPENT MUSHROOM SUBSTRATES INFLUENCE SOIL QUALITY AND**
3 **NITROGEN AVAILABILITY IN A SEMIARID VINEYARD SOIL**

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25 **SPENT MUSHROOM SUBSTRATE INFLUENCE SOIL QUALITY AND N**
26 **AVAILABILITY IN A SEMIARID VINEYARD SOIL**

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42 **Keywords:** spent mushroom substrate; labile organic matter fractions; β -Glucosidase
43 activity; soil aggregation; N availability; soil CO₂ emissions

44
45
46

47 **Abstract**

48

49 Two spent mushroom substrates (fresh, F-SMS, and composted in aerobic conditions,
50 C-SMS) were annually applied for four years at two different rates (8 and 25 Mg ha⁻¹ as
51 dry matter) to a vineyard soil (Typic Haploxerept) located near Logroño (Ebro Valley,
52 La Rioja, Spain). Soil samples were collected at three depths (0–5, 5–15 and 15–25 cm),
53 and analysed for soil organic C (SOC), water-soluble carbon (WSC), potentially
54 mineralizable N (PMN), N-NO₃⁻ and exchangeable N-NH₄⁺, microbial biomass (MBC),
55 soil CO₂ evolution (SR), β -glucosidase activity (GLU) and soil mean weight diameter
56 (MWD) of aggregates and water-stable aggregates (WSA). The highest rate of F-SMS
57 and C-SMS (25 Mg ha⁻¹) increased SOC, Total N and labile organic forms (WSC and

58 PMN) at 0-5 and 5-15 cm soil depth and microbiological activity (MBC and GLU) at 5-
59 15 cm soil depth. There were no differences in soil CO₂ emissions between F-SMS and
60 C-SMS amendments. Applications of SMS did not increase the soil aggregation but
61 increased the content of inorganic N in the soil surface (0-5 cm depth) layer. Factor
62 analysis showed the positive relationship between labile organic matter fractions and N
63 availability. Finally, application of SMS to semiarid vineyard soils facilitates SMS
64 recycling with simultaneous improvement in soil quality indicators related to labile
65 organic matter and microbiologic activity. However, increased PMN and soil N-NO₃⁻
66 indicate a potential risks for N leaching from SMS amendments that should be further
67 evaluated.

68

69 Abbreviations: Fs, factorial component, GLU, β-glucosidase activity; MBC, microbial
70 biomass carbon; MWD, mean weight diameter; N_{inorg}, N-NO₃⁻+N-NH₄⁺; PMN,
71 potentially mineralizable nitrogen; SOC, soil organic carbon; SMS, spent mushroom
72 substrate; SOM, soil organic matter; SR, soil CO₂ emissions; WSC, water soluble
73 carbon; WSA, water stable aggregates percentage.

74

75

INTRODUCTION

76

77 Mushroom producers in La Rioja region (NW, Spain) use a formulated substrate
78 with straw (50 %), poultry manure (40-35 %), gypsum (10 %), and some N additives
79 like urea, ammonium sulphate or ammonium nitrate (1 %) to grow the white button
80 mushroom *Agaricus bisporus*. This material is considered “spent” when the mushroom
81 yield diminishes. 300,000 Mg per year of spent mushroom substrate (SMS) are
82 generated in La Rioja region and finally disposed of in landfills, thereby creating a risk

83 of groundwater pollution. La Rioja winegrowing region has approximately 60,000 ha of
84 vineyards representing 34 % of the total cultivated area. The majority of this vineyard
85 surface is generally tilled (0-15 cm soil depth) every 4 to 6 weeks as weed control
86 demands during the grapevine growth cycle. Soil tillage buries residues, disrupts
87 macroaggregates, increases aeration, stimulates microbial breakdown of soil organic C
88 (SOC) (Reeves 1997) and increases CO₂ release into the atmosphere. Therefore soil of
89 vineyards in La Rioja area have a low soil organic matter (SOM) content (<1 %) and a
90 loamy texture type (Peregrina et al. 2010a). The application of SMS to these soils as an
91 organic amendment would be a beneficial way of SMS recycling, improving the soil
92 quality due to its capacity to supply organic matter.

93 Recently, the application of various types of organic waste with a high organic
94 matter content (e.g., composted urban wastes, sewage sludge, composted plant materials
95 derived from municipal waste) to semiarid soils has been a common practice for
96 increasing SOM, reclaiming degraded soils, and supplying plant nutrients (Moreno et al.
97 1999; Ros et al. 2003; Walker 2003). Spent mushroom substrate has also been used
98 previously as a soil amendment (Wang et al. 1984; Maynard 1994; Courtney and
99 Mullen 2008) because of its high organic matter content and because it contains plant
100 nutrients such as N, P, K, Ca and Mg. The effects of sewage sludge compost
101 (Korboulewsky et al. 2002), biowaste compost (Nendel et al. 2007) or spent mushroom
102 compost (Morlat and Chausod 2008) on vineyards soils has been studied under climates
103 other than semiarid. In addition, scarce information exists about spent mushroom
104 substrate applications in semiarid vineyard soils and its implications on the soil quality
105 and N availability.

106 Evaluation of soil quality is a complex task, requiring the determination of
107 various physical, chemical and biological properties (Bastida et al. 2008). Under

108 semiarid climatic conditions, the labile organic matter fractions are more sensitive than
109 SOC to changes in soil management and, therefore, they could be used as indicator of
110 soil quality (Haynes 2005). Among these labile fractions, water soluble C (WSC) is a
111 readily available substrate for microbial activity and is considered representing the most
112 dynamic soil C fraction representing (McGill et al. 1986). Potentially mineralizable N
113 (PMN) is an indicator of soil N availability (Waring and Bremner 1964; Soon et al.
114 2007), or a bioindicator of labile organic matter as the indigenous microbial biomass
115 release N from soil SOM (Haynes 2005). Water stable aggregates percentage (WSA) is
116 related to SOM content (Tisdall and Oades 1982; Franzluebbers and Arshad 1996;
117 Ternan et al. 1996) and is a sensitive indicator of soil susceptibility to erosion (Barthès
118 and Roose 2002). The mean weight diameter (MWD) of soil aggregates has also been
119 used to detect changes in aggregation (Álvaro-Fuentes et al. 2008). However physical
120 properties change slowly compared to biochemical and microbiological properties [e.g.,
121 microbial biomass C (MBC), β -Glucosidase activity (GLU) and Soil respiration] which
122 are more sensitive to management (Dick and Tabatabai 1993; Scholter et al. 2003;
123 Coleman et al. 2004).

124 Therefore, this study evaluated the effects of adding fresh-SMS and composted-
125 SMS on both the soil quality and inorganic N content in a semiarid vineyard
126 agroecosystem. We monitored soil organic C, total N, labile organic matter fractions,
127 soil microbiological properties, soil aggregation and inorganic N. We hypothesized that
128 SMS addition to a semiarid vineyard soil could have beneficial effects on soil quality,
129 especially on soil biochemical properties and microbiological parameters.

