

# The complexity of soil biological sustainability

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## Abstract

**Additions of organic amendments to soil not only compensate for decreased soil C, but also contribute to energy requirements for conserving biological activity levels. The soil microbial biomass displays some highly conserved, and possibly unique, characteristics that do not permit a classic interpretation of microbial metabolic parameter data. The resilience of soil microbial biomass and the role of soil organic matter in sustaining microbial biomass under practically zero C inputs were assessed in two long term incubation experiments using soils from the Broadbalk experiment at Rothamsted (UK). Soils with low organic C contents, showed the greatest decline in biomass C during the first 30 d of incubation. The ATP concentration of this rapidly declining microbial biomass did not change during the prolonged incubation period, confirming this peculiar character of the soil microbial biomass. Specific respiration rate did not depend upon substrate availability, being higher in soils that had received the lowest C inputs. Qualitative and quantitative changes observed in humic fractions suggest that humified soil organic matter is a much more dynamic soil fraction than is normally considered and provides a utilizable energy reserve for soil microorganisms. Carbon levels can be successfully restored in soils through practices such as incorporation of crop residues, re-vegetation and application of manures, biosolids and composts. Some amendments, such as olive mill waste compost, promote incorporation of altered lignin structures, N-containing compounds and carbohydrates into humic acids. The mineral-bound fraction of humic C also increases, after their addition, and contributes to the accumulation of the most inert soil C pools.**

**Keywords:** soil sustainability, soil resilience, organic matter, biological properties

## INTRODUCTION

Soil sealing by roads and buildings, etc., is nowadays the most rapidly growing limitation for soil functions (<http://eusoiils.jrc.ec.europa.eu/InternationalCooperation/SEPLS/>). Across the EU, the total sealed surface area in 2006 was greater than the size of Portugal (100,000 km<sup>2</sup>) and around 500 km<sup>2</sup> of land are sealed each year (European Commission, 2011). Soil sustainability has therefore become a pressing priority. It is a complex issue which needs to be considered relatively to various contexts, according to the wide variety of ecosystem services that are provided (Smith and Powlson, 2007). These services can be of considerable value (Blum, 2005; Clothier et al., 2011) and include natural soil functions such as storing and releasing water, decomposing plant and animal residues, transforming and recycling nutrients, sequestering and detoxifying pollutants as well as food security and productivity of agricultural systems, biological resource use, continued quality of the environment, support to housing, infrastructure, recreation uses and waste disposal, protection and preservation of cultural heritage (Blum, 2005).

The IUCN Environmental Policy and Law Paper No. 45 (Hannan and Boer, 2002) makes a distinction between two different conceptual ways to define sustainability which are associated with two different time frames: a short-term human-centred view that equates sustainability with food sufficiency and a long-term ecological sustainability concept. Short-term sustainability focuses on maximizing long-term production and sets production targets to be met by combining different resources, aiming at a greater productive efficiency. On the



contrary, long-term sustainability focuses on a broader view of maintaining the capacity of soil to sustain future needs (Smith and Powlson, 2007). Whatever definition we may choose, e.g., sustainable soil management, measures and practices needed to achieve the aim of either short- or long-term soil sustainability must be based on a precise knowledge of concepts and issues related to soil degradation and soil quality. Sustainable soil use has been defined as “the use of soil as a natural resource in a way that does not exert any negative effects – that are irreparable under rational conditions – either on the soil itself or any other systems of the environment” (Tóth et al., 2007). Sustainable soil use is therefore aimed at minimizing or eliminating soil degradation. The Thematic Strategy for Soil Protection (European Commission, 2006) states that in order to achieve sustainable development, soil functions need to be protected from degradation.

