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1 **Early warning indicators of changes in soil ecosystem functioning**

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24 **Abstract**

25 In the last decades soil are facing numerous environmental threats and climatic changes that are
26 causing a rapid decline of soil fertility and biodiversity. Soil organic matter (SOM), has the most
27 widely recognized influence on soil quality, but it hardly puts in evidence processes associated to the
28 new soil threats, because of its insensitivity in assessing soil quality changes in the short-term. A series
29 of chemical and biochemical analyses were carried out in agricultural and forestry soil ecosystems
30 subjected to different threats, to identify the parameters that better evidence changes in soil
31 characteristics in a short term, but the identification of basic universal indicators and the choice of the
32 number of estimated measures are still under investigation and discussion. The main aim of this paper
33 was to identify biochemical markers to be used routinely and applicable to different soil ecosystems, as
34 early warning indicators of alteration in soil ecosystem functioning. The results obtained allowed to
35 identify three indicators, microbial biomass (MBC), water soluble phenols (WSP), and fluorescein
36 diacetate hydrolase (FDA), as effective tools in the evaluation of soil quality changes in the short term,
37 showing also a threat-indicator specificity. MBC reflected changes mainly induced by abiotic stress,
38 FDA displayed modification caused by climate, and WSP pointed out alteration due to the organic
39 amendment.

40

41 **Keywords** Agriculture ecosystem. Biological indicators. Forest ecosystem. Soil organic matter. Soil
42 quality.

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49 **1. Introduction**

50 Soils are the most significant non-renewable geo-resource we have and that are facing numerous
51 environmental threats while trying to resist to climatic changes. Interest in evaluating the quality and
52 health of our soils has been stimulated by increasing awareness that soil is a critical important
53 component of the earth's biosphere, functioning not only in the production of food and fiber but also in
54 the maintenance of global sustainability and environmental balance (Glanz, 1995). Soil is also the basis
55 of agricultural and of natural plant communities. Thus, the thin layer of soil covering the surface of the
56 earth represents the difference between survival and extinction for most land-based life (Doran et al.,
57 1996). Whilst the majority of countries have criteria to evaluate the quality of the air and water, the
58 same does not occur for the quality of the soil. Traditionally, soil quality is associated with
59 productivity (Karlen et al., 1997), but recently it has been defined in terms of sustainability (Toth et al.,
60 2007), that is, the capacity of the soil to absorb, store and recycle water, minerals and energy in such a
61 way that the production of the crops can be maximized and environmental degradation minimized.
62 Nevertheless, a significant decline in soil quality has occurred throughout the entire world as a result of
63 adverse changes in its physical, chemical and biological properties, caused by human activity and
64 climate changes (Van Camp et al., 2004; EC 2006). According to Steer (1998), in the last decades of
65 the last century, about 2 billion of the 8.7 billion agricultural lands, permanent pastures, forests and
66 wild native lands have been degraded. Soil degradation processes constitute a serious problem on a
67 worldwide basis, with significant environmental, social and economic consequences. Many economic
68 activities such as agriculture, industry and tourism depend both directly and indirectly on soil quality,
69 which has been proposed as a prime indicator for characterizing and defining management factors
70 contributing to soil degradation. Many constraints cause short-term disturbances that are detrimental to
71 soil quality (IPCC 2007; EEA-JRC-WHO, 2008) as they increase the emissions of greenhouse gases
72 (i.e., CO₂, NO, or N₂O), cause nitrate accumulation and leaching, and/or modify soil microbial
73 community structure in a way that decreases the retention of organic C and N (Liu et al., 2006).
74 Generally, soil quality has been related to the SOM (Gao et al., 2013), microbial activity, total
75 nitrogen, and C/N ratio (Molope and Page, 1986; Eash et al., 1994; Roberson et al., 1995; Murphy et
76 al., 2011), but these soil parameters not necessarily change as a result of changing external conditions
77 or use (Muscolo et al., 2014 in press), and hardly address short term changes in soil processes
78 associated to the new environmental threats. To rise the challenge of soil resource degradation, there is
79 an urgent need to develop common, simple and transparent method to identify changes in soil

80 characteristics in response to the main environmental constraints. Soil-quality assessment, based on
81 inherent soil factors and focused on dynamic aspects of soil system (Paz-Ferreiro and Fu, 2014 in
82 press; Muscolo et al., 2014 in press) is an effective method for evaluating the environmental
83 sustainability (Hamblin, 1991) of land use and management activities. In these scenarios, the overall
84 goal of this paper was to compare data on soils subjected to different types of use and environmental
85 constraints, in order to find out biochemical markers to be used routinely and applicable to different
86 soil ecosystems, as early warning indicators of changes in soil ecosystem functioning. A series of
87 chemical and biochemical analyses were carried out in forest managed soils, amended agriculture soils,
88 soil irrigated with brackish water and forest soil influenced by seasonal variation to identify the
89 parameters that better reflect changes in soil quality, in the short term. The assessment was
90 comparative because of the lack of specific criteria or guidelines available in the literature for
91 interpretation of most soil property indices measured. The starting hypothesis was that natural soils
92 have developed, over time, an equilibrium with the environment reaching the maximum quality and
93 the greatest degree of balance in their properties (Fedoroff, 1987), but soil use and the new
94 environmental constraints alter this balance by affecting soil biochemical properties even in a short
95 time. (Fedoroff, 1987).

