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Changes in biological properties and antioxidant capacity of an agricultural soil amended with sewage sludge

Cardelli Roberto, Arduini Iduna, Marchini Fausto, Masoni Alessandro and Saviozzi Alessandro
Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

Abstract

The effects of applying sewage sludge (SS) to agricultural soil (at low rate of 22.5, LRS, and at high rate of 45 t ha^{-1} dry basis, HRS) were monitored over a 120-d experimental period. Total organic carbon (TOC), water-soluble organic carbon (WSOC), alkali-soluble phenols, basal respiration, specific enzyme activity, dehydrogenase activity (DH-ase), metabolic potential (MP) and FDA-hydrolytic activity (FDA) were strongly increased by both rates of SS applications. In the SS amended soil, about 70% of the organic C added with the material remained at the end of the experiment. Basal respiration increased with increasing SS doses. The specific enzyme activity and the MP indicate an increase in the enzyme activity in soil.

The addition of SS led to higher values than the control of all the tested parameters up to the end of the experimental period. The antioxidant capacity (trolox equivalent antioxidant capacity, TEAC) was influenced by SS addition only when applied at HRS. After 120 days only HRS value of TEAC (5.13 mM g^{-1}) was higher than control (4.09 mM g^{-1}). The pattern of TEAC did not enable any link to be established between antioxidant capacity and both alkali-soluble phenols and basal respiration in soil.

Keywords: Soil basal respiration, dehydrogenase activity, specific enzyme activity, metabolic potential, antioxidant capacity.

Introduction

Understanding the management factors affecting soil quality is crucial for planning farming systems that maintain soil fertility. Intensive agriculture negatively affects soil quality, principally because of the loss in soil organic matter (Carter 2002; Cardelli et al. 2012). Organic matter plays a major role in maintaining soil fertility because of its influence on soil structure, stability, water retention, biodiversity, and plant nutrients. Soil organic matter is also considered as one of the most important factors in protecting soil from erosion and desertification (Senesi et al. 2007). Thus, the recovery of depleted soil organic matter and its maintenance at adequate levels is critical.

Sustainable practices providing organic amendments, such as sewage sludge, could be a useful tool to maintain or increase organic matter content in agricultural soils, improving their fertility (Liu et al. 2013; Saviozzi & Cardelli 2014). These practices in agriculture provide a source of waste disposal which is gaining popularity due to the reduction in available disposal sites.

Sewage sludge generally contains useful concentrations of organic matter, N, P and K and to a lesser extent, Ca, S and Mg (Singh & Agrawal 2008; Mondal et al. 2015). With the increased cost and shortage of N fertilizers, there is increased emphasis on using sewage sludge for its nutrient content and, therefore, the material may be considered as an important resource for sustainable agriculture. Liu et al. (2013) reported that the total organic carbon (TOC) and labile organic carbon fractions increased significantly under fertilisation regimes, especially in treatments with amendments. Sewage sludge also increases soil organic matter levels (Soriano-Disla et al., 2010), and thus helps to sequester carbon and mitigate greenhouse gas emissions (Pitombo et al., 2015).

Several short- and long-term experiments have reported that sewage sludge applications affect soil biological activity (García-Gil et al. 2004; Mondal et al. 2015; Yada et al. 2015). A number of biological and biochemical properties, such as dehydrogenase and phosphatase activities, and basal respiration, may be early and sensitive indicators of organic matter transformations and dynamics, and have been found to provide a reliable tool with which to estimate early changes in soil microbial processes. Also, soil enzymes are good markers of soil biological fertility, because of

58 their rapid response to changes in soil management such as the use of fertilizers (Gianfreda &
59 Bollag 1996).
60 The activities of soil enzymes are closely related to changes in rates of soil respiration and turnover
61 patterns of pools of soil organic matter (Xu et al. 2015; Veres et al. 2015). The respiration rate is
62 another valid biological measurement, which has been used to assess soil management effects on
63 soil quality (Saviozzi et al. 2007). The correct identification of the ‘‘mineralisable’’ soil C pool is
64 essential as it is an important component in modeling soil C dynamics and ecosystem responses to
65 changing environmental factors (Stewart et al. 2008).
66 Soil incubation is a more direct approach to quantifying mineralisable soil C than various
67 procedures using chemical extraction or organic compound class analysis. The mathematical
68 description of the dynamics of C mineralisation in incubation studies may also be of great interest
69 for the prediction of the ability of soils to supply potentially mineralisable organic C and, more
70 generally, for the organic matter balance (Aslam et al. 2008; Ahn et al. 2009). These mathematical
71 descriptions of C release patterns may also provide useful indices and facilitate the testing of
72 hypotheses concerning the mechanisms involved (Alvarez & Alvarez 2000).
73 Rimmer (2006) hypothesized that the protection of organic matter from oxidation is linked to the
74 soil antioxidant capacity, the production of antioxidant molecules. The mechanism explaining this
75 effect may be the antioxidant activity of the phenol compounds in soil organic matter, which slow
76 the rate of oxidation, thus controlling the rate of breakdown in more labile and easily degradable
77 fractions. However, Rimmer and Abbott (2011) demonstrated that the amount of antioxidants
78 reflects soil water-soluble organic carbon and total organic carbon contents, and concluded that the
79 phenolic compounds measured were not important contributors to the antioxidant capacity of the
80 soil extracts.
81 In view of the development of new strategies for soil conservation, achievable through the
82 maintenance and improvement of soil organic carbon (carbon sink), the use of organic fertilizers,
83 different on their antioxidant capacity, able to slow down the mineralisation process, can be

