Early warning indicators of changes in soil ecosystem functioning

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Abstract

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

Keywords Agriculture ecosystem. Biological indicators. Forest ecosystem. Soil organic matter. Soil quality.

1. Introduction

Soils are the most significant non-renewable geo-resource we have and that are facing numerous environmental threats while trying to resist to climatic changes. Interest in evaluating the quality and health of our soils has been stimulated by increasing awareness that soil is a critical important component of the earth's biosphere, functioning not only in the production of food and fiber but also in the maintenance of global sustainability and environmental balance (Glanz, 1995). Soil is also the basis of agricultural and of natural plant communities. Thus, the thin layer of soil covering the surface of the earth represents the difference between survival and extinction for most land-based life (Doran et al., 1996). Whilst the majority of countries have criteria to evaluate the quality of the air and water, the same does not occur for the quality of the soil. Traditionally, soil quality is associated with productivity (Karlen et al., 1997), but recently it has been defined in terms of sustainability (Toth et al., 2007), that is, the capacity of the soil to absorb, store and recycle water, minerals and energy in such a way that the production of the crops can be maximized and environmental degradation minimized. Nevertheless, a significant decline in soil quality has occurred throughout the entire world as a result of adverse changes in its physical, chemical and biological properties, caused by human activity and climate changes (Van Camp et al., 2004; EC 2006). According to Steer (1998), in the last decades of the last century, about 2 billion of the 8.7 billion agricultural lands, permanent pastures, forests and wild native lands have been degraded. Soil degradation processes constitute a serious problem on a worldwide basis, with significant environmental, social and economic consequences. Many economic activities such as agriculture, industry and tourism depend both directly and indirectly on soil quality, which has been proposed as a prime indicator for characterizing and defining management factors contributing to soil degradation. Many constraints cause short-term disturbances that are detrimental to soil quality (IPCC 2007; EEA-JRC-WHO, 2008) as they increase the emissions of greenhouse gases (i.e., CO_2, NO, or N_2O), cause nitrate accumulation and leaching, and/or modify soil microbial community structure in a way that decreases the retention of organic C and N (Liu et al., 2006). Generally, soil quality has been related to the SOM (Gao et al., 2013), microbial activity, total nitrogen, and C/N ratio (Molope and Page, 1986; Eash et al., 1994; Roberson et al., 1995; Murphy et al., 2011), but these soil parameters not necessarily change as a result of changing external conditions or use (Muscolo et al., 2014 in press), and hardly address short term changes in soil processes associated to the new environmental threats. To rise the challenge of soil resource degradation, there is an urgent need to develop common, simple and transparent method to identify changes in soil

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

2. Material and Method

2.1 Experiments and soil sampling

Four separate experiments were carried out to identify early warning indicators that better reflect changes in soil chemistry and biochemistry parameters related to soil quality. The experiments were conducted both in forest and agriculture soils underwent to different management practices and climate. The first experiment (named **Case study 1**) was conducted in field, in order to evaluate the effects of artificial brackish water at different concentrations (0; 0.5%; 1%; 1.5%) on chemical and biochemical properties of a haplic Kastanozem (IUSS, 2006) located in the Agricultural Farm of ‘’Mediterranea University’’, Reggio Calabria, Southern Italy. Soil during the dry season (June, July and August), have been irrigated, three time a week, with synthetic brackish water (EC 4 dS m^{-l}) prepared using $NaHCO_3$, NaCl, Na_2SO_4, and $MgSO_4$ with $Cl:SO_4$ ratio of 1:1 and Ca:Mg ratios of 4:1 to maintain the 70% of field capacity. Three months after the irrigations with brackish water, soil samples were collected and analyzed for the chemical and biochemical parameters. Six composite soil

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

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

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

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

The average seasonal precipitation are typically highest during the winter (1100) and autumn (1500) compared to spring (900) and summer (600). The soil were Haplic Phaeozem (IUSS, 2006) with a xeric soil regime moisture and a vegetal cover of *Pinus laricio* Poiret ssp. Calabrica. The effects of seasons (autumn, winter, spring and summer) were evaluated on soil chemical and biochemical parameters as described below. Soil profiles were carefully excavated, different (layers) horizons were thoroughly separated from the top to the bottom of the profile on the basis of morphological differences that could be perceived by the naked eye. Every15 days, soil samples (1 kg) were taken from each horizon over a year (24 times in a year). The samples were brought to the laboratory on the same day of the collection, and kept in the refrigerator at 4 °C for up to 24 h until processing. Prior to the soil analysis, except for FDA hydrolysis and MBC, all the soil samples were air-dried, sieved (<2 mm), and visible roots were removed. Data presented are the means of three replicate determinations.