96 **2. Material and Method**

97 *2.1 Experiments and soil sampling*

98 Four separate experiments were carried out to identify early warning indicators that better reflect
99 changes in soil chemistry and biochemistry parameters related to soil quality. The experiments were
100 conducted both in forest and agriculture soils underwent to different management practices and
101 climate. The first experiment (named **Case study 1**) was conducted in field, in order to evaluate the
102 effects of artificial brackish water at different concentrations (0; 0.5%; 1%; 1.5%) on chemical and
103 biochemical properties of a haplic Kastanozem (IUSS, 2006) located in the Agricultural Farm of
104 ‘‘Mediterranea University’’, Reggio Calabria, Southern Italy. Soil during the dry season (June, July
105 and August), have been irrigated, three time a week, with synthetic brackish water (EC 4 dS m⁻¹)
106 prepared using NaHCO₃, NaCl, Na₂SO₄, and MgSO₄ with Cl:SO₄ ratio of 1:1 and Ca:Mg ratios of 4:1
107 to maintain the 70% of field capacity. Three months after the irrigations with brackish water, soil
108 samples were collected and analyzed for the chemical and biochemical parameters. Six composite soil

109 samples (0-20 cm) for each treatment were taken from the Agricultural farm of Mediterranea
110 University of Reggio Calabria Italy. The samples were brought to the laboratory on the same day of the
111 collection, and kept in the refrigerator at 4 °C for up to 24 h until processing. Prior to the soil analysis,
112 except for FDA hydrolysis and MBC, all the soil samples were air-dried, sieved (<2 mm), and visible
113 roots were removed.

114 The second experiment (named **Case study 2**) was performed in climatic chamber for 40 days, in
115 plastic pots (10 cm diameter×7 cm height). The soil (Haplic Kastanozem) used was taken from the
116 Agricultural farm of Mediterranea University of Reggio Calabria Italy, in spring. Each pot was filled
117 with 350 g of soil, in order to evaluate the effects of amendment with digestate at different
118 concentrations (0, 25, 50, 75 %) on soil chemical and biochemical properties. The digestate was
119 obtained by a bio-gas energy plant with 998 kW_{el} of installed power, supplied with animal manure
120 (poultry, cow and sheep), milk serum, maize silage and in minor amount with olive waste and citrus
121 pulp. During the experiment, the soil humidity was maintained at 70% of the field capacity in all
122 treatments. The soils differently treated (6 replicates), were air-dried and sieved (<2mm) prior to the
123 chemical analysis. Soil samples for the biochemical determination (microbial biomass and enzyme
124 activities) were stored in the refrigerator at 4 °C for up to 24h until processing.

125 The third experiment (named **Case study 3**) was carried out in field, in the Calabrian Apennine Forest,
126 Southern Italy, to investigate if artificial gaps and in particular the size of the gaps affected the soil
127 chemical and biochemical parameters related to natural forest regeneration. The research area was in
128 the Regional Park of Serre (Calabrian Apennines, Southern Italy at an elevation of 900–940 m. Soils,
129 were classified as Haplic Phaeozem (IUSS, 2006). The natural forest is dominated by silver fir (*Abies*
130 *alba* Mill) and beech (*Fagus sylvatica* L). In this forest, three small (185 m²) and three medium (410
131 m²) gaps were created by felling trees and removing boles. The treatments were named as follow: A=
132 medium gaps; B= canopy cover sites; C= small gaps. Gap sites were paired with an adjacent site under
133 canopy cover. Soil were sampled 3 months after gap opening and were analyzed for chemical and
134 biochemical properties. Soil samples were collected from 0 to 30 cm depth in each gap and in its
135 adjacent forest canopy cover site. Each soil sample consisted of a mixture of six sub-samples taken at
136 random. Prior to the soil analysis, except for soil moisture content, microbial biomass and FDA, all soil
137 samples were air-dried and sieved (<2 mm).

138 The fourth experiment (named **Case study 4**) was carried out in field. The study area was located in
139 the Peripoli Mountain (San Lorenzo) of Aspromonte Mountains (Calabria, Southern Italy), 1270 m
140 above sea level. The climate is predominantly Mediterranean, with dry hot summers and cold winters.

141 The average seasonal precipitation are typically highest during the winter (1100) and autumn (1500)
142 compared to spring (900) and summer (600). The soil were Haplic Phaeozem (IUSS, 2006) with a
143 xeric soil regime moisture and a vegetal cover of *Pinus laricio* Poiret ssp. Calabrica. The effects of
144 seasons (autumn, winter, spring and summer) were evaluated on soil chemical and biochemical
145 parameters as described below. Soil profiles were carefully excavated, different (layers) horizons were
146 thoroughly separated from the top to the bottom of the profile on the basis of morphological
147 differences that could be perceived by the naked eye. Every 15 days, soil samples (1 kg) were taken
148 from each horizon over a year (24 times in a year). The samples were brought to the laboratory on the
149 same day of the collection, and kept in the refrigerator at 4 °C for up to 24 h until processing. Prior to
150 the soil analysis, except for FDA hydrolysis and MBC, all the soil samples were air-dried, sieved
151 (<2 mm), and visible roots were removed. Data presented are the means of three replicate
152 determinations.