84 strategically important. This work thus investigated and compared the responses of soil biological
85 and biochemical properties during a 120-d experiment, as well as the antioxidant capacity, to the
86 application of sewage sludge at different rates in agricultural soil.
87
88 **Materials and Methods**
89
90 ***Soil***
91 Main soil characteristics were: 54.9% sand (2 - 0.05 mm), 33.5% silt (0.05 - 0.002 mm), 11.6% clay
92 (< 0.002 mm) (sandy-loam soil), 8.1 pH, 5.0 g kg^{-1} total organic carbon (TOC), 0.7 g kg^{-1} total
93 nitrogen, 4.4 mg kg^{-1} available P, 69.3 mg kg^{-1} available K, 10.3 cmol (+) kg^{-1} cation exchange
94 capacity (CEC). Soil type was a Hortic Anthrosol, resulting from deep cultivation, intensive
95 fertilisation and/or continued application of organic residues.
96
97 ***Sewage sludge***
98 The sewage sludge was collected at the wastewater plant, Piombino Ferriere, (Livorno, Italy).
99 Main sewage sludge characteristics are reported in Table 1.
100
101
102 ***Site description and experimental design***
103 The research was carried out at the research centre of the Department of Agriculture, Food and
104 Environment of the University of Pisa, Italy, which is located at a distance of approximately 4 km
105 from the sea (43°40′N, 10°19′E) and 1 m above sea level. The climate of the area is hot-summer
106 Mediterranean (Csa) with mean annual maximum and minimum daily air temperatures of 20.2 and
107 9.5 °C respectively, and a mean rainfall of 971 mm per year.
108 Four fertiliser treatments were tested: a) control, without N or organic fertiliser; b) mineral N as
109 urea, applied at 120 kg N ha^{-1}; c) sewage sludge (SS), at the highest rate that can be applied to
110 agricultural soils (Legislative Decree n. 99, 1992) LRS (equivalent to 22.5 t ha^{-1} dry matter and

111 1786 kg ha^{-1} total N) and d) at a double HRS rate (equivalent to 45 t ha^{-1} dry matter and 3572 kg ha^{-1} total N).

113 The research was carried out in an open-air facility consisting of 72 pots (4 fertiliser treatments x 6 sampling times x 3 replications) of 29-L volume (60 cm long by 25 cm diameter) spaced 20 cm apart and embedded in expanded clay to prevent daily fluctuations in soil temperature. The pots were made from polyvinyl chloride (PVC) tubes fitted with a PVC base, serving as a bottom, and filled with 35 kg of soil.

118 Sewage sludge was mixed with the soil in a concrete mixer before pot filling. For the urea treatment, nitrogen was applied at the rate of 120 kg ha^{-1} and, in order to simulate a fertilisation scheme for crops, the total amount was split into two applications: 20 and 100 kg N ha^{-1}.

121 All pots were watered near to field capacity throughout the experimental period.

122 Six sampling times were selected to monitor soil parameters: at the beginning (T_1), and 21 (T_2), 41 (T_3), 55 (T_4), 80 (T_5) and 120 (T_6) days after the application. The soil samples from the top 5-15 cm and consisting of 12 cores (Dia.: 2.5 cm, L: 10 cm) per sampling time measuring , were air-dried and passed through a 2 mm sieve.

126

127 ***Analytical methods***

128 *Soil analyses*

129 The particle-size distribution of the soils was obtained by the pipette method. The pH was determined according to the SISS (1995); $CaCO_3$ was determined with a Scheibler apparatus; TOC was determined by dry combustion after removing carbonate-C (Nelson & Sommers, 1982) (induction furnace 900 CS, ELTRA); total Kjeldahl nitrogen was determined by the method of Bremner and Mulvaney (1982); available P was measured on the 0.5 N $NaHCO_3$ extract at pH 8.5±0.1 (Olsen et al. 1954); exchangeable K was determined on the 1 N CH_3COONH_4 extract at pH 7.0 (Thomas 1982); cation exchange capacity (CEC) was determined by Ba^{2+} saturation and subsequent complete replacement of Ba^{2+} with Mg^{2+}, according to Bascomb (1964).

137

138 *Sewage sludge (SS) analyses*

139 The pH, TOC, total N, total P, humification degree, phenolic compounds and total heavy metals
140 were analyzed according to IRSA-CNR (1985) methods.