130

131

MATERIALS AND METHODS

132

133 Site description, experimental layout and treatments

134

135 The experiment was established in 2006 at the experimental farm “La Grajera”
136 property of La Rioja regional government, located in northern Spain (latitude, 42° 26′34
137 18′′N; longitude 2° 30′53 07′′W). Field slope was about 10.2 % with a west-east
138 orientation. Soil was classified as Fine-loamy, mixed, thermic Typic Haploxerepts
139 according to the USDA soil classification (Soil Survey Staff 2006) and contained 230 g
140 kg⁻¹ clay, 433 g kg⁻¹ silt, and 337 g kg⁻¹ sand, 9.3 g kg⁻¹ organic matter, and 149 g kg⁻¹
141 carbonates; pH was 8.62 and electrical conductivity 0.17 dS m⁻¹ in the Ap horizon (0-15
142 cm).

143 Climate in the area is semiarid according UNESCO aridity index (UNESCO,
144 1979), with high-intensity winter rains and summer drought conditions. For the 2006–
145 2009 period, the average annual precipitation was 494.4 mm, the average annual
146 temperature was 13.2 °C, and the reference evapotranspiration (ET_o) calculated
147 according to the FAO Penman-Monteith method was 1117 mm.

148 The vineyard had been established in 1996 planting *Vitis vinifera* L.
149 “Tempranillo”, grafted on 110-R rootstock. Vine spacing was 1.1 m in row and 2.1 m
150 between the east-west facing rows, with a total planting density of 3,135 vines per
151 hectare. Vines were trained on a VSP trellis system.

152 Fresh-SMS (SMS without any processing) and composted-SMS (fresh-SMS
153 composted in aerobic conditions) were employed as these two are the available types of
154 SMS from the residue treatment plant present in La Rioja. The two different application
155 rates (8 Mg of dry matter ha⁻¹ and 25 Mg of dry matter ha⁻¹) corresponded to two
156 different strategies for vineyard management. The lower rate served as a fertilization for

157 the grape wine crop, and the higher rate served as an organic amendment to improve the
158 low organic matter content in vineyard soils.

159 The experimental layout was a randomized complete block design with five
160 treatments, three replicates per treatment and one replicate per block. The plot size was
161 35 m² (2.9 m width x 12.1 m length) and included 10 vines. During four years the
162 treatments were the following: (1) Control: non-amended control plot; (2) F8: soil
163 amended with F-SMS (8 Mg of dry matter ha⁻¹); (3) C8: soil amended with C-SMS (8
164 Mg of dry matter ha⁻¹); (4) F25: soil amended with F-SMS (25 Mg of dry matter ha⁻¹);
165 and (5) C25: soil amended with C-SMS (25 Mg ha⁻¹ of dry matter). Spent mushroom
166 substrate was applied homogeneously by hand on the surface in May 2006, April 2007,
167 February 2008 and March 2009. After each application the soil was tilled with a
168 cultivator (0-15 cm). In addition, soil tillage (0-15 cm depth) to eliminate weeds was
169 carried out every 4 to 6 weeks during the growing season (February to August). No
170 additional inorganic fertilization was applied throughout the experiment.

171

172 **Description of the Spent Mushroom Substrate**

173

174 Two different SMS were used: F-SMS and C-SMS from *A. bisporus* cultivation;
175 they were supplied by “Intraval, TRADEBE Environmental Group, S.L.” (La Rioja,
176 Spain) and applied as explained above. In general SMS is a pasteurized mixture of
177 cereal straw, poultry manure, ammonium nitrate, urea and minerals (gypsum and/or
178 calcium carbonate).

179 F-SMS was obtained after plastic bags and calcic gravel were separated from
180 SMS. To form C-SMS, F-SMS was placed in compost piles (2.5 m high) and further
181 composted for 90 days under aerobic conditions. During this period, the SMS material

182 was mixed with woodchips for aeration and turned periodically to allow its maturation
183 and decomposition, which increased the uniformity and stability level of the C-SMS.
184 Finally, the residual woodchips were eliminated and the SMS was sieved, crumbled,
185 and homogenized. The physical-chemical properties of the F-SMS and C-SMS are
186 presented in Table 1.

187

188 **Sampling procedure**

189

190 Soil sampling was carried out in June 2009 at depths of 0–5, 5–15 and 15–25
191 cm. At each plot, six soil cores were taken to make a composite sample representative
192 of each plot and depth. At the 0-5 cm depth, soil cores were taken with stainless steel
193 cylinders (height 51 mm, diameter 50 mm, volume 100 cm³) and at 5-15 and 15-25 cm
194 depths with an Edelman type auger.

195

196 **Sample Preparation and Laboratory Analyses**

197

198 Once in the laboratory, the field moist soil was sieved (2 mm) and divided into
199 two subsamples. One was immediately stored at 4 ° C and analyzed within 4 weeks. The
200 other was air-dried for chemical analysis.

201

202 SOC was determined by the wet oxidation method of Walkley and Black
(Nelson and Sommers 1982). Water soluble C (WSC) was extracted by deionized water
203 (1:2 w/v extractant ratio) for 1 hour, after shaking 10 g of air dried soil in 20 ml of
204 deionized water in a 50 ml centrifuge tube for 30 minutes, centrifuging it for 30 min. at
205 3500 rpm and filtering all supernatant solution through a Whatman n°. 42 filter. The
206 organic carbon in the filtrate was determined by the dichromate oxidation method of

207 Burford and Bremer (1975). Total N content was determined by dry combustion with a
208 LECO CNS elemental analyzer (LECO Corporation, St. Joseph, MI, USA).

209 Potentially mineralizable N (PMN) was determined with the anaerobic
210 incubation method (Burt 2004). Exchangeable N-NH_4^+ was extracted in 1:5 ratio of soil
211 to 2 N KCl. The N-NH_4^+ contents were determined by the colorimetric Berthelot
212 method (Mulvaney 1996). The exchangeable N-NH_4^+ was subtracted from the PMN
213 results to obtain the N-NH_4^+ during the anaerobic incubation. N-NO_3^- extractable
214 contents were determined in 1:3 soil to saturated calcium sulfate solution extract by
215 second derivative method (Sempere et al. 1993).

216 To determine the water stable aggregates (WSA), dried aggregates between 1
217 and 2 mm were separated from the <2 mm soil fraction using a 1 mm sieve. The WSA
218 was measured using the Kemper and Rosenau procedure (1986). Briefly, 4 g of 1–2 mm
219 of air-dried aggregates were placed on the top of a 0.25 mm sieve and sieved in distilled
220 water during 3 min with a stroke length of 1.3 cm and a frequency of 34 strokes $\cdot\text{min}^{-1}$.
221 Soil retained on each sieve was transferred to an aluminium pan, dried and weighed.
222 Sand correction was done in all samples by dispersing the stable aggregates with a
223 sodium hexametaphosphate solution and sieving again through a 0.25 mm sieve.