2.2 Soil Chemical Analysis

Organic C was estimated by the Walkley–Black procedure (Nelson and Sommers, 1982) and was converted to organic matter by multiplying the percentage of C by 1.72; total N was measured by the Kjeldahl method (Bremner and Mulvaney, 1982). Humic substances were extracted with 0.1 N NaOH (solid:liquid ratio 1:10); the suspension was shaken for 16 h at room temperature and centrifuged at 5,000 rpm for 30 min; the extract was dialysed by Wisking tubes against distilled water to pH 6.0. Subsequently, the solution was filtered through a column of Amberlite IR 120 H^+. The fractionation of humic substances was carried out as follows: aliquots of extracts were acidified to pH 2.0 with dilute H_2SO_4; the humic acids precipitated and were removed by centrifugation, while the fulvic acids corresponded to the supernatants (Bettany et al. 1980). The C content of humic and fulvic acids was determined by dichromate oxidation (Nelson and Sommers 1982). Phenols were extracted with distilled water as this is the most realistic extractant in allelopathic studies (Kaminsky and Muller 1977, 1978). Thirty grams of dry weight samples were mixed in 200 ml distilled water and shaken at 75 rev min)1 for 20 h at room temperature. Solutions were filtered through Whatman's No 1 paper. All samples were extracted in triplicate. Total water-soluble phenols (monomeric and polyphenols) were determined by using the Folin–Ciocalteau reagent, following the method of Box (1983). Tannic acid was used as a standard and the concentration of water-soluble phenolic compounds was expressed as tannic acid equivalents (µg TAE g^{-1} D.W.).

2.3 Soil Biochemical Analysis

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

Enzymatic assay: Dehydrogenase (DH) activity was determined by the method of von Mersi and Schinner (1991). Briefly, to a sample of fresh soil equivalent to 1 g of oven dried (105° C) soil were 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 No I 8377), and the suspension was kept at 40 C for 1 h. Then 10 ml of extractant (methanol) was added and the samples were mixed using a vortex mixer, and then left in the dark for 10 min. Finally, the solids were filtered out (Whatman's no 40 paper), and the absorbance of the filtrate was determined at 490 nm.

Alkaline and acid phosphatase (AlPh, AcPh) activities were determined on 1 g (fresh weight) aliquots of soil, according to the method of Tabatabai (1982). Enzyme activities are expressed as µg *p*-nitrophenol produced by 1 g of dry soil in one hour (µg p-nitrophenol g1 h1).

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

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

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

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

2.4 Statistical analysis

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

Index Y = Y x Depth (cm)/100

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

Y= SOM, MBC, FDA, WSP

3. Results

3.1 Case study 1

Table 1 shows the chemical properties related to the soil treated with different salinity concentrations. There were no significant changes related to organic matter and its fraction (humic and fulvic carbon) between the soil irrigated with freshwater and the soil irrigated with different concentrations of brackish water. No significant variations were observed for total nitrogen, for C/N ratio and for WSP 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 µS 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

3.4 Case study 4

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

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

4. Discussion

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

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

Conclusion

There are several biological soil properties that can be used as soil quality indicators, alone or in combination with other chemical or physical properties. However they are far from being universal and should be chosen according to the situation under consideration. On the other hand there are several soil properties sensitive to management changes but difficult to determine and to interpret. The basic indicators and the number of estimated measures are still under investigation. In this study, we have identified MBC, WSP, and FDA as effective tools in the evaluation of soil quality to understand soil performance and processes in the short term, putting in evidence for the first time a threat-indicator 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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Table 1. Chemical characteristics of brackish-water irrigated soils. Organic Matter (OM %); Humic Carbon (HC %); Fulvic Carbon (FC%); Total Nitrogen (N %); Total Phenols (WSP µg TAE g^{-1} dry soil). Numbers denote the standard errors (n=6) Means with the same letters are not significantly different (Tukey's test. $p \leq 0.05$)