141

142 *Incubation experiment*

143 A short-term (30 day) aerobic incubation was used on the samples collected at the beginning and
144 120 days after SS and urea applications to determine the potential to mineralize organic C. The CO_2
145 evolution was monitored daily in 50 g of soil which were placed in 250-ml glass containers closed
146 with rubber stoppers, moistened with 50% of the maximum water holding capacity, and incubated
147 at 25±1°C. The CO_2 evolved was trapped in NaOH solution and the alkali excess was titrated with
148 HCl. The results, normalized with respect to time, were expressed as mg of C mineralised / 100 g of
149 dry soil.

150

151 *Monitoring experiment*

152 The water soluble organic carbon (WSOC) was determined by stirring samples of soil with distilled
153 water (soil/H_2O 1:20) for 24 h at room temperature, centrifuging the suspension at 10,000 rpm for
154 10 min, and filtrating through a 0.4 mm glass fiber. In this extract, the WSOC was determined with
155 a C analyzer for liquid samples (Hach QbD1200).
156 The alkali-soluble phenols were measured on a 2M NaOH solution extract (soil : solution 1:5). The
157 NaOH extraction was performed under N_2 for 16 hours at room temperature. After centrifuging
158 (6000rpm x 15min), the centrifuged extract was filtered on cellulose acetate (pore size 0.2 μm) and
159 treated with a 10% solution of TCA to remove proteins. The water- and alkali- extracted phenols
160 were determined using a Folin-Ciocalteu reagent, following Kuwatsuka and Shindo (1973).
161 The trolox equivalent antioxidant capacity (TEAC) was determined on the 2M NaOH solution
162 extract used for phenols. Before the TEAC assay, the NaOH extract was neutralized from

163 approximately pH 13 to pH 7±0.2 using 2M HCl. The antioxidant capacity of the extracts was
164 measured using the TEAC method (Re et al. 1999).
165 The fluorescein diacetate (FDA)-hydrolytic activity was determined as the absorbance at 490 nm of
166 the filtrate from the soil suspension incubated with fluorescein diacetate at 24°C for 60 min
167 (Schnurer & Rosswall, 1982).
168 The dehydrogenase activity (DH-ase) was assayed on fresh sieved soil stored at 4 °C, within one
169 week after sampling, following Casida et al. (1964), based on a colorimetric assay of 2,3,5
170 triphenylformazan (TPF) produced by the microorganism reduction of 2,3,5 triphenyltetrazolium
171 chloride (TTC). Results were expressed as mg of TPF g^{-1} soil h^{-1}.
172 The specific enzyme activity was calculated by dividing the dehydrogenase activity by total organic
173 C (Trasar-Cepeda et al. 2008).
174 The metabolic potential (MP) (Masciandaro et al. 1998) was calculated as follows: MP =
175 dehydrogenase activity / 10^{-3} WSOC.
176
177 ***Statistical methods***
178 Data were expressed on the basis of the oven-dry weight of the soil.
179 Results were subjected to two-way analysis of variance. The effect of fertilisation treatment,
180 sampling time, and their interaction were analysed using a split-plot design with fertilisation
181 treatment designed as whole plots and sampling time as sub-plots. Significantly different means
182 were separated at the 0.05 probability level by the Tuckey's test (Steel et al., 1997). Statistica 7.0
183 software (StatSoft Inc., Tulsa, Oklahoma, USA) was used for the statistical analysis. Results (*P*
184 *values*) of statistical analysis in ANOVA test for the main effect of fertiliser, sampling time, and
185 fertiliser x sampling time interaction are shown in Table 2.
186 A non-linear least squares regression analysis (Graph Pad Prism 2007) was used to calculate the
187 parameters from the cumulative data of C-mineralisation. The C mineralisation kinetics were
188 determined following a first-order model [$C_m = C_0 (1-e^{-kt})$], where C_m is the cumulative value of

189 mineralised C during t (days); C_0 is the potentially mineralisable carbon, and k is the rate constant
190 of C mineralisation. The coefficient of determination (R^2) was used for the evaluation of the model
191 fit.
192
193 **Results and Discussion**
194
195 ***Sewage sludge characteristics***
196 Table 1 shows the properties of the sewage sludge. SS can be applied to agricultural land if the
197 sludge and soil meet the criteria established by Italian legislation (Legislative Decree n. 99, 1992),
198 adapted to conform to the E.C. directive 86/278 concerning sludge application. According to the
199 maximum permitted amount of heavy metals for the final disposal of sewage sludge, the SS tested
200 in this experiment can be considered as a safe material for soil treatment.
201
202 ***Influence of sewage sludge on pH, TOC and WSOC***
203 Since the SS added to soil had a pH of 6.4, the value of soil pH (8.1) would be expected to decrease
204 because of sludge amendment. Indeed, Alva et al. (2011) reported that at a 44.8 Mg ha^{-1} SS
205 treatment, soil pH was 5.3 compared with 6.4 in the unamended treatment. However, during the
206 experiment, the pH was not affected by the addition of SS, probably because of the buffer power of
207 this soil (data not shown). As expected, the pH of the soil increased from 8.1 to 8.3 after the
208 addition of urea (T_1), after which the pH of the samples rapidly declined. The drop in pH observed
209 could result from the increase in nitrate formation.
210 Figure 1 presents the changes of the total and water-soluble organic carbon in the soil during the
211 experiment. The addition of SS to the soil increased the TOC content ($p < 0.05$) in LRS- and HRS-
212 treated soils at T_1, which was almost proportional to the doses applied. There are reports in the
213 literature that SS added to soil increased the organic matter content (Liu et al. 2013, Saviozzi &
214 Cardelli 2014). No significant differences between the control and urea-fertilised soil were observed