224 The mean weight diameter (MWD) of soil aggregates was determined only for
225 the 0-5 cm depth because sampling with auger can modify the macroaggregates size
226 distribution at 5-15 and 15-25 cm depths. MWD was measured by placing 200 g of air-
227 dried soil (previously passed through an 8 mm sieve) on top of a vertical
228 electromagnetic sieve apparatus (FRITSCH Analysette 3 PRO) equipped with a stack of
229 seven sieves with the following screens: 4, 2, 1, 0.85, 0.50, 0.25 and 0.05 mm. The
230 sieving time was 5 min and the amplitude was 0.1 mm as reported by Álvaro-Fuentes
231 (et al. 2008). Dry soil remaining on each sieve was collected and weighed. The mean

232 weight diameter was used to express dry aggregate size distribution (Youker and
233 McGuinness 1957).

$$234 \quad MWD = \sum_{i=1}^8 X_i W_i$$

235 Where X_i is the mean diameter of the size fraction, W_i is the proportion of total sample
236 weight retained on each sieve and 8 is the number of size fractions.

237 Soil microbial biomass was determined using the CHCl_3 fumigation–extraction
238 method (Vance et al. 1987). The method used to determine measure β -glucosidase was
239 based on the colorimetric determination of saligenin released by β -glucosidase where
240 5 g of soil was incubated for 3 h at 37 °C with acetate buffer (pH 6.2), and salicin (β -
241 glucosido-saligenin) (Tabatabai 1994). For each analysis of enzymatic activity, two
242 subsamples and one control were carried out. Results were reported on an oven-dry-
243 weight basis, determined by drying the soils for 24 h at 105 °C..

244 Soil CO_2 emissions was monitored in winter, spring, summer and autumn of
245 2009, the first sampling (February 25) was carried out prior to the SMS application. The
246 second sampling (March 22) was taken 1 week after the amendments application. Soil
247 respiration was determined by two measurements per replication. All measurements
248 were taken between 9:00 A.M. and 12:00 P.M. to reduce variability in CO_2 emissions
249 due to diurnal changes in temperature (Parkin and Kaspar 2003). The CO_2 flux was
250 measured using an Environmental Gas Monitor chamber attached to a data logger
251 (model EGM-4, PP System, Haverhill, MA). The chamber was 15 cm in height, 10 cm
252 in diameter, and was able to measure CO_2 emissions ranging from 0 to 9.99 g CO_2 m^{-2}
253 h^{-1} as well as the percent of humidity in chamber's head space. In each plot, the soil
254 chamber was placed on the soil surface avoiding disturbance for 150 seconds until the
255 CO_2 emissions measurement was recorded in the data logger. At the time of the CO_2

256 measurements, soil temperature was monitored next to the soil chamber with a soil
257 digital thermometer (Multi-Thermometer) at 5 cm depth.

258

259 **Statistical Analysis**

260

261 A mixed-effects ANOVA with fixed factors (treatment, and depth) and random
262 factor (block) was carried out on the data. Also, in order to determine pair-wise
263 differences by post-hoc tests, a one-way ANOVA was performed at each depth. The
264 post-hoc test applied was Fisher's least significant difference (LSD) method. Factorial
265 analysis (FA) was carried out in order to reduce the original set of variables into a
266 smaller set of non-correlated components, or factorial components (Fs), which represent
267 most of the information found in the original variables. Generally, only the first few Fs
268 in a descending order explain the maximum of the total variance of all original
269 variables. The factorial loadings of the data were analysed and three principal
270 components were established (F1, F2 and F3). This multivariate method was applied to
271 all soil parameters determined at the 0-5, 5-15, 15-25 cm soil depth. All statistical
272 procedures were carried out using Statgraphics Plus for Windows (1998).

273

274 **RESULTS**

275

276 **SOC, Total N and Labile organic matter fractions**

277

278 SOC, Total N, WSC and PMN were influenced by treatments and SOC, Total N
279 and PMN by depth (Table 2). There was an interaction between treatment and

280 depth for SOC, Total N, WSC and PMN, and an interaction between treatment and
281 block for SOC and Total N (Table 2).

282 Spent mushroom substrate treatments with higher rates (F25 and C25) increased
283 SOC with respect to the control at 0-5, 5-15 and 15-25 cm soil depth (Fig. 1). For the
284 lower SMS rates (F8 and C8) the increase was significant only at 0-5 cm depth for the
285 F8 treatment.

286 Total N tended to increase in comparison to the control with SMS applications at
287 all soil depths. The differences were significant for the higher rates (F25 and C25),
288 while for SMS lower rates (F8 and C8) the differences were significant only for F8 at 0-
289 5 cm soil depth and C8 at 15-25 cm soil depth (Fig. 1).

290 Water soluble C increased with respect to the control in all F-SMS and C-SMS
291 treatments at 0-5 cm soil depth, while at 5-15 cm depth only C8, F25 and C25
292 treatments increased WSC with respect to the control (Fig 1.). WSC increased in the
293 SMS higher rates (F25 and C25) in comparison to SMS lower rates (F8 and C8) at 0-5
294 cm soil depth (Fig. 1). At 15-25 cm soil depth no differences existed between
295 treatments.

296 Potentially mineralizable N values were higher in all SMS treatments than in the
297 control at 0-5 and 5-15 cm soil depths. At these soil depths the highest SMS rates (F25
298 and C25) presented greater PMN with respect to the lower rates (F8 and C8) (Fig. 1).
299 While at 15-25 cm soil depth, C8, F25, and C25 treatments increased PMN with respect
300 to the control.

301

302

Microbiological properties

303

304 MBC, MBC/SOC were influenced by treatments and GLU and GLU/SOC were
305 affected by depth (Table 2). There was interaction between treatment and soil
306 depth for MBC, and there were interactions between soil depth and block for MBC
307 and MBC/SOC.

308 Microbial biomass C in the F25 and C25 treatments was two-fold greater than
309 control at 5-15 cm depth (Fig. 2). At 0-5 cm and 15-25 cm soil depths, no significant
310 differences were found between treatments.

311 Microbial biomass C to soil organic C ratio tended to be lower in SMS
312 treatments with respect to the control at 0-5 cm depth, while significant differences were
313 only observed for C8, F25 and C25 treatments (Fig. 2). At the 5-15 cm depth, SMS
314 treatments tended to decrease MBC/SOC ratio although significant differences were not
315 observed. Finally, MBC/SOC ratio in SMS treatments tended to decrease with respect
316 to the control at 15-25 cm depth, with significant differences for C8 and F25 treatments.

317 β -Glucosidase activity at 0-5 cm depth increased in the F8 treatments with
318 respect to the control (Fig. 2). SMS treatments also tended to increase GLU with respect
319 to the control at the 5-15 cm depth, in this case only the higher rates treatments (F25 and
320 C25) had significant increases with respect to the control. Finally, at 15-25 cm soil
321 depth, no significant differences were found between treatments.