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

Table 2. Microbial Biomass (MBC µg C g^{-1} soil), fluorescein diacetate (FDA) hydrolysis (fluorescein released, µg g-1 dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. µg p-nitrophenol g^{-1} h^{-1}) and urease (URE µg NH_4^+-N g^{-1}dry soil 2 h^{-1}), dehydrogenase (DH µg INTF g^{-1} dry soil h^{-1}) β-glucosidase (β-GLU µg p-nitrophenol g^{-1} h^{-1}) activities, in brackish-water irrigated soils. Numbers denote the standard errors (n=6) Means with the same letters are not significantly different (Tukey's test. $p \leq 0.05$).

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

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 µg 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 µg C g^{-1} soil), fluorescein diacetate (FDA) hydrolysis (fluorescein
560 released, µg g^{-1} dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. µg *p*-nitrophenol
561 g^{-1} h^{-1}) and urease (URE µg NH_4^+-N g^{-1}dry soil 2 h^{-1}), dehydrogenase (DH µg INTF g^{-1} dry soil h^{-1})
562 β-glucosidase (β-GLU µg *p*-nitrophenol g^{-1} 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±1.5[a]	59.5±1.5[a]	78±4.2[a]	87.9±2.2[a]	248±3.5[a]	339±3.0[a]	860±3.4[a]
25%	43.5±2.5[a]	59.4±1.6[a]	82±2.5[a]	89.4±2.0[a]	248±3.0[a]	340 ±2.0[a]	865±3.0[a]
50%	41.9±2.1[a]	62.1±1.8[a]	81±3.1[a]	91.1±2.3[a]	247±3.5[a]	344±3.0[a]	860±5.0[a]
75%	43.9±2.8[a]	63.5±2.5[a]	84±4.0[a]	92.4±2.5[a]	246±3.0[a]	345±3.0[a]	858±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 µg 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.

572

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

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Table 6. Microbial Biomass (MBC µg C g^{-1} soil), fluorescein diacetate (FDA) hydrolysis (fluorescein released, µg g^{-1} dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. µg *p*-nitrophenol g^{-1} h^{-1}) and urease (URE µg NH_4^+-N g^{-1}dry soil 2 h^{-1}), dehydrogenase (DH µg INTF g^{-1} dry soil h^{-1}) β-glucosidase (β-GLU µg *p*-nitrophenol g^{-1} h^{-1}) activities, in gaps and under canopy cover sites. Numbers denote the standard errors (n=9) Means with the same letters are not significantly different (Tukey's test. $p \leq 0.05$) A: medium gaps, B: canopy cover sites, C: small gaps.

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

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

Season	Horizon	Depth (cm)	OM	HC	FC	N	C/N	WSP
	Oi	5-0	34.4^{a}	6.23^{a}	1.10^{b}	0.87^{a}	23^{a}	285^{a}
Autumn	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}
	Oi	5-0	34.1^{a}	6.15^{a}	1.06^{b}	0.81^{a}	24^{a}	287^{a}
Winter	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}
	Oi	5-0	33.0^{a}	3.90^{b}	1.50^{a}	0.80^{a}	24^{a}	299^{a}
Spring	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}
	Oi	5-0	33.9^{a}	3.81^{b}	1.45^{a}	0.83^{a}	24^{a}	290^{a}
Summer	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 µg C g^{-1} soil), fluorescein diacetate (FDA) hydrolysis (fluorescein
599 released, µg g^{-1} dry soil; acid phosphatase and alkaline phosphatase (Ac and Ak. P. µg *p*-nitrophenol
600 g^{-1} h^{-1}) and urease (URE µg NH_4^+-N g^{-1}dry soil 2 h^{-1}), Dehydrogenase (DH µg INTF g^{-1} dry soil h^{-1})
601 β-glucosidase (β-GLU µg *p*-nitrophenol g^{-1} 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}	43l^{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}

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

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