According to Lal (2002) soil degradation refers to the decline in a soil’s inherent capacity to produce economic goods and perform ecological functions, a definition which implies the maintenance of soil quality. Soil quality was defined by the Soil Science Society of America’s Committee on soil quality as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation” (Karlen et al., 1997). Soil quality is generally associated with suitability for specific land uses, whereas soil health is often used in a broader sense to indicate the capacity of soil to function and perform its many diverse functions. In this sense, maintenance of soil health has been considered synonymous with sustainability (Doran and Zeiss, 2000). From an operational point of view, a well-defined quantitative evaluation of soil health or soil quality is essential for the implementation of sustainable soil use. To do this, these complex concepts need to be translated into a weighted combination of measurable descriptors. Islam and Weil (2000) emphasized that soil quality is best assessed by soil properties that are neither so permanent as to be insensitive to management, nor so easily changeable as to give little indication of long-term alterations. To be useful as an indicator of the sustainability of land management practices, a soil parameter must respond sensitively to changes in management (Doran and Parkin, 1996). Soil organism and biotic parameters (e.g., abundance, diversity, food web structure or community stability) meet most of the criteria for useful indicators of soil quality (Doran and Zeiss, 2000). Soil microorganisms in particular are well correlated with beneficial soil functions and are responsible for decomposition and nutrient cycling as well as detoxification of xenobiotics. They not only respond sensitively to anthropogenic disturbance (Pankhurst et al., 1997; Wolters and Schaefer, 1994), but also because, in spite of the variability of soil types and climatic environments, they are characterized by a conservative set of important biological properties.

Biological soil properties are therefore an optimum choice and provide a wide set of parameters which are both easily measured and directly linked to both agricultural productivity and environmental functions.

### **SOIL RESISTANCE AND RESILIENCE**

Different soils do not undergo degradation in the same way when exposed to degrading factors of the same intensity, but will display different degrees of resistance and resilience. Resistance is defined as the degree to which a system is insensitive to a disturbance, whereas resilience is the rate at which a system returns to a pre-disturbance condition. The “soil resilience” concept has been introduced into soil science only recently, mainly to address soil ecology and sustainable land use issues. Both resistance and resilience are usually quantified in relation to a normal level of intrinsic variability.

The use of predictive models is an essential tool in the individuation of threats and the quantification of their long-term impacts. They are also essential in the determination of threshold limits. Thresholds were defined by Dumanski et al. (1992) as “levels beyond which a system undergoes significant change; points at which stimuli provoke response”. Thus threshold limits are closely related to resistance and resilience: they represent values of a variable beyond which rapid and even exponential damage occurs (Pieri et al., 1995;

Schjønnning, 2004).

Assessing soil quality is not sufficient to achieve sustainability: the real issue being the management of soil quality. Indicators of soil quality must therefore be viewed with respect to their response to soil management. Regarding soil biological activity, for instance, soil microbial biomass C (Bc) is a soil quality indicator for which a threshold can be established, while the rate of C inputs (e.g., t organic C ha<sup>-1</sup> year<sup>-1</sup>) required to maintain the soil microbial biomass at some prescribed level represents the management threshold. The ratio between soil Bc and total soil organic C (Corg) (Table 1) is a sensitive index of soil changes, since microbial biomass responds more rapidly to changes than organic matter (Powlson et al., 1987). A value of Bc/Corg of 2.2 reflects a good equilibrium for cultivated soils (Jenkinson and Ladd, 1981).

Table 1. Organic C, annual C inputs, microbial biomass C and biomass C/Corg ratios of selected soils of the Broadbalk long-term experiment at Rothamsted (see text for acronyms).

	Corg (mg C g <sup>-1</sup> )	C Input (t C ha <sup>-1</sup> y <sup>-1</sup> )	Biomass C (mg C g <sup>-1</sup> )	Biomass C/Corg
Paths	9.2	-	115	1.19
NIL	9.6	1.1	151	1.72
NPK	11.6	1.7	184	1.59
NPK+Straw	10.8	3.0	264	2.44
Stubbed	40.2	4.0	1289	3.21
Woodland	42.4	4.1	1281	3.02