322 β -Glucosidase activity to soil organic C ratio tended to decrease with respect to
323 the control in the 0-5 cm depth, with the higher SMS rates, significant only for C25
324 treatment (Fig. 2). At 5-15 cm depth no differences were found between treatments. In
325 the 15-25 cm depth only C25 treatment had a lower GLU to SOC ratio respect to the
326 control. At 15-25 cm soil depth GLU/SOC for all treatments was two-fold greater than
327 at 0-5 y 5-15 cm soil depths.

328

Soil CO₂ emissions

329

330

331 Soil CO₂ emissions (SR) in the control treatment presented a pattern with two
332 peaks at the end of spring-beginning of summer (5-7-2009) and at beginning of autumn
333 (25-10-2009). These results agreed with both the highest air humidity and soil
334 temperatures higher than 20 ° C (Table 3). All SMS treatments had a similar SR pattern
335 in comparison to the control, except in March when F25 treatment had a significantly
336 greater SR with respect to the control, F08 and C8 treatments, and had no significant
337 differences with respect to C25.

338

Water stable aggregates and dry mean weight diameter of soil aggregates

339

340

341 Percentage of water soluble aggregates was affected by depth (Table 2). All
342 SMS treatments tended to decrease WSA with respect to the control, with significant
343 differences for C25 treatment at 0-5 cm soil depth and for C8 treatment at 5-15 cm soil
344 depth (Fig. 3). Mean weight diameter value ranged between 3.22 and 2.72 mm and no
345 significance differences between treatments were noted.

346

Nitrogen availability

347

348

349 N-NO₃⁻ and N-NH₄⁺ were affected by both treatment and soil depth and
350 WSC/N_{inorg} was affected by soil depth. There was interaction between treatment
351 and soil depth for N-NO₃⁻ (Table 2). F8, F25 and C25 treatments increased N-NO₃⁻
352 content at 0-5 cm soil depth with respect to the control (Fig. 4) with F25 and C25 were
353 four times greater than control and F8 and C8 were two-fold greater than control at 0-5

354 cm soil depth. However, only F25 and C25 treatments increased soil N-NO₃⁻ with
355 respect to the control at 5-15 cm soil depth. Finally, no differences were found at 15-25
356 cm soil depth.

357 Regarding N-NH₄⁺, a similar trend to N-NO₃⁻ occurred; at 0-5 cm soil depth N-
358 NH₄⁺ content was higher in F8, F25 and C25 treatments with respect to the control (Fig.
359 4). In addition, only F25 treatment increased N-NH₄⁺ with respect to the control both at
360 5-15 and 15-25 cm soil depths.

361 Spent mushroom substrate tended to decrease WSC/N_{inorg} ratio with respect to
362 the control at 0-5, 5-15, and 15-25 cm soil depths although significant differences
363 appeared only in C25 at 0-5 cm soil depth (Fig. 4).

364

365

Factor analysis

366

367 A graphical representation in two dimensions of the 12 studied properties,
368 projected on the plane defined by factor 1 and factor 2, is shown in Figure 5. The first
369 principal component was strong and positively correlated with SOC, Total N, WSC,
370 PMN, N-NO₃⁻, N-NH₄⁺ and MBC, this later component was also negatively correlated
371 to the GLU/SOC, GLU/MBC and WSC/N_{inorg} ratios. The second principal component
372 was strong and positively correlated to GLU and the GLU/SOC and WSC/N_{inorg} ratios.
373 Finally the third principal, component was highly correlated with MBC, the MBC/SOC
374 ratio, and GLU (Table 4). In the principal component analysis the first, second and third
375 principal components explained respectively 45.5 %, 20.9 % and 10.9 % of the total
376 variance (Table 4).

377

Discussion

378

379 We can conclude that the two SMS types studied (F-SMS and C-SMS) had a
380 similar positive effect on SOC, labile organic matter fractions and N availability.
381 Moreover, neither of the SMS types had a positive effect on the soil aggregation.

382 One of the most important aspects of incorporating organic amendments into
383 degraded soils is the increase of SOC and total N. In our study, both SMS types applied
384 at the higher rates (25 Mg ha⁻¹) increased SOC and Total N at 0-15 cm soil depth (where
385 SMS and soil was mixed with a cultivator). This is an important result as organic matter
386 levels in vineyards with conventional tillage in La Rioja region are normally below 1 %
387 (Peregrina et al. 2010a). Increments in SOC were similar to those observed in other
388 degraded soils under semiarid conditions with sewage sludge applications (Bastida et al.
389 2008; 2010) or with SMS applications (Courtney and Mullen 2008; Morlat and
390 Chaussod 2008). In addition, similar Total N increases were found in studies where
391 SMS was applied (Morlat and Chaussod 2008) or where winery and distillery wastes in
392 a semiarid calcareous vineyard soil were employed (Bustamante et al. 2010).

393 Factor analysis showed a strong relationship between SOC increases and the
394 increases of the labile organic matter (WSC and PMN). Bastida et al. (2008) reported
395 WSC increase after sewage sludge applications. Water soluble C (WSC) is considered
396 the most dynamic C fraction in soil and represents a readily available substrate for
397 microbial activity (McGill et al. 1986). Potential mineralizable N represents the
398 proportion of soil organic N subjected to mineralization which is available to plants in
399 the short to medium term. It is a sensitive bioassay to management practices. Moreover,
400 correlations between PMN and both SOC and MBC have been found (Brejda et al.
401 2000) and also between PMN and the soil respiration rate and microbial biomass
402 (Carpenter-Boggs et al. 2003). Given the relationships between labile organic matter

403 fractions (WSC and PMN) and different microbiological properties of soils, the
404 increases of WSC and PMN can be considered as improvements in soil quality.

405 Our results suggest that WSC has a positive influence on the N mineralization
406 capability of microbial soil fauna. Correlation between WSC and PMN was also
407 reported by Peregrina et al. (2010b; 2012) in this vineyard soil with the use of cover
408 crops. The relationships between N-NO_3^- and N-NH_4^+ with WSC, vary in accordance
409 with simultaneous increments of WSC and N-NH_4^+ under conventional tillage in
410 vineyard soils as reported by Steenwerth and Belina (2008a,b). In addition, dissolved
411 organic carbon directly regulates N mineralization and nitrification, in Mediterranean
412 vineyard soils under conventional tillage (Christou et al. 2006)

413 Microbial biomass C in control treatments was similar to values shown by
414 Lagomarsino et al. (2011) in Mediterranean vineyard soil under conventional tillage.
415 Moreover the SMS applications, particularly at the higher rates, increased MBC and
416 GLU within 5-15 cm soil depth. Organic manure applications increased MBC in
417 semiarid climatic conditions (Bastida et al. 2010), and also SMS applications increased
418 MBC in vineyard soil with conventional tillage (Morlat and Chaussod 2008).
419 Consequently, SMS is linked to an increase of MBC which indicates an improvement of
420 soil quality.

421 Increases in GLU with the application of organic manure had been reported by
422 various authors (Tejada et al. 2006; Bastida et al. 2008; Liu et al. 2010; Dinesh et al.
423 2010). The β -glucosidase activity of soil reflects the ability of soil microorganisms to
424 decompose cellulose to its low molecular-weight derivatives, such as cellobiose to
425 glucose, and to support diverse microbial populations (Turner et al. 2002). Thus greater
426 GLU activities at 5-15 cm soil in the higher SMS treated soils suggest enrichment in
427 cellulolytic substrates.