153

154 2.2 Soil Chemical Analysis

155 Organic C was estimated by the Walkley–Black procedure (Nelson and Sommers, 1982) and was
156 converted to organic matter by multiplying the percentage of C by 1.72; total N was measured by the
157 Kjeldahl method (Bremner and Mulvaney, 1982). Humic substances were extracted with 0.1 N NaOH
158 (solid:liquid ratio 1:10); the suspension was shaken for 16 h at room temperature and centrifuged at
159 5,000 rpm for 30 min; the extract was dialysed by Wisking tubes against distilled water to pH 6.0.
160 Subsequently, the solution was filtered through a column of Amberlite IR 120 H⁺. The fractionation of
161 humic substances was carried out as follows: aliquots of extracts were acidified to pH 2.0 with dilute
162 H₂SO₄; the humic acids precipitated and were removed by centrifugation, while the fulvic acids
163 corresponded to the supernatants (Bettany et al. 1980). The C content of humic and fulvic acids was
164 determined by dichromate oxidation (Nelson and Sommers 1982). Phenols were extracted with
165 distilled water as this is the most realistic extractant in allelopathic studies (Kaminsky and Muller
166 1977, 1978). Thirty grams of dry weight samples were mixed in 200 ml distilled water and shaken at
167 75 rev min⁻¹ for 20 h at room temperature. Solutions were filtered through Whatman's No 1 paper. All
168 samples were extracted in triplicate. Total water-soluble phenols (monomeric and polyphenols) were
169 determined by using the Folin–Ciocalteu reagent, following the method of Box (1983). Tannic acid
170 was used as a standard and the concentration of water-soluble phenolic compounds was expressed as
171 tannic acid equivalents ($\mu\text{g TAE g}^{-1} \text{ D.W.}$).

172 *2.3 Soil Biochemical Analysis*

173 The amount of microbial biomass C (MBC) was determined by using the chloroform fumigation–
174 extraction procedure (Vance et al. 1987) with field moist samples (equivalent to 20 g D.W.). The
175 filtered soil extracts of both fumigated and unfumigated samples were analyzed for soluble organic C
176 using the methods of Walkley and Black (1934). MBC was estimated on the basis of the differences
177 between the organic C extracted from the fumigated soil and that from the unfumigated soil, and an
178 extraction efficiency coefficient of 0.38 was used to convert soluble C into biomass C (Vance et al.
179 1987).

180 Enzymatic assay: Dehydrogenase (DH) activity was determined by the method of von Mersi and
181 Schinner (1991). Briefly, to a sample of fresh soil equivalent to 1 g of oven dried (105° C) soil were
182 added 1.5 ml of 1 M Tris–HCl buffer of pH 7.5 followed by 2 ml of 0.5% INT solution (Sigma product
183 No I 8377), and the suspension was kept at 40 C for 1 h. Then 10 ml of extractant (methanol) was
184 added and the samples were mixed using a vortex mixer, and then left in the dark for 10 min. Finally,
185 the solids were filtered out (Whatman’s no 40 paper), and the absorbance of the filtrate was determined
186 at 490 nm.

187 Alkaline and acid phosphatase (AlPh, AcPh) activities were determined on 1 g (fresh weight) aliquots
188 of soil, according to the method of Tabatabai (1982). Enzyme activities are expressed as $\mu\text{g } p\text{-nitrophenol}$
189 produced by 1 g of dry soil in one hour ($\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ h}^{-1}$).

190 FDA hydrolysis reaction was determined according to the methods of Adam and Duncan (2001).
191 Briefly, to 2 g of soil (fresh weight, sieved <2 mm) 15 ml of 60 mM potassium phosphate pH 7.6 and
192 0.2 ml 1000 mg FDA ml^{-1} were added. The flask was then placed in an orbital incubator at 30 °C for
193 20 min. Once removed from the incubator, 15 ml of chloroform/methanol (2:1 v/v) was added to
194 terminate the reaction. The content of the flask was centrifuged at 2000 rpm for 3 min. The supernatant
195 was filtered and the filtrates measured at 490 nm on a spectrophotometer (Shimadzu UV–Vis 2100,
196 Japan). Hydrolysing coefficient (Hc): mmol of fluorescein diacetate hydrolysed/mmol of total
197 fluorescein diacetate before hydrolysis (Perucci, 1992).

198 Urease (URE) was determined according to the method of Kandeler and Gerber (1988). Soil (5 g fresh
199 weight) was mixed with 2.5 ml of urea (80 mM) and 20 ml 0.1 M borate buffer pH (10.0). The mixture
200 was allowed to react for 2 h in an orbital shaker at 37 °C. After incubation, pipette 2.5 ml of urea to the
201 control, add 30 ml of KCl (2 M) to both sample and control, and shake for 30 min. Filter the contents
202 of the flasks through folded filters. Aliquots of 1 ml of the filtered solution were mixed with 9 ml of

203 distilled water, 5 ml of sodium/salicylate solution, and 2 ml of dichloroisocyanuric acid (Na⁺ salt). The
204 colour intensity of the solution was measured at 690 nm. Ammonium concentrations were determined
205 by using a calibration curve of ammonium chloride standard solution.

206 Beta-glucosidase activity was detected according to the method of Valášková et al. (2007). Soil (1 g
207 fresh weight) was placed into a plastic tube and treated with 4 mL of modified universal buffer (MUB,
208 pH 6). The reaction mixture contains 0.16 ml of 1.2mM PNP-substrate (p-nitrophenyl-β-d-glucoside)
209 in 50mM sodium acetate buffer (pH 5.0) and 0.04 ml of the sample. Reaction mixtures were incubated
210 at 40°C for 20–120 min. After incubation the reaction was stopped and the yellow color from the
211 p-nitrophenol was developed by the addition of 0.1 ml of 0.5M sodium carbonate, The p-nitrophenol
212 was measured by absorption on a spectrophotometer at a wavelength of 400 nm and quantified by
213 comparison with a standard curve.