Other biological parameters are useful in the evaluation of soil resilience, e.g., the specific respiration of the soil microbial biomass. Soil pollution by heavy metals causes increased specific respiration of the soil microbial biomass and depressed biosynthesis (Chander and Brookes, 1991). In a study on microbiological resilience of soils contaminated with crude oil, Franco et al. (2004) observed that evaluation of soil resilience by crude oil degradation rates was difficult as less than 2% of crude oil was mineralized to CO<sub>2</sub>. However, crude oil contamination resulted in large and significant variations in soil microbial biomass. Grassland soils, generally, support larger microbial populations in comparison with otherwise similar arable soils. However, soil microbial biomass displays some highly conserved peculiar characteristics that do not allow a classic interpretation of microbial metabolic parameters data.

#### **CONSERVED BIOLOGICAL PROPERTIES OF THE SOIL MICROBIAL BIOMASS AND ITS RESISTANCE TO LACK OF SUBSTRATE INPUTS IN SOILS OF DIFFERENT ORGANIC MATTER LEVELS**

The low energy requirement (Anderson and Domsch, 1990) and the slow turnover time calculated for soil microbial biomass (Jenkinson and Ladd, 1981) indicate that the microbial biomass mainly exists in a dormant state. This is considered to be an adaptation for survival in the soil environment where utilizable C is normally severely limited and fresh substrate inputs very scarce and intermittent. Yet, the soil microbial biomass maintains an adenosine 5'-triphosphate (ATP) concentration typical of microorganisms undergoing exponential growth *in vitro*. This high concentration (around 10-12 μmol ATP g<sup>-1</sup> Bc) is unchanged irrespective of soil organic matter content and soil management or if readily decomposable substrates, e.g., glucose or plant residues, have recently been given, which can trigger intense microbial activity (Contin et al., 2001; De Nobili et al., 1996). Literature data support the concept of the biomass having, for all practical considerations, throughout the world, an ATP concentration around 10-12 μmol ATP g<sup>-1</sup> Bc (Table 2) (Contin et al., 2001).

Table 2. Microbial biomass ATP concentrations in soils under different management (modified from Contin et al., 2001).

Management	Range of soil organic C concentrations (mg C org kg <sup>-1</sup> soil)	Range of biomass ATP concentrations (μmol ATP g <sup>-1</sup> biomass C)	Mean biomass ATP concentration
Arable	6.8-82.0	6.71-23.95	11.13±2.78
Grassland	12.0-200.0	4.25-28.02	11.59±3.95
Woodland	11.8-399.0	6.25-17.79	10.25±2.97
All	6.8-399.0	4.25-28.02	10.98±3.26

Zero C inputs are quite frequent in cultivated soils, which may be kept fallow for relatively long periods, receiving hardly any C input, until the crop grows again. Under such conditions, the biomass will require energy and the main source will be soil organic matter (Joergensen et al., 1990; Powlson and Johnston, 1994). The resistance of soil microbial biomass and the role of soil organic matter in sustaining microbial biomass at zero C inputs were assessed in two long-term incubation experiments by De Nobili et al. (2006, 2008) on soils from the Broadbalk experiment at Rothamsted (UK). The Broadbalk experiment was established in 1843 to study the effects of inorganic fertilizers and organic manures on the nutrition and yield of winter wheat (Jenkinson, 1991). In 1883, the Stubbed grassland, where trees were not allowed to grow, and the Woodland sections of the Broadbalk experiment were left to revert to wilderness, after wheat was sown. The arable plots were as follows: a plot given 35 t ha<sup>-1</sup> y<sup>-1</sup> farmyard manure since 1843 (FYM), a plot that had not received fertilizers since 1843 (NIL) and two plots receiving the same amount of mineral fertilizers (98 kg N ha<sup>-1</sup> y<sup>-1</sup>) – one receiving mineral fertilizer only (N5PK) and the other where straw had also been incorporated in the soil since 1986 (N5PK+Straw). Soil samples were also collected from three 3 m wide strips left bare as paths to separate the different plots (Paths).