215 in this work. Figure 1 shows that, in spite of a generally constant decrease in TOC, about 70% of
216 the organic C added with the SS remained at T_6, and its content was still significantly greater than
217 the control. These results, found in a short time experiment, suggest that SS can contribute to
218 increasing the organic matter in the soil and thus lessen its decline in intensively cultivated areas.
219 The substantial reduction in TOC in the soil during the experimental period could be due to both the
220 temperature and moisture levels which were probably in the optimum range for microbial activities,
221 thus producing an accelerated rate of substrate mineralisation. Although our soil was coarsely
222 sieved, some organic materials may also have been susceptible to rapid mineralisation. In the SS
223 amended soil, the decline in TOC was greater in HRS than in LRS, perhaps due to a possible
224 priming effect because of the induction by SS of the proliferation of microorganisms active in the
225 decomposing native organic matter (Singh et al. 2011). This was particularly the case in the last
226 stage of the experiment, to such an extent that, at T_6, the parameter values were statistically similar
227 in the two amendment rates.
228 Most of the water-soluble organic carbon (WSOC) in soil solution is composed of organic acids and
229 soluble carbohydrates (Cook & Allan, 1992), and aliphatic acids and other simple molecules may
230 also be present (Fletcher & Beckett, 1987). The immediate effect of the application of an organic
231 material to soil is an increase in WSOC (Figure 1), which may pass into the soil solution, thus
232 enhancing the native soluble organic fraction of the soil (Liu et al. 2013). Consequently, in our
233 study a significant increase in WSOC was observed in the samples treated with SS (Figure 1). The
234 highest value was obtained when the sludge was applied at the highest rate. In the later stages, a
235 progressive decrease was found, due to the use of WSOC as an energy source by the microbial
236 community (Iqbal et al. 2010) (Figure 1). At T_6 and irrespective of the application rate, WSOC in
237 the SS amended soil was still significantly higher than the control.
238
239 ***Effects of sewage sludge on C mineralisation***

240 The usefulness of an organic material as fertilizer is affected by the rate at which it is decomposed
241 by microorganisms when added to soil. Figure 2 shows the trends in cumulative CO_2 evolution
242 from the control and the differently treated-soils, in samples collected at the beginning (T_1) and
243 after 120 days (T_6), during the 30-d incubation.
244 Our experiment shows that the incorporation of the sludge increased C_m with respect to the control
245 and urea-treated soils, suggesting that SS can be actively metabolized in soil (Table 3). The C_m
246 values, expressed on the basis of soil TOC, were higher in SS than in the control, suggesting that
247 there is a large fraction of readily decomposable organic matter in sludge. Other studies (Jiménez et
248 al. 2007; Pascual et al. 2007; Yada et al. 2015) have reported that the SS amendment may stimulate
249 C mineralisation and microbial activity as a result of the amount of C added to the soil, particularly
250 the readily-available C in the easily decomposing compounds. Applying large amounts of organic
251 material often inhibits soil microorganisms, which could be masked by an increase in CO_2
252 evolution. In our study, C_m increased with the incremental addition of SS. The C_m over the
253 incubation period amounted to 13.6% and 14.8% of the organic carbon in SS in LRS and HRS,
254 respectively (C_m%TOC, Table 3). This indicates the increasing efficiency of respiration with
255 increasing application rates and/or a lack of inhibitory effects of soluble compounds from SS which
256 could have lowered the microbial activity and mineralisation in the soil.
257 The direct relationship between the amounts of CO_2 evolved during the incubation period (C_m)
258 (Table 3) and the WSOC values (Figure 1) highlights that soluble carbon controls the oxidation rate,
259 and then the decomposition, of organic C in the soil. As reported by Marschner and Kalbitz (2003),
260 substrates must pass through the dissolved phase to reach and pass through microbial membranes.
261 Thereafter, the lower decomposition rates reflect the greater resistance of the recalcitrant materials.
262 All samples taken at T_1 showed a greater amount of C_m than the samples collected at T_6 (Table 3).
263 This is probably because the organic matter in soil and sewage sludge becomes proportionally
264 richer in compounds that are more resistant to degradation, thus decreasing microbial activity and
265 respiration.