214 *2.4 Statistical analysis*

215 One-way ANOVA was used to test the effects of the factors (treatments) on soil indexes for each case
216 study separately. Treatment means were compared using Tukey's test (Sokal and Rohlf, 1981). All
217 statistical analyses were performed using Systat v. 8.0 software package (SPSS Inc, Evanston, Ill,
218 USA). In order to calculate the correlation coefficients between SOM and MBC, FDA and WSP
219 indexes of the SOM, MBC, FDA and WSP were calculated as follows:

220 $\text{Index Y} = Y \times \text{Depth (cm)} / 100$

221 $\text{Index } Y_{\text{whole profile}} = \text{the sum of the individual index}$

222 $Y = \text{SOM, MBC, FDA, WSP}$

223

224 **3. Results**

225 *3.1 Case study 1*

226 Table 1 shows the chemical properties related to the soil treated with different salinity concentrations.
227 There were no significant changes related to organic matter and its fraction (humic and fulvic carbon)
228 between the soil irrigated with freshwater and the soil irrigated with different concentrations of
229 brackish water. No significant variations were observed for total nitrogen, for C/N ratio and for WSP
230 content between the control soil and the treatments. The brackish water treatments affected only in part

231 the enzymatic tissue of the soil. In particular, as shown in Table 2, dehydrogenase activity decreased in
232 soil irrigated with brackish water at the concentrations of 1 and 1.5 %, while the AcP activity
233 decreased only at the highest salinity concentration (1.5%). No significant differences were observed in
234 the activities of the other enzymes between the control and the treatments. Significant differences were
235 instead observed in the MBC contents not only between the treatments and the control, but also among
236 the treatments themselves. Increasing the salinity percentage, the MBC amount significantly and
237 gradually decreased, reaching a 76% reduction in the presence of 1.5 % brackish water.

238

239 *3.2 Case Study 2*

240 The digestate used, had the following characteristic: total solid 25%, total solid volatile 79%. pH 8.4,
241 electric conductivity 1707 $\mu\text{S cm}^{-1}$, total carbon 43 % ss, organic matter 74 % ss, total nitrogen 5.3%
242 ss, C/N 8.1 (Table 3). 40 days after the application of the different amounts of digestate to the soil, no
243 significant differences were observed in the organic matter, HC, FC, N contents, and in the values of
244 C/N ratio. The quantity of water soluble phenols significantly increased increasing the percentage of
245 digestate added to the soil, putting well in evidence significant differences among the treatments
246 themselves. The data related to the biological parameters, enzyme activities and MBC, did not change
247 between the treated and untreated soils (Table 4).

248

249 *3.3 Case study 3*

250 The data of soil chemical properties (Table 5) evidenced significant differences in organic matter trend,
251 between gaps and the adjacent under canopy cover sites, but not between medium and small gaps.
252 Within small and medium gaps a lower amount of organic matter compared to the adjacent sites under
253 canopy cover was observed. A similar trend was observed for FC, no significant differences between
254 the gaps of different sizes and forest were observed for HC content. The amount of total nitrogen was
255 significantly higher in the medium gaps. The values of C/N ratio were quite similar between the gaps
256 of different sizes but it was significantly different between the gaps and under canopy cover sites. No
257 significant variation in the content of WSP between managed and unmanaged soils was observed.
258 FDA (Table 6) was the biological parameter that changed on the basis of the management, showing
259 significant variations among medium gap, small gap and under canopy cover site. Conversely, no
260 significant differences were observed in the activities of the other enzymes among the gaps themselves
261 and between gaps and under canopy cover site.

262

263 3.4 Case study 4

264 In each season, variations in SOM, HC, FC, N, C/N and WSP along the soil profiles were observed:
265 the greatest amount of these soil properties were detected in the litter layer, and then they declined
266 consistently with the soil depth. Comparing each horizon with its counterpart in the different seasons
267 no significant differences were observed for SOM, N, C/N and WSP. No significant variation in HC
268 and FC were observed in the horizons between the adjacent seasons, e.g. between summer and spring
269 or between winter and autumn. Significant differences were appreciable comparing the data detected in
270 winter or autumn with those detected in summer or spring. (Table 7). A similar trend was observed for
271 the parameters reflecting the biochemical properties of soils (Table 8), in each season, variations in soil
272 enzyme activities and MBC contents along the profiles were observed, the greatest enzymatic activities
273 and the higher MBC content were detected in the litter layer and then they declined, with depth,
274 consistently. No significant differences in the biochemical properties were observed comparing each
275 horizon with its counterpart between the contiguous seasons. The differences were noticeable only
276 between the very different seasons. FDA was the only biochemical parameter able to put in evidence
277 the variations in soil due not only to the soil depth, but moreover to the seasons, in particular FDA
278 evidenced differences between summer and spring or winter and autumn that all the other parameters
279 considered have not been able to show.

280 In addition, in each case study, our data showed a highly significant correlation between SOM and
281 MBC, FDA and WSP. (Table 9). In agriculture soils (case studies 1 and 2) the correlations were
282 positive between SOM, FDA and WSP and negative between SOM and MBC. Conversely, in forest
283 soils, the correlations were positive between SOM, MBC and FDA, and negative between SOM and
284 WSP. The results of ANOVA, showed also that the biological parameter most affected in each case
285 study was also more correlated to the SOM than the other ones. In the case study 1 MBC was the
286 parameter most affected by salinity and most correlated with SOM (Table 9), in the case study 2 WSP,
287 was the parameter most affected by digestate treatment, and most correlated with SOM, in the case
288 studies 3 and 4 FDA was the parameter most affected by management and seasonal changes and most
289 correlated with SOM (Table 9).