During incubation, Bc (Figure 1) and soil ATP (Table 3) declined markedly. The sharpest decline was in the non-fertilized continuous wheat (NIL) soil and in the paths where biomass decreased by more than 30% during the first 30 d. Percentage losses were larger than those observed in all the other soils which, after 30 d, only declined on average by about 14%. However, after 316 d, Bc had declined in all soils to about 60-70% of the initial value, without significant differences among soils (Table 3). Our expectation that soil microbial biomass of cultivated soils would be better able to survive substrate limitations when the soil had not been excessively depleted of organic matter was only partly verified. Although more biomass survived in soils of larger soil organic matter (SOM) content, abundance of organic C alone was not sufficient to permit the survival of a larger percentage of soil microorganisms.

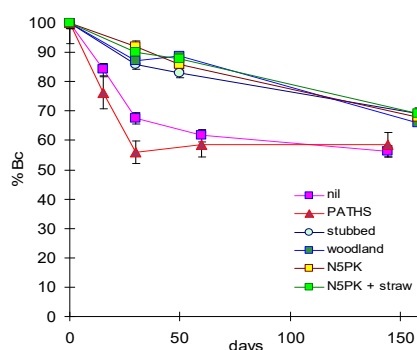


Figure 1. Percentage microbial biomass C decline during incubation of Broadbalk soils at zero C input (see text for acronyms).

Table 3. Bc, ATP and ATP concentration of soil microbial biomass, in selected Broadbalk soils during long-term incubation at zero C inputs (see text for acronyms).

Soil	Incubation time								
	0 days			60 days			158 days		
	Bc ( $\mu\text{g g}^{-1}$ )	ATP ( $\text{nmol g}^{-1}$ )	ATP/Bc ( $\text{nmol g}^{-1}$ )	Bc ( $\mu\text{g g}^{-1}$ )	ATP ( $\text{nmol g}^{-1}$ )	ATP/Bc ( $\text{nmol g}^{-1}$ )	Bc ( $\mu\text{g g}^{-1}$ )	ATP ( $\text{nmol g}^{-1}$ )	ATP/Bc ( $\text{nmol g}^{-1}$ )
Stubbed	1289±37	12.40	9.5	1068±27	9.45	8.8	578±57	7.70	8.6
Woodland	1281±72	13.63	12.4	1135±17	7.58	6.7	748±28	8.84	8.2
FYM	404±18	3.29	8.1	406±21	2.83	7.0	386±25	3.17	8.2
N3PK	241±23	2.46	10.2	185±13	2.21	11.9	193±16	1.72	8.9
N5PK	220±11	2.09	9.5	169±5	1.75	10.3	118±15	1.77	11.9
N5PK+Straw	264±17	3.11	11.7	232±4	2.27	9.8	129±3	2.32	12.6
NIL	151±16	1.41	9.3	102±11	1.16	11.3	85±5	1.01	11.9
Paths	124±12	1.19	9.0	81±16	1.03	12.7	79±9	0.94	11.8
Mean			9.8±1.4			10.4±2.3			10.6±2.0

In soils with low organic C inputs, microbial biomass should either display a metabolic activity similar to that of the high C input soils, if in equilibrium, or a lower C mineralization if adapted to a starvation-survival strategy based on reduced C requirements. On the contrary, specific respiration rates of soil microbial biomass were not initially related to substrate availability, but were much larger in soils that received abnormally low C inputs (NIL and Paths), even compared with intensive monoculture management (Table 4). Paradoxically, this difference was largest during the first day of incubation, when soil respiration was maximum due to utilization of substrates made available by soil aggregate disruption. In fact, the specific respiration rate of the microbial biomass was much larger in depleted soils. These soils maintained a specific respiration ( $1.45\text{-}1.49 \mu\text{g CO}_2\text{-C mg}^{-1} \text{Bc h}^{-1}$ ) rate for up to 50 d of incubation that was much larger than that of the other soils ( $0.71\text{-}0.91 \mu\text{g CO}_2\text{-C mg}^{-1} \text{Bc h}^{-1}$ ). Specific respiration rates measured between 2 and 50 d in the arable soils, excluding the NIL soil, were all very close and in very good agreement with the data of Anderson and Gray (1991) for similar soils under monoculture. However, there was no difference between the specific respiration rate of arable soils under monoculture and the soils of the same experiment which were left to revert to woodland or stubbed grassland, which, according to the report of Anderson and Domsch (1990), should have evolved much less  $\text{CO}_2$  per unit of Bc. This can probably be explained, because the Broadbalk Wilderness soil has neutral pH, whereas woodland soils examined by Anderson and Domsch (1990) were strongly acidic.