266 Although N addition generally decreases soil microbial respiration (Treseder 2008; Ramirez et al.
267 2010), the form of N seems to affect the process. For example, the co-addition of reduced C when
268 adding urea may counterbalance the inhibition (Kelliher et al. 2005). Ramirez et al. (2010) reported
269 that urea addition decreased respiration in aspen and pine soils but increased respiration rates in
270 grassland soil. Bhattacharya et al. (2013) found that CO_2 evolution reached the highest peak within
271 15 days of the experiment and then gradually started to decrease. In our study, in samples collected
272 at T_1, the CO_2 evolving over the 30-d incubation period from the urea-treated soil was slightly
273 higher than the control (Table 3). The total losses of soil organic carbon as CO_2 at T_6 were 7.2% in
274 soil treated with urea, against 5.8% in the control soil. These results may be due to the C in urea
275 being labile and quickly used and/or of the lack of the inhibition of urea nitrogen when added at a
276 low rate (20 kg N ha^{-1}). This is confirmed by the respiration results observed in samples collected at
277 T_6: where urea was present in a more consistent way (100 kg N ha^{-1}), the N inhibition of microbial
278 respiration was noticeable (Table 3).
279
280 ***Modeling of soil C mineralisation***
281 The parameters calculated according to the first-order model are reported in Table 3. R^2 values of
282 the first-order model were highly significant for all samples, with values that were always greater
283 than 0.97. The data in Table 3 show that the rate constants of C mineralisation (k) fell within a wide
284 range, suggesting that microbial respiration metabolized organic compounds that were quite
285 different or of a different degree of availability. K values were lower in the control and urea-treated
286 soils (0.083 and 0.081 day^{-1}, respectively) than in the SS amended soil (about 0.1 day^{-1}), indicating
287 that sludge applied with the two doses strongly influenced the CO_2-C evolution rates. However, in
288 samples collected at T_6, K values were similar for all doses (0.074-0.079 day^{-1}). As expected, the
289 amount of C_0 increased with increasing levels of sludge. The C_0 values ranged from a minimum of
290 29.3 for control soil to a maximum of 144.3 mg $100g^{-1}$ for HRS (Table 3). Not surprisingly, there
291 was a close association between C_0 and the amount of C_m.

292

293 ***Effects of sewage sludge on soil antioxidant capacity***

294 The presence of antioxidants such as phenolic compounds in an organic material is believed to slow
295 soil respiration, thus controlling the breakdown of soil organic matter. To understand the possible
296 influence of SS on soil respiration, phenol compounds and TEAC were checked at T_1 and T_6. Table
297 4 shows that the application of SS led to a marked increase in alkali-soluble phenols, with values
298 which were almost proportional to the amounts applied. For both doses, the values of the alkali-
299 soluble phenols remained higher than the control. Saviozzi and Cardelli (2014) reported significant
300 direct relationships between TEAC and alkali-soluble phenols in soil amended with cow manure,
301 peat, municipal solid waste compost, wet olive husk compost and green waste compost. In this
302 study, the SS addition did not exert a marked influence on soil TEAC. On samples collected at T_1, a
303 significant increase of TEAC was observed only for HRS, while no noticeable difference was found
304 for LRS. Lower TEACs than at the start were observed after 120 days in each trial and, once again,
305 only the HRS value remained higher than the control. To better evaluate the differences in TEAC
306 between treatments, the dominant influence of soil C content was removed by normalizing the data
307 (Table 4). TEAC values of SS, expressed on a TOC basis, were lower than the control. This
308 confirms the patterns of soil respiration in C_m, C_m/TOC and K, which were greater in SS than in the
309 control.

310 These results do not highlight an important role of the phenol compounds of SS in determining the
311 pattern of antioxidant capacity of soil. Rimmer and Abbott (2011) and Saviozzi and Cardelli (2014)
312 showed that the trend of soil antioxidant capacity (TEAC) was related with the TOC and WSOC
313 contents of soils. However, in the present study, TEAC values were not related to either TOC or
314 WSOC (Figure 1). Thus it is likely that other sludge compounds are involved in the antioxidant
315 system.

316 Rimmer (2006) hypothesized that TEAC protects the soil organic matter from degradation by
317 slowing the rate of oxidation. In our study, no inverse relationships were found between TEAC