290

291 4. Discussion

292 Interest in evaluating the quality and health of our soil resources has been stimulated by increasing
293 awareness that soil is a critically important component of the earth's biosphere, functioning not only in
294 the production of food and fiber but also in the maintenance of local, regional, and global
295 environmental quality (Glanz, 1995). In a changing world, assessment of soil quality/health is needed
296 to identify problem in the production areas (Thomsen et al., 2012), to monitor changes in sustainability
297 and environmental quality related to agricultural management for making realistic estimates of food
298 production, and to assist government agencies in formulating and evaluating sustainable agricultural
299 and land-use policies (Granatstein and Bezdicek, 1992). Use of current methods for assessing or
300 indexing soil quality is fraught with complexity and precludes its practical or meaningful use by land
301 managers or policy makers (Harris et al., 1996). Our results evidenced that soil organic matter, most
302 widely recognized influencing soil quality and, typically used as a measure of soil health, hardly
303 evidenced the processes associated to the new soil constraints, because of its insensitivity to assess soil
304 quality changes in the short-term. However, SOM has a number of fractions (phenolic compounds,
305 microbial biomass, and enzymes) with different functional roles in soil (Zagal et al., 2009) which could
306 provide a measure of subtle, or early changes in soil quality. Our results showed that MBC, WSP, and
307 FDA are highly correlated to the SOM and are sensitive to external changes, in the short term, much
308 more than SOM itself, thus they are suitable to be used as early indicator of changes in soil ecosystem.
309 Our results, in agreement with Ji et al. (2014) showed an inverse correlation between SOM and MBC
310 in agriculture soils, due to the tillage practice that decreased the amount of soil microorganisms much
311 more quickly than organic matter. The inverse correlation observed in forest soils between SOM and
312 water soluble phenols independently by treatments, evidenced that the humification process prevailed
313 in these sites. It is the first time, that MBC, WSP, and FDA may be directly related to changes caused
314 by specific external factors (soil management practices and/or environmental conditions), showing an
315 index-factor specificity. FDA was the only biological parameter that changed in forest soils over
316 seasons, and in respect to gap opening, showing a particular sensitivity to the climatic and/or
317 pedoclimatic variations (temperature and moisture). These results are fully in agreement with previous
318 findings showing that FDA is the soil biological parameter most affected by environmental factors
319 (Sicardi et al., 2004; Pesaro et al., 2004; Son et al., 2006; Sumalan et al., 2010; Muscolo et al., 2014 in
320 press). Additionally, our data didn't show a relationship between FDA and MBC amount, suggesting
321 that FDA does not reflect the amount of total microbial biomass, but the amount of the active biomass
322 of the soils (Schnurer and Rosswall, 1982; Araujo et al., 2003), that is stimulated by soil moisture and
323 temperature. As reported by Smit et al. (2001) environmental constraints and seasonal variations

324 influence qualitatively soil community composition, causing considerable fluctuations in the bacteria
325 community (Terry, 1980; Kara and Bolat, 2009), the main component of the active microbial biomass
326 and the main producers of hydrolytic soil enzymes (Emimol et al., 2012). Our results evidenced that
327 MBC was the only soil biological property that rapidly changed under increasing salinity. Many
328 authors (Pankhurst et al., 2001; Mamilov et al., 2004; Yuan et al., 2007; Corstanje et al., 2007;
329 Chowdhury et al., 2011) have already demonstrated that MBC responded to salinity stress,
330 irrespectively of the soil C content. The simple explanation for this was that high salt concentrations in
331 the soil solution increase the external osmotic potential (Harris, 1980), the ion toxicity (Keren, 2000),
332 and the ion competition causing a negative impact on the size and on the activity of soil microbial
333 biomass (Rietz and Haynes, 2003; Tripathi et al., 2006; Yuan et al., 2007; Mavi et al., 2012). WSP,
334 component of the light fraction of the organic matter (Nierop and Buurman, 1998; Riffaldi et al.,
335 2003), changed in a short time after the addition of digestate, pointing out soil alteration due to the
336 organic amendments that other chemical and biochemical soil parameters were not able to evidence
337 quickly. The changes in the size of WSP may have caused significant shifts in the structure and in the
338 function of the microbial community, not reflected in the total MBC, which in turn influence the SOM
339 mineralization and the viability of the soil for agriculture. In short this light fraction of soil organic
340 matter can be consider a useful early indicator of management-related carbon (C), and can be used to
341 describe the effects of compost amendments to soil (Grandy et al., 2002; Carter et al., 2004; Lynch et
342 al., 2005; Dale et al., 2008).

343

344 **Conclusion**

345 There are several biological soil properties that can be used as soil quality indicators, alone or in
346 combination with other chemical or physical properties. However they are far from being universal and
347 should be chosen according to the situation under consideration. On the other hand there are several
348 soil properties sensitive to management changes but difficult to determine and to interpret. The basic
349 indicators and the number of estimated measures are still under investigation. In this study, we have
350 identified MBC, WSP, and FDA as effective tools in the evaluation of soil quality to understand soil
351 performance and processes in the short term, putting in evidence for the first time a threat-indicator
352 specificity. The use of specific and appropriate indicators, is useful to predict the dynamic behavior of

353 soil processes and the impact of management practices and/or climate in the short term, saving time
354 and money, helping to develop management strategies to shift soil conditions in a positive direction.