428 The ratios between microbiological parameters and SOC can be considered as
429 indicators of the changes in these soil properties. Thus, in the current study lower
430 MBC/SOC ratios in the SMS treatments near the soil surface (0-5 cm) are probably due
431 to lower availability and/or degradation of organic compounds from SMS, and hence
432 the lower microbial growth and C turnover. Moreover, SMS applications did not
433 increase GLU/SOC ratio, therefore in SMS, the relation between cellulolytic substrates
434 and total organic matter could be too low to influence the GLU/SOC ratio in the soil.

435 Soil CO₂ emissions values in the control and the SMS treatments showed a
436 similar annual trend to that reported by Steenwerth et al. (2010) and Carlisle et al.
437 (2006) in Californian vineyards where, under Mediterranean climatic conditions, soil
438 respiration is limited by the soil moisture in summer and by temperature in winter
439 (Steenwerth et al. 2010). In a study by Mariscal-Sancho et al. (2010) the soil CO₂
440 emissions increased with soil organic carbon and β-glucosidase activity under different
441 soil management and in Mediterranean climatic conditions. However in our conditions,
442 it seems that soil CO₂ emissions were not affected by SOC and GLU increases due to
443 SMS applications. This difference with the afore mentioned results could be due to the
444 fact that the highest GLU values in our conditions was four times lower than the highest
445 GLU values reported by Mariscal-Sancho et al. (2010).

446 In our study the applications of SMS increased N availability especially within
447 the first 5 cm of soil. N-NO₃⁻ and N-NH₄⁺ increases have been found in soils including
448 vineyards soils amended with sewage sludge (Korboulewsky et al. 2002; Tarrasón et al.
449 2008), wine distillery wastes (Bustamante et al. 2010) and SMS (Morlat and Chaussod
450 2008). Factor analyses showed that N-NO₃⁻ and N-NH₄⁺ were related to the labile
451 organic matter fractions (WSC, and PMN) and MBC. Positive correlations of SOC with

452 MBC, N-NO₃⁻, and N-NH₄⁺ after sewage sludge application in semiarid soils have been
453 reported by Bastida et al. (2010), which agrees with our results.

454 The higher N mineralization rates in the 0-5 cm depth with SMS results in a
455 lower WSC/N_{inorg} ratios at this depth. DeLuca and Keeney (1993) reported that a low
456 WSC/N_{inorg} ratio indicates high potential N mineralized rates.

457 In addition, in Spanish Mediterranean vineyards amended with cattle manure, N
458 inorganic losses by surface runoff may be twice as high as untreated soils due to the
459 higher inorganic N on the soil surface (Ramos and Martínez-Casasnovas 2006), so the
460 N-NO₃⁻ and PMN increases with SMS applications to soil surface could increase the
461 risk of N leaching.

462 The improvement of the soil surface resistance is particularly important in
463 semiarid vineyards soils where high erosion rates can occur (Ramos and Martínez-
464 Casasnovas 2004). Various authors reported soil aggregation increases due to organic
465 amendments applications (Bipfubusa et al. 2008; Wortmann and Shapiro 2008; Ojeda et
466 al. 2008). However SMS applications did not increased WSA and MDW even at the
467 higher SMS rates. The SMS at high rates (e.g. 25 Mg ha⁻¹ year⁻¹) in this vineyard soil
468 increased electrical conductivity and concentrations of Na⁺, K⁺ and Mg²⁺ (Larrieta et al.
469 2010a;2010b). Increased ionic concentration can enhance the susceptibility to dispersion
470 and decrease the aggregate stability (Bronick and Lal 2005). This process could thus
471 counter the positive effects in soil aggregation due to SOC increases with SMS
472 applications.

473

474

CONCLUSIONS

475 Under semiarid climatic conditions and conventional soil management the
476 application of F-SMS or C-SMS at the higher studied doses increased soil organic

477 matter content, Total N, labile organic matter forms and improved microbiological
478 activity. Consequently, the addition of spent mushroom substrate may be considered as
479 a good strategy for rehabilitating the soil quality of semiarid vineyard soils with low
480 organic matter content. However, SMS application did not increase soil aggregation
481 regardless of application rate, suggesting that utilization of SMS does not decrease
482 erosion risk.

483 Regarding the N availability, SMS applications increased soil N-NO₃⁻,
484 particularly at 0-5 cm soil depth. The increases of potentially mineralizable nitrogen and
485 N-NO₃⁻ suggest that more research must be carried out to evaluate the risks of N
486 leaching from soil amendment with SMS.