318 (Table 4) and any of the C mineralisation parameters, Cm, C_0, and k (Table 3). This suggests that
319 the mineralisation process in soil amended with SS is due to the amount of labile C pools as the
320 controller of the oxidation rate, and thus the decomposition of organic C rather than the antioxidant
321 capacity.
322
323 ***Influence of sewage sludge on enzyme activities***
324 The rate of hydrolysis of fluorescein diacetate (FDA) by soil samples has been considered as an
325 index of the overall microbial activity (Schnurer & Rosswall 1982), because such hydrolysis is
326 carried out by active cells and is due to a variety of enzymes. Figure 3 shows that no noticeable
327 influence of the urea treatment on FDA was observed over the experimental period. Conversely, at
328 T_1 the SS treatment showed twice the level of values compared to the control, without any
329 differences between the application rates. Due to a constant increase in the FDA, the maximum
330 value was found in HRS at T_3, after which values tended to decrease. However, the LRS and HRS
331 showed higher values of FDA up to the T_6, indicating that SS may improve and maintain the
332 biological activity in soil over time.
333 Figure 3 shows the soil dehydrogenase activity. There were no significant differences between the
334 control and urea-treated soil throughout the experimental period. DH-ase of the soil without SS
335 amendment was lower than in the SS-treated soil which was observed throughout the experimental
336 period. Similar results were reported by Pascual et al. (2007) in soil treated with high amounts of SS
337 (140 t ha^{-1}) over 64 days. The initial high dehydrogenase activity recorded (Figure 3) in both LRS
338 and HRS may be ascribed to the organic short acting C substrates added to SS plots, i.e. the high
339 content of WSOC, which can increase the synthesis of these enzymes and/or the possible presence
340 in the SS of enzymes that promote enzyme activity in amended soil (García-Gil et al. 2004). At T_1,
341 the soil amended with the high dose of sludge (HRS) showed the highest dehydrogenase activity,
342 followed by the soil with the low dose of sludge (LRS).

343 HRS patterns enabled us to distinguish between two phases as the experiment progressed (Figure
344 3). The first phase, up to T_3, was characterized by a marked increase in enzyme activity, probably
345 due to a stimulation of the metabolic activity because of the degradation of easily available organic
346 substrates by microorganisms. Similarly to FDA, DH-ase of HRS increased up to the maximum
347 observed in HRS at T_3, after which the enzyme activity decreased sharply and remained practically
348 stable during the last stages of the experimental period. The stabilization of the dehydrogenase
349 activity suggests that most of the easily available organic matter had been decomposed. Although
350 the values of dehydrogenase activity in relation to TOC were considered (specific enzyme activity,
351 i.e. the values of activity per unit of carbon), no significant differences between the control and
352 urea-treated soil were found (Figure 3). As for the absolute values of DH-ase, the specific enzyme
353 activities were always higher in the SS-treated soil than in the urea- and control soils. Parallel with
354 the results reported for the absolute values of dehydrogenase, the specific enzyme activity was
355 almost twice as high in HRS than in LRS during the experimental period. These results indicate that
356 SS increased the level of organic C in soil, but the increase in enzyme activity was higher. The
357 logical result of these variations is a higher quantity of DH-ase per unit C, i.e. soil amendment with
358 sewage sludge causes a relative increase in enzyme activity. Similarly to findings for DH-ase, the
359 MP showed two distinct phases for LRS and HRS, characterized by an initial increase in the activity
360 followed, after about a month, by a decrease and stabilization of the index values. This result was
361 probably due to a reduction in hydrolytic activities and microbial activity induced by the
362 disappearance of easily decomposable organic compounds.
363 The dynamics of this process can also be described by an index that links the activity of the viable
364 microbial community (dehydrogenase activity) and the energy sources for microorganisms
365 (WSOC). The Metabolic Potential index (Masciandaro et al. 1998) confirmed the general pattern of
366 DH-ase (Figure 3). However, the increase in the index in the SS-treated soil with respect to the
367 control was even more marked than the specific enzyme activity. This thus indicates that the water-
368 soluble organic carbon of sewage sludge is particularly effective in stimulating enzyme activity.

Conclusions

The addition of SS led to higher values than the control of WSOC, basal respiration, dehydrogenase activity, specific enzyme activity, metabolic potential and FDA up to the end of the experimental period. This indicates that SS may improve and maintain the biological activity in soil over time.

The specific enzymatic activity and the MP showed a parallel trend of DH-ase but highlighted further differences between treatments.

Urea nitrogen added at a low rate (20 kg N ha^{-1}) slightly increased soil respiration, while at 100 kg N ha^{-1} an N inhibition of microbial respiration was noticeable.

The SS addition increased the content of alkali-soluble phenols, but it raised the level of TEAC only at the HRS.

Therefore, there is a need to conduct research on the identification of compounds in SS, other than phenols, that may be responsible of the antioxidant capacity of the material. The TEAC pattern did not establish any link between antioxidant capacity and basal respiration in soil.

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521 Table 1. Selected characteristics of sewage sludge.

pH	6.4	
Total Organic C	38.5	%
Total N	7.94	%
Total P	1.20	%
Humification degree	1.87	%
Total phenolic compounds	0.6	g kg^{-1}
Cr^{VI}	<1	mg kg^{-1}
As	<5.0	mg kg^{-1}
Cd	<2.0	mg kg^{-1}
Cr^{III}	16	mg kg^{-1}
Hg	<0.1	mg kg^{-1}
Ni	25	mg kg^{-1}
Pb	12.5	mg kg^{-1}
Cu	72.4	mg kg^{-1}
Zn	185.1	mg kg^{-1}

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Table 2. Results (*P values*) of statistical analysis in ANOVA test for the main effect of fertiliser treatment, sampling time, and fertiliser treatment x sampling time interaction.