355

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361

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532 **Table 1.** Chemical characteristics of brackish-water irrigated soils. Organic Matter (OM %); Humic
 533 Carbon (HC %); Fulvic Carbon (FC%); Total Nitrogen (N %); Total Phenols (WSP $\mu\text{g TAE g}^{-1}$ dry
 534 soil). Numbers denote the standard errors (n=6) Means with the same letters are not significantly
 535 different (Tukey's test. $p \leq 0.05$)

Treatment	OM	HC	FC	N	C/N	WSP
0%	2.25 \pm 0.1 ^a	0.78 \pm 0.2 ^a	0.50 \pm 0.2 ^a	0.12 \pm 0.009 ^a	10.90 \pm 2.3 ^a	44 \pm 2.5 ^a
0.5%	2.36 \pm 0.2 ^a	0.81 \pm 0.1 ^a	0.51 \pm 0.1 ^a	0.13 \pm 0.008 ^a	10.55 \pm 2.4 ^a	45 \pm 3.1 ^a
1.0%	2.49 \pm 0.1 ^a	0.79 \pm 0.2 ^a	0.49 \pm 0.1 ^a	0.12 \pm 0.003 ^a	12.0 \pm 1.8 ^a	42 \pm 3.5 ^a
1.5%	2.51 \pm 0.2 ^a	0.84 \pm 0.2 ^a	0.53 \pm 0.2 ^a	0.12 \pm 0.003 ^a	13.1 \pm 1.0 ^a	50 \pm 4.5 ^a

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543 **Table 2.** Microbial Biomass (MBC $\mu\text{g C g}^{-1}$ soil), fluorescein diacetate (FDA) hydrolysis (fluorescein
 544 released, $\mu\text{g g}^{-1}$ dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. $\mu\text{g p-nitrophenol}$
 545 $\text{g}^{-1} \text{h}^{-1}$) and urease (URE $\mu\text{g NH}_4^+\text{-N g}^{-1}\text{dry soil } 2 \text{ h}^{-1}$), dehydrogenase (DH $\mu\text{g INTF g}^{-1}$ dry soil h^{-1}) β -
 546 glucosidase (β -GLU $\mu\text{g p-nitrophenol g}^{-1} \text{h}^{-1}$) activities, in brackish-water irrigated soils. Numbers
 547 denote the standard errors (n=6) Means with the same letters are not significantly different (Tukey's
 548 test. $p \leq 0.05$).

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Treatment	FDA	DH	β-GLU	URE	Ac.P	Ak.P	MBC
0%	42.0 \pm 1.4 ^a	57 \pm 1.5 ^a	71 \pm 3.5 ^a	84.30 \pm 1.9 ^a	250 \pm 3.9 ^a	332 \pm 3.9 ^a	862.2 \pm 3.7 ^a
0.5%	44.1 \pm 0.9 ^a	55 \pm 1.3 ^a	69 \pm 2.8 ^a	80.95 \pm 1.5 ^a	255 \pm 3.5 ^a	334 \pm 2.8 ^a	631.5 \pm 2.9 ^b
1.0%	44.2 \pm 1.4 ^a	48 \pm 1.2 ^b	69 \pm 3.1 ^a	81.40 \pm 0.9 ^a	251 \pm 1.9 ^a	340 \pm 5.1 ^a	401.2 \pm 1.6 ^c
1.5%	43.7 \pm 1.3 ^a	35 \pm 1.0 ^c	67 \pm 2.3 ^a	82.15 \pm 0.5 ^a	195 \pm 2.1 ^b	341 \pm 2.6 ^a	201.5 \pm 1.4 ^d

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555 **Table 3.** Chemical characteristics of soil treated with different concentration of digestate. Organic
 556 Matter (OM %); Humic Carbon (HC %); Fulvic Carbon (FC%); Total Nitrogen (N %); Total Phenols
 557 (WSP $\mu\text{g TAE g}^{-1}$ dry soil). Numbers denote the standard errors (n=9) Means with the same letters are
 558 not significantly different (Tukey's test. $p \leq 0.05$)

Treatment	OM	HC	FC	N	C/N	WSP
0	2.29±0.2 ^a	0.77±0.2 ^a	0.52±0.2 ^a	0.12±0.008 ^a	11.1±2.3 ^a	41±2.5 ^d
25%	2.30±0.1 ^a	0.75±0.2 ^a	0.58±0.2 ^a	0.12±0.009 ^a	11.1±2.0 ^a	55±3.5 ^c
50%	2.33±0.3 ^a	0.62±0.1 ^a	0.60±0.2 ^a	0.13±0.007 ^a	10.4±1.8 ^a	66±2.9 ^b
75%	2.36±0.2 ^a	0.63±0.1 ^a	0.68±0.1 ^a	0.15±0.005 ^a	9.2±2.5 ^a	98±2.9 ^a

559 **Table 4.** Microbial Biomass (MBC $\mu\text{g C g}^{-1}$ soil), fluorescein diacetate (FDA) hydrolysis (fluorescein
560 released, $\mu\text{g g}^{-1}$ dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. $\mu\text{g } p\text{-nitrophenol}$
561 $\text{g}^{-1} \text{h}^{-1}$) and urease (URE $\mu\text{g NH}_4^+\text{-N g}^{-1}\text{dry soil } 2 \text{ h}^{-1}$), dehydrogenase (DH $\mu\text{g INTF g}^{-1}$ dry soil h^{-1})
562 β -glucosidase (β -GLU $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{h}^{-1}$) activities, in soil treated with different concentration
563 of digestate. Numbers denote the standard errors (n=9) Means with the same letters are not
564 significantly different (Tukey's test. $p \leq 0.05$)