487

488

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489

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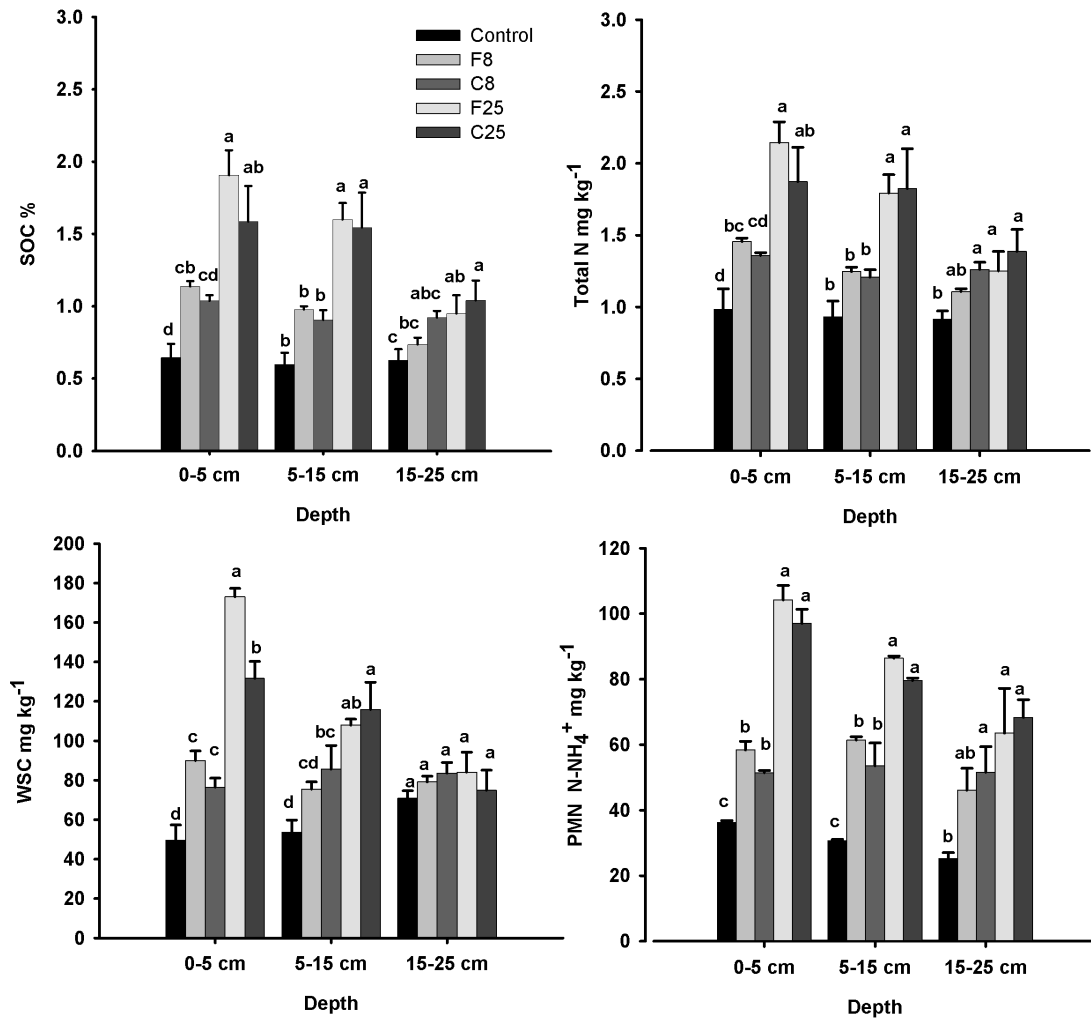
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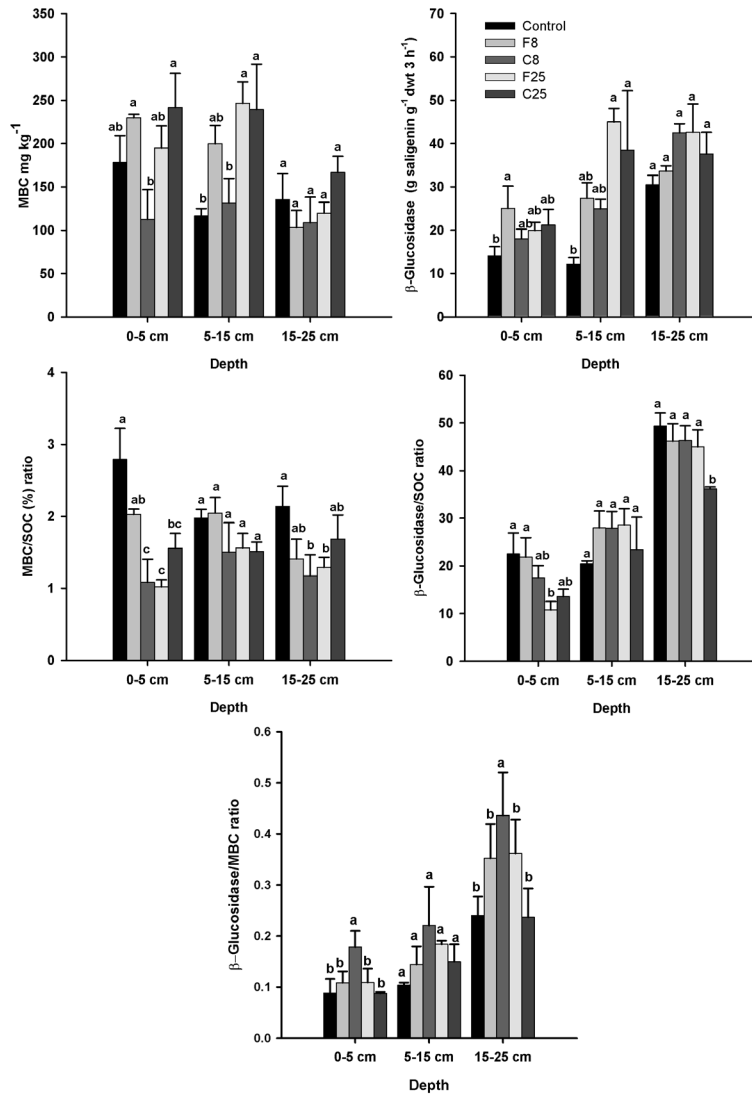
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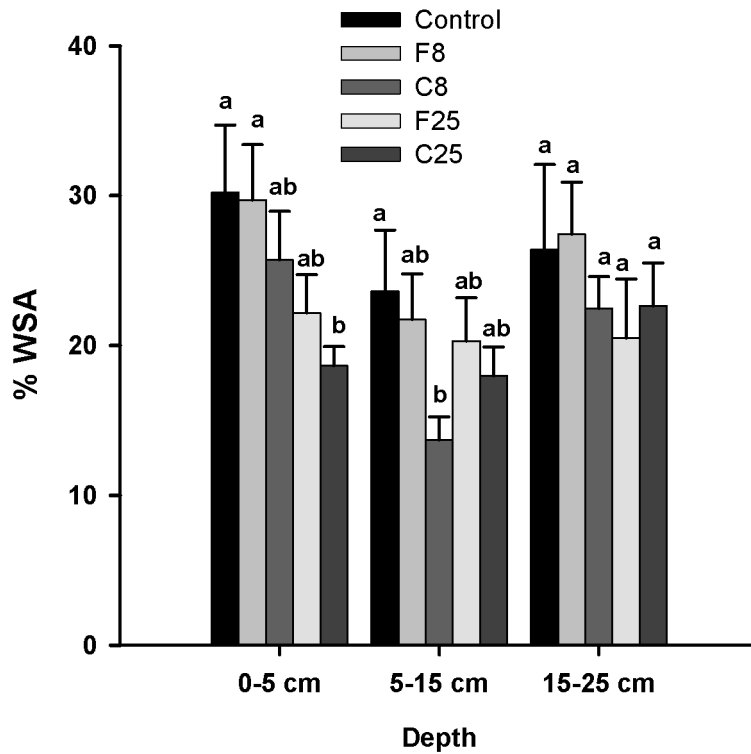
676 Figure 1. Soil organic carbon (SOC), total nitrogen (Total N), water soluble carbon
 677 (WSC) and potentially mineralizable nitrogen (PMN) at different soil depths for each
 678 treatment. The letters represent the comparisons by L.S.D. test ($p < 0.05$) among
 679 treatments within each depth. Bars represent standard error. Control: non-amended
 680 control plot; F8: soil amended with fresh-SMS (8 Mg of dry matter ha⁻¹); C8: soil
 681 amended with composted-SMS (8 Mg of dry matter ha⁻¹); F25: soil amended with

682 fresh-SMS (25 Mg of dry matter ha⁻¹); C25: soil amended with composted-SMS (25
 683 Mg ha⁻¹ of dry matter)