Soil properties	P values (10^{-3})		
	Fertliser treatment	Sampling time	TxS interaction
pH	130	140	110
TOC	0.114	<0.01	0.118
WSOC	0.014	<0.01	<0.01
Alkali-soluble phenols	0.012	<0.01	31.664
TEAC	<0.01	<0.01	31.892
FDA	<0.01	<0.01	<0.01
DH-ase	<0.01	<0.01	<0.01
Speecific enzyme activity	<0.01	<0.01	<0.01
MP	0.037	<0.01	<0.01
Cm	<0.01	<0.01	<0.01

Table 3. Cumulative amount of CO_2-C and parameter estimates according to the first-order model for the C mineralisation in control, urea-treated soil (Urea) and sewage sludge-treated soil (low rate, LRS and high rate, HRS) at T_1 and T_6.

Fertiliser treatment	Sampling time	C_m mg kg^{-1}	C_m % TOC	C_0 mg kg^{-1}	k	R^2
Soil control	T_1	29.2 ef	5.8 de	29.3	0.083	0.98
	T_6	26.5 fg	5.7 de	27.8	0.079	0.99
Urea	T_1	36.0 cd	7.2 c	39.4	0.081	0.99
	T_6	21.3 g	4.4 e	22.5	0.074	0.98
LRS	T_1	107.3 b	13.6 b	109.7	0.099	0.98
	T_6	32.6 de	5.0 ef	34.3	0.078	0.98
HRS	T_1	139.1 a	14.8 a	144.2	0.105	0.98
	T_6	41.8 c	6.1 d	44.5	0.076	0.99

C_m = cumulative amount of mineralised carbon during 30 days of incubation; C_0 = potentially mineralisable carbon; k= rate constant; R^2 = quality of fit.

Means followed by the same letter in a column were not significantly different ($P<0.05$) according to Tuckey's test.

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578 Table 4. Alkali-soluble phenols and soil antioxidant capacity (TEAC) as affected by
579 "fertiliser treatment x sampling time" interaction.

Sampling time	Fertiliser treatment			
	Soil control	Urea	LRS	HRS
	Alkali-soluble phenols (μg p-coumaric acid g^{-1})			
T_1	154 d	153 d	199 b	235 a
T_6	98 f	103 f	127 e	167 c
	TEAC (mM g^{-1})			
T_1	4.09 b	3.80 b	3.88 b	5.13 a
T_6	2.28 d	2.49 d	2.52 d	3.20 c
	TEAC (mM g^{-1} TOC^{-1})			
T_1	8.2 a	7.6 a	4.9 c	5.5 b
T_6	5.1 bc	5.2 bc	3.9 d	4.7 c

580 Means followed by the same letter were not significantly different ($P<0.05$) according to
581 Tuckey's test.

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588 Figure captions:

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591 Figure 1. Total organic carbon (TOC) and water-soluble carbon (WSOC) concentration as affected
592 by "fertiliser treatments x sampling times" interaction.
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595 Figure 2. Cumulative CO_2-C in studied treatments after 30-d incubation of samples collected at the
596 beginning (T_1) of the experiment and 120 days (T_6) after the addition of the sewage sludge.

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598 Figure 3. FDA-hydrolytic activity, dehydrogenase activity (DH-ase), absolute values and specific
599 activity and metabolic potential (MP) as affected by "fertiliser treatments x sampling times"
600 interaction.

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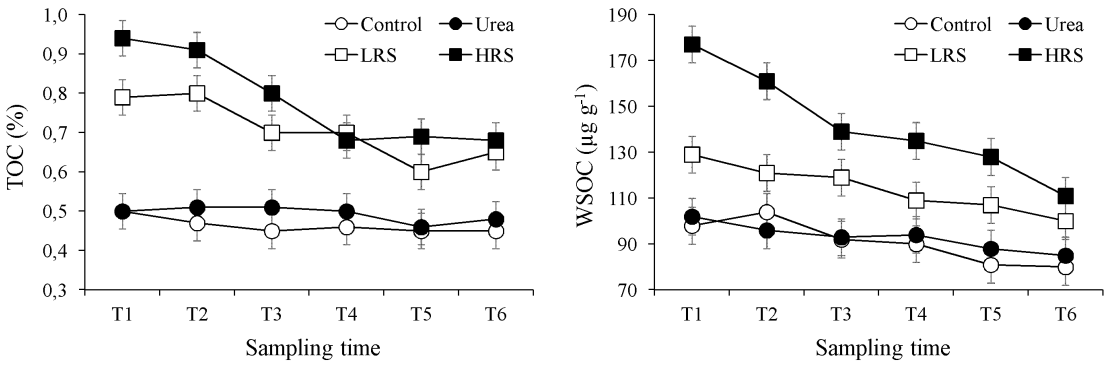
608 Figure 1