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Treatment	FDA	DH	β-GLU	URE	Ac.P	Ak.P	MBC
0%	42.4 \pm 1.5 ^a	59.5 \pm 1.5 ^a	78 \pm 4.2 ^a	87.9 \pm 2.2 ^a	248 \pm 3.5 ^a	339 \pm 3.0 ^a	860 \pm 3.4 ^a
25%	43.5 \pm 2.5 ^a	59.4 \pm 1.6 ^a	82 \pm 2.5 ^a	89.4 \pm 2.0 ^a	248 \pm 3.0 ^a	340 \pm 2.0 ^a	865 \pm 3.0 ^a
50%	41.9 \pm 2.1 ^a	62.1 \pm 1.8 ^a	81 \pm 3.1 ^a	91.1 \pm 2.3 ^a	247 \pm 3.5 ^a	344 \pm 3.0 ^a	860 \pm 5.0 ^a
75%	43.9 \pm 2.8 ^a	63.5 \pm 2.5 ^a	84 \pm 4.0 ^a	92.4 \pm 2.5 ^a	246 \pm 3.0 ^a	345 \pm 3.0 ^a	858 \pm 3.4 ^a

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568 **Table 5.** Chemical characteristics of soil in gaps and under canopy cover sites. Organic Matter (OM %);
 569 Humic Carbon (HC %); Fulvic Carbon (FC%); Total Nitrogen (N %); Total Phenols (WSP $\mu\text{g TAE g}^{-1}$ dry
 570 soil). Numbers denote the standard errors (n=9) Means with the same letters are not significantly different
 571 (Tukey's test, $p \leq 0.05$) A: medium gaps; B: canopy cover sites; C: small gaps.

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Treatment	OM	HC	FC	N	C/N	WSP
A	7.0 \pm 0.1 ^b	2.70 \pm 0.2 ^a	1.36 \pm 0.2 ^b	0.50 \pm 0.008 ^a	8.1 \pm 2.3 ^b	255 \pm 2.7 ^a
B	11 \pm 0.5 ^a	3.08 \pm 0.2 ^a	2.03 \pm 0.2 ^a	0.35 \pm 0.009 ^b	18.2 \pm 2.0 ^a	252 \pm 2.2 ^a
C	7.2 \pm 0.3 ^b	2.62 \pm 0.1 ^a	1.39 \pm 0.2 ^b	0.38 \pm 0.007 ^b	11.0 \pm 1.8 ^b	254 \pm 2.6 ^a

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576 **Table 6.** Microbial Biomass (MBC $\mu\text{g C g}^{-1}$ soil), fluorescein diacetate (FDA) hydrolysis (fluorescein
577 released, $\mu\text{g g}^{-1}$ dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. $\mu\text{g } p\text{-nitrophenol}$
578 $\text{g}^{-1} \text{h}^{-1}$) and urease (URE $\mu\text{g NH}_4^+\text{-N g}^{-1}\text{dry soil } 2 \text{ h}^{-1}$), dehydrogenase (DH $\mu\text{g INTF g}^{-1}$ dry soil h^{-1})
579 β -glucosidase (β -GLU $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{h}^{-1}$) activities, in gaps and under canopy cover sites.
580 Numbers denote the standard errors (n=9) Means with the same letters are not significantly different
581 (Tukey's test, $p \leq 0.05$) A: medium gaps, B: canopy cover sites, C: small gaps.

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Treatment	FDA	DH	β -GLU	URE	Ac.P	Ak.P	MBC
A	0.701 \pm 0.05 ^b	55.5 \pm 1.0 ^a	80 \pm 2.1 ^a	133 \pm 2.3 ^a	448 \pm 3.5 ^a	366 \pm 3.0 ^a	1258 \pm 4.5 ^a
B	0.950 \pm 0.03 ^a	56.4 \pm 1.3 ^a	82 \pm 2.0 ^a	135 \pm 3.0 ^a	451 \pm 3.0 ^a	369 \pm 2.0 ^a	1266 \pm 5.0 ^a
C	0.805 \pm 0.01 ^c	54.1 \pm 1.5 ^a	83 \pm 1.9 ^a	131 \pm 2.8 ^a	447 \pm 3.5 ^a	365 \pm 3.0 ^a	1259 \pm 5.0 ^a

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594 **Table 7.** Changes in forest soil chemical characteristics under *Pinus laricio* plantation over season.
 595 Organic Matter (OM %); Humic Carbon (HC %); Fulvic Carbon (FC%); Total Nitrogen (N %); Total
 596 Phenols (WSP $\mu\text{g TAE g}^{-1}$ dry soil). Numbers denote the standard errors (n=18) Means with the same
 597 letters are not significantly different (Tukey's test. $p \leq 0.05$)