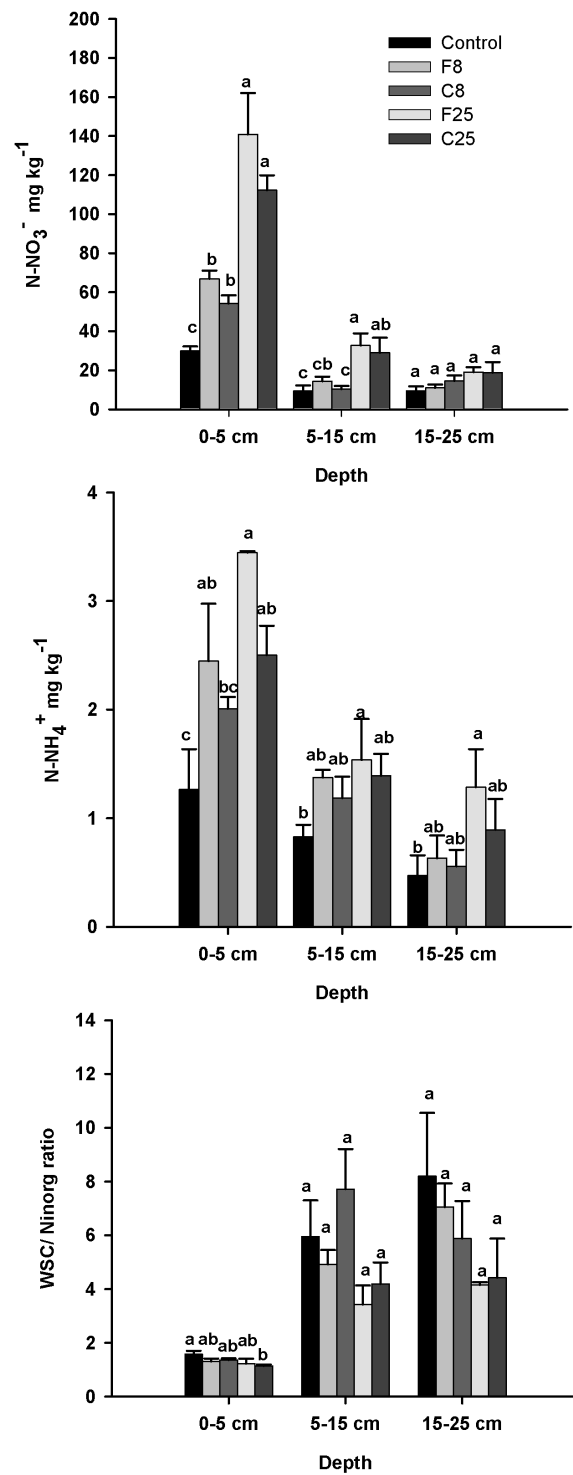


684
 685 Figure 2. Microbial biomass carbon (MBC), β-Glucosidase activity (GLU), MBC to
 686 SOC ratio (MBC/SOC), and β-Glucosidase to SOC ratio (GLU/SOC) values at different
 687 soil depths for each treatment. The letters represent the comparisons by L.S.D. test (*p*
 688 <0.05) among treatments within each depth. Bars represent standard error. Control: non-
 689 amended control plot; F8: soil amended with fresh-SMS (8 Mg of dry matter ha⁻¹); C8:
 690 soil amended with composted-SMS (8 Mg of dry matter ha⁻¹); F25: soil amended with

691 fresh-SMS (25 Mg of dry matter ha⁻¹); C25: soil amended with composted-SMS (25
 692 Mg ha⁻¹ of dry matter)



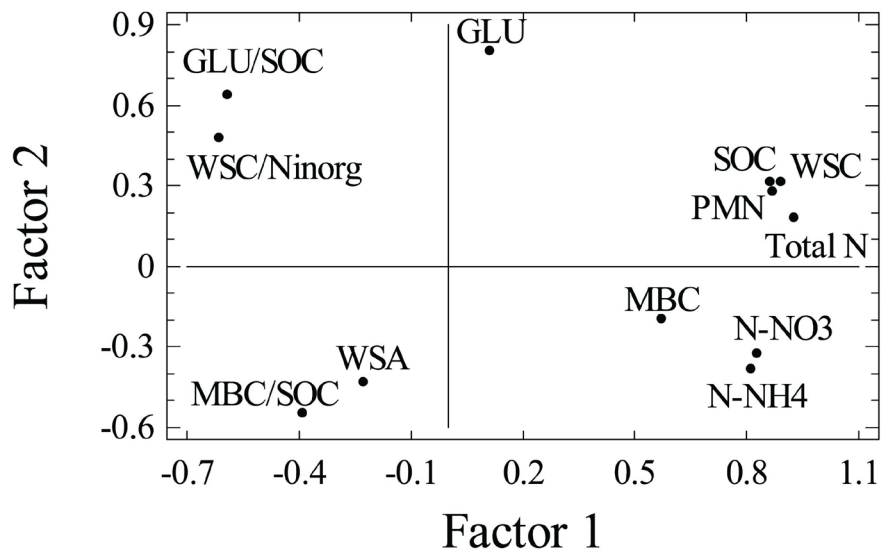
693
 694 Figure 3. Water stable aggregates (WSC) values at different soil depths of each
 695 treatment. The letters represent the comparisons by L.S.D. test ($p < 0.05$) among
 696 treatments within each depth. Bars represent standard error. Control: non-amended
 697 control plot; F8: soil amended with fresh-SMS (8 Mg of dry matter ha⁻¹); C8: soil
 698 amended with composted-SMS (8 Mg of dry matter ha⁻¹); F25: soil amended with
 699 fresh-SMS (25 Mg of dry matter ha⁻¹); C25: soil amended with composted-SMS (25
 700 Mg ha⁻¹ of dry matter)



701

702 Figure 4. $N-NO_3^-$, $N-NH_4^+$ and ratio the WSC/N_{inorg} values at different soil depths for
 703 each treatment. The letters represent the comparisons by L.S.D. test ($p < 0.05$) among
 704 treatments within each depth. Bars represent standard error. Control: non-amended
 705 control plot; F8: soil amended with fresh-SMS (8 Mg of dry matter ha⁻¹); C8: soil

706 amended with composted-SMS (8 Mg of dry matter ha⁻¹); F25: soil amended with
 707 fresh-SMS (25 Mg of dry matter ha⁻¹); C25: soil amended with composted-SMS (25
 708 Mg ha⁻¹ of dry matter)



709
 710 Figure 5. Factor analysis. Graphical representation of the loadings of the soil properties
 711 projected on the plain defined by the two first factor loadings. SOC: soil organic carbon;
 712 Total N, total nitrogen; WSC, water soluble carbon; PMN, potentially mineralizable
 713 nitrogen; MBC, microbial biomass carbon; GLU, β -Glucosidase enzymatic activity;
 714 N_{inorg}, N-NH₄⁺+N-NO₃⁻; WSA, percentage of water stable aggregates.

715
 716 Table 1. Chemical properties of Fresh-Spent mushroom compost (F-SMC) and
 717 Composted-Spent mushroom compost (C-SMC) employed in each year of the
 718 experiment. All values are expressed on a dry weight basis.