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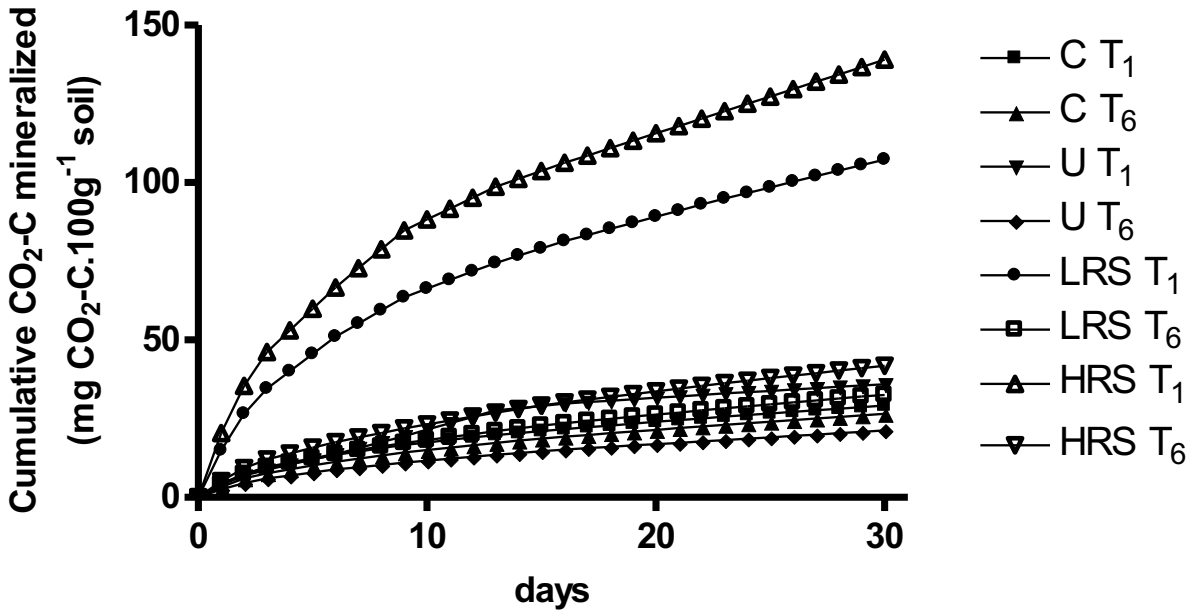
614 Figure 2



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628 Figure 3

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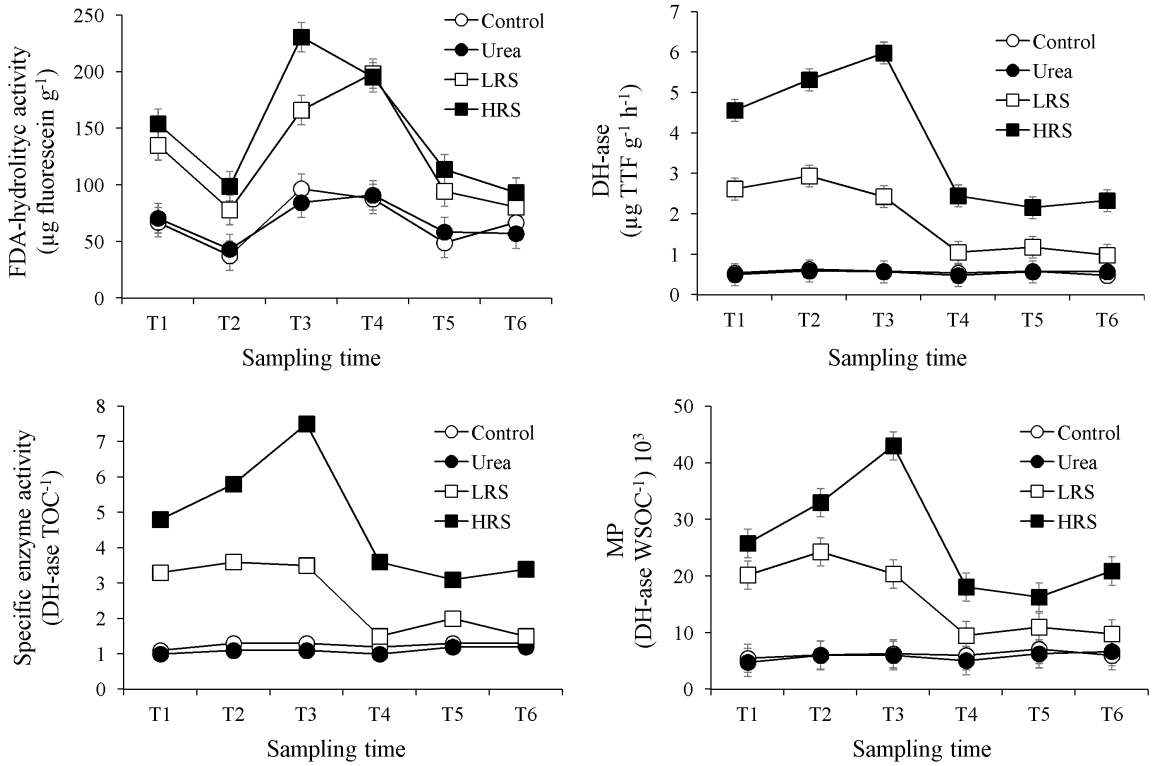

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