Season	Horizon	Depth (cm)	OM	HC	FC	N	C/N	WSP
Autumn	Oi	5-0	34.4 ^a	6.23 ^a	1.10 ^b	0.87 ^a	23 ^a	285 ^a
	Ah ₁	0-30	6.3 ^b	2.60 ^c	0.95 ^c	0.23 ^b	16 ^b	108 ^b
	Ah ₂	30-50	4.9 ^c	2.50 ^c	0.91 ^c	0.18 ^c	16 ^b	84 ^c
Winter	Oi	5-0	34.1 ^a	6.15 ^a	1.06 ^b	0.81 ^a	24 ^a	287 ^a
	Ah ₁	0-30	5.9 ^b	2.61 ^c	0.94 ^c	0.21 ^b	16 ^b	103 ^b
	Ah ₂	30-50	4.7 ^c	2.51 ^c	0.90 ^c	0.17 ^c	16 ^b	87 ^c
Spring	Oi	5-0	33.0 ^a	3.90 ^b	1.50 ^a	0.80 ^a	24 ^a	299 ^a
	Ah ₁	0-30	6.0 ^b	1.05 ^d	0.78 ^d	0.22 ^b	16 ^b	99 ^b
	Ah ₂	30-50	5.0 ^c	0.88 ^f	0.62 ^e	0.17 ^c	17 ^b	88 ^c
Summer	Oi	5-0	33.9 ^a	3.81 ^b	1.45 ^a	0.83 ^a	24 ^a	290 ^a
	Ah ₁	0-30	6.5 ^b	0.99 ^e	0.80 ^d	0.22 ^b	17 ^b	99 ^b
	Ah ₂	30-50	5.2 ^c	0.87 ^f	0.65 ^e	0.18 ^c	17 ^b	89 ^c

598 **Table 8.** Microbial Biomass (MBC $\mu\text{g C g}^{-1}$ soil), fluorescein diacetate (FDA) hydrolysis (fluorescein
599 released, $\mu\text{g g}^{-1}$ dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. $\mu\text{g p}$ -nitrophenol
600 $\text{g}^{-1} \text{h}^{-1}$) and urease (URE $\mu\text{g NH}_4^+\text{-N g}^{-1}$ dry soil 2h^{-1}), Dehydrogenase (DH $\mu\text{g INTF g}^{-1}$ dry soil h^{-1})
601 β -glucosidase (β -GLU $\mu\text{g p}$ -nitrophenol $\text{g}^{-1} \text{h}^{-1}$) activities, in forest soil over seasons. Numbers denote
602 the standard errors (n=18) Means with the same letters are not significantly different (Tukey's test. p
603 ≤ 0.05)

Season	Horizon	Depth cm	FDA	DH	β -GLU	URE	Ac.P	Ak.P	MBC
Autumn	Oi	5-0	0.555 ^c	123 ^b	211 ^b	152.3 ^a	1204 ^b	894 ^b	2170 ^b
	Ah ₁	0-30	0.301 ⁱ	45 ^c	142 ^d	46.7 ^c	513 ^c	212 ^d	1007 ^e
	Ah ₂	30-50	0.121 ^l	17 ^d	49 ^f	34.1 ^d	127 ^e	99 ^f	431 ^f
Winter	Oi	5-0	0.400 ^f	119 ^b	199 ^b	102.7 ^b	1190 ^b	851 ^b	1950 ^c
	Ah ₁	0-30	0.107 ^g	47 ^c	138 ^d	25.6 ^e	497 ^c	201 ^d	999 ^e
	Ah ₂	30-50	0.091 ^m	19 ^d	41 ^f	23.7 ^e	135 ^e	103 ^f	430 ^f
Spring	Oi	5-0	0.899 ^a	199 ^a	333 ^a	156.2 ^a	2771 ^a	2468 ^a	2611 ^a
	Ah ₁	0-30	0.530 ^d	109 ^b	168 ^c	51.3 ^c	914 ^b	397 ^c	1514 ^d
	Ah ₂	30-50	0.358 ^g	44 ^c	69 ^e	26.9 ^e	207 ^d	184 ^e	449 ^f
Summer	Oi	5-0	0.834 ^b	205 ^a	320 ^a	151.6 ^a	2754 ^a	2563 ^a	2573 ^a
	Ah ₁	0-30	0.421 ^e	115 ^b	170 ^c	46.7 ^c	934 ^b	401 ^c	1036 ^e
	Ah ₂	30-50	0.330 ^h	50 ^c	77 ^e	22.1 ^c	199 ^d	170 ^e	439 ^f

605 **Table 9.** Correlation coefficients between SOM and MBC, FDA and WSP indexes in forest and
 606 agriculture soils subjected to different management practices and climate. Case study 1: effects of
 607 artificial brackish water at different concentrations (0; 0.5%; 1%; 1.5%) on agriculture soil. Case
 608 study 2: effects of amendment with digestate at different concentrations (0, 25, 50, 75 %) on
 609 agriculture soil. Case study 3: effects of gap size on forest soil under *Fagus sylvatica* and *Abies*
 610 *Alba*. Case study 4: effects of seasonal variation on forest soil under *Pinus laricio*.

		MBC index	FDA index	WSP index
<i>Case study 1</i>				
	<i>r</i>	-0.936	0.836	0.211
SOM index	<i>p</i> -value	<0.001	<0.001	<0.001
	R ²	0.877	0.700	0.044
<i>Case study 2</i>				
	<i>r</i>	-0.635	0.373	0.977
SOM index	<i>p</i> -value	<0.01	<0.01	<0.01
	R ²	0.403	0.139	0.955
<i>Case study 3</i>				
	<i>r</i>	0.518	0.927	-0.677
SOM index	<i>p</i> -value	<0.001	<0.001	<0.001
	R ²	0.269	0.860	0.459
<i>Case study 4</i>				
	<i>r</i>	0.545	0.852	-0.184
SOM index	<i>p</i> -value	<0.001	<0.001	<0.001
	R ²	0.297	0.725	0.034