	2006		2007		2008		2009	
	F-SMC	C-SMC	F-SMC	C-SMC	F-SMC	C-SMC	F-SMC	C-SMS
General chemical properties								
Dry weight (%)	49.8	51.9	73.3	50.0	66.2	41.2	37.8	49.8
pH (H ₂ O)	6.7	8.1	6.6	7.8	6.7	8.1	8.4	7.7
E.C. 1:5 (mS cm ⁻¹)	6.0	5.4	5.6	7.1	5.8	6.9	8.3	9.2
Organic matter (%)	48.9	39.1	65.4	36.2	63.5	50.2	54.6	48.3
Organic C (%)	28.4	22.7	37.9	21	37.4	28.8	31.7	28.0

Total N (%)	2.0	1.4	2.9	1.6	2.2	1.8	2.8	2.4
C/N ratio	14.2	16.2	13.1	13.1	17.0	16.0	11.4	11.4
				<i>Macroelements g kg⁻¹</i>				
P	8.5	6.0	9.4	6.4	7.3	8.5	7.6	8.6
K	20.5	19.1	24.6	18.0	27.0	22.0	36.7	32.9
Mg	7.4	24.5	9.6	9.7	8.7	12.8	9.2	9.5
Na	2.4	2.1	4.1	1.7	2.9	3.1	2.4	2.0
				<i>Microelements mg kg⁻¹</i>				
Cr	10	13	5	89	11	517	11	45
Pb	3	6	1	10	2	22	4	19
Zn	202	145	258	163	188	216	353	314
Cd	0.15	0.21	0.13	0.21	0.26	0.52	0.20	0.40
Ni	7	10	5	7	4	15	6	8
Cu	81	73	60	46	36	64	61	74
Hg	0.01	0.01	0.05	0.07	0.00	0.03	0.03	0.04
Mn	260	295	359	255	229	395	263	325

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738 Table 2. Results of mixed-effects ANOVA (treatment and depth as fixed factors and
 739 block as random factor) for the soil parameters determined at 0-5, 5-15 and 15-25 cm
 740 soil depth.
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	SOC	Total N	WSC	PMN	MBC	GLU	MBC/SOC	GLU/SOC	WSA	N-NO ₃ ⁻	N-NH ₄ ⁺	WSC/Ninorg
	P values											
treatment	0.001	0.002	0.000	0.000	0.001	0.106	0.002	0.109	0.080	0.000	0.013	0.130
depth	0.001	0.002	0.069	0.004	0.110	0.029	0.800	0.000	0.032	0.001	0.005	0.002
block	0.160	0.121	0.646	0.495	0.185	0.325	0.690	0.761	0.479	0.818	0.742	0.414
treatment x soil depth	0.001	0.002	0.000	0.000	0.023	0.201	0.056	0.318	0.863	0.000	0.097	0.305
treatment x block soil depth x block	0.006	0.003	0.904	0.455	0.717	0.140	0.372	0.675	0.759	0.651	0.097	0.304
treatment x soil depth x block	0.792	0.610	0.354	0.880	0.016	0.239	0.007	0.984	0.824	0.126	0.082	0.569

742 Parameters: SOC, soil organic C; Total N, total nitrogen; WSC, water soluble C; PMN,
 743 potentially mineralizable N; MBC, microbial biomass C; GLU, β-glucosidase activity; GLU/SOC, β-
 744 glucosidase activity to soil organic C ratio; WSA, % water stable aggregates; WSC/Ninorg, water soluble
 745 C to inorganic N ratio
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Table 3. Soil CO₂ emissions (SR), soil temperature at 5 cm depth, % air humidity in soil chamber, during year 2009.

Treatment	Calendar date						
	25-2-2009	23-3-2009	1-6-2009	5-7-2009	16-9-2009	25-10-2009	17-11-2009
	Soil CO ₂ emissions g CO ₂ m ² h ⁻¹						
Control	0.12a†	0.22b	0.21a	0.35a	0.15a	0.29a	0.29a
F8	0.14a	0.21b	0.21a	0.29a	0.13a	0.29a	0.19a
C8	0.15a	0.22b	0.21a	0.24a	0.14a	0.27a	0.26a
F25	0.14a	0.41a	0.29a	0.37a	0.18a	0.36a	0.31a
C25	0.16a	0.25ab	0.29a	0.37a	0.18a	0.36a	0.31a
L.S.D. 95 %	0.16	0.17	0.13	0.19	0.08	0.18	0.20
	Soil temperature at 0-5 cm depth (° C)						
Control	8.0a	20.4a	24.8a	24.3a	15.3a	18.8a	11.2a
F8	7.8a	18.9a	24.2a	24.9a	15.1a	18.3a	11.1a
C8	7.8a	18.4a	24.2a	24.1a	15.4a	17.5a	11.4a
F25	7.9a	20.0a	26.4a	24.1a	15.1a	17.9a	11.1a
C25	7.8a	22.1a	24.3a	25.0a	14.7a	17.1a	11.4a
L.S.D. 95 %	1.2	4.2	3.9	2.0	1.9	2.5	1.3
	Air humidity in soil chamber (%)						
Control	12.6a	14.0a	17.0a	25.8a	17.9a	26.4a	16.2a
F8	13.0a	14.0a	18.3a	22.9a	17.9a	26.3a	16.0a
C8	13.3a	13.2a	19.2a	24.7a	17.3a	25.6a	16.3a
F25	13.5a	14.0a	19.9a	24.4a	17.5a	25.2a	15.8a
C25	13.5a	14.0a	19.9a	24.4a	17.5a	25.2a	15.8a
L.S.D. 95 %	1.7	3.9	3.7	5.5	2.5	2.6	1.6

†Different letters indicate significant differences among treatments by L.S.D. test ($p < 0.05$).
Control: non-amended control plot; F8: soil amended with fresh-SMS (8 Mg of dry matter ha⁻¹); C8: soil amended with composted-SMS (8 Mg of dry matter ha⁻¹); F25: soil amended with fresh-SMS (25 Mg of dry matter ha⁻¹); C25: soil amended with composted-SMS (25 Mg ha⁻¹ of dry matter)

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Table 4. Factor loadings and communalities of a three-factor model of the soil properties selected.

	Factor			Communalities
	F1	F2	F3	
	48.200	19.708	11.170	
SOC	0.894	0.314	0.117	0.911
Total N	0.929	0.180	0.122	0.910
WSC	0.871	0.280	-0.079	0.844
PMN	0.863	0.315	-0.007	0.845
MBC	0.574	-0.196	0.762	0.948
GLU	0.111	0.801	0.414	0.826
MBC/SOC	-0.389	-0.546	0.676	0.906
GLU/SOC	-0.590	0.640	0.165	0.785
N-NO ₃ ⁻	0.829	-0.325	-0.201	0.833
N-NH ₄ ⁺	0.812	-0.383	-0.127	0.822
WSC/N _{inorg}	-0.615	0.477	-0.046	0.609
WSA	-0.228	-0.433	0.106	0.251

792 SOC: soil organic carbon; Total N, Total nitrogen; WSC, water soluble carbon; PMN, potentially
793 mineralizable nitrogen; MBC, microbial biomass carbon; GLU, β -Glucosidase enzymatic activity; N_{inorg},
794 N-NH₄⁺+N-NO₃⁻; WSA, percentage of water stable aggregates.
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