Table 4. Specific respiration rate of soil microbial biomass, in selected Broadbalk soils during incubation at 25°C and zero C inputs.

Soil	Biomass specific respiration ( $\mu\text{g CO}_2 \text{mg}^{-1} \text{Bc h}^{-1}$ ) $\times 10^{-4}$				
	1 day	2-50 days	50-158 days	158-218 days	218-316 days
Stubbed	25.2	8.7	3.8	3.3	4.3
Woodland	21.3	7.8	4.4	3.8	3.9
FYM	24.1	9.1	4.1	-	-
N3PK	24.6	8.4	3.91	-	-
N5PK	22.8	11.4	2.9	3.1	1.9
N5PK+Straw	24.7	10.7	4.7	4.2	3.1
NIL	66.7	14.5	7.3	-	-
Paths	79.5	14.6	7.2	4.0	3.9
Mean	39.7±25	10.7±2.9	4.6±1.5	3.5±0.4	3.4±0.9

In soil, the ATP concentration of a rapidly declining microbial biomass does not decrease: the decrease in ATP matches the decrease in Bc so that the ATP concentration of microbial biomass remains constant (Table 3). This had been observed also by Brookes (1997) and by Joergensen et al. (1990), and confirms that ATP measurements at optimum moisture and temperature do reflect microbial biomass size but not biomass activity. The high ATP levels are therefore part of a precise survival strategy which is also adopted by marine oligotrophic microorganisms (Morita, 1988).

### RESISTANCE AND RESILIENCE OF SOIL MICROBIAL BIOMASS TO DRYING AND REWETTING.

Soil drying causes a dramatic cessation of microbial activity, which is readily reversible under natural conditions, but whose effects are not yet completely understood. Drying and rewetting events can induce significant changes in microbial C and N dynamics (Franzluebbers et al., 1996, 2000), but their actual effects on decomposition processes is still controversial (Mamilov and Dilly, 2002). Resistance and resilience of soil microbial biomass to the frequent and diffuse phenomena of soil drying and rewetting is another example of its peculiar functioning. According to De Nobili et al. (2006), varying amounts of ATP can be extracted from recently air-dried soil samples, ranging from about 4.0 to 0.20 nmol ATP g<sup>-1</sup> soil, corresponding to about 10 to 35% of the initial soil ATP concentration before drying (Figure 2a). Proportionately much more ATP remains in soils rich in organic C. Two days after rewetting, soil ATP recovers to between 60-80% of the original amount (Figure 2b). The amount of ATP synthesized in 48 h in the soil that had originally contained the largest amount of ATP and Bc both before and after the drying stage (stubbed soil) was much larger than in the other soils. However, at the same time, this soil displayed the lowest percentage recovery of ATP, and more ATP was actually synthesized per unit of ATP remaining after air-drying in the arable soils. At 7 d after re-wetting, the percentage recovery of ATP ranged from 83 to 100%. Organic matter, however, preserves the viability of soil microorganisms during prolonged storage under dry conditions. In a series of soils stored for up to 80 years, a significant difference was observed after 12 d where, in all FYM soils but the oldest one, the ATP concentration reached about 20% of the original value. The microbial biomass ATP concentration (about 11-14 μmol ATP g<sup>-1</sup> Bc) by 12 d of incubation was the same as in fresh soil. Therefore, a fraction of the microbial biomass that had survived storage (about 20% by 47 years of storage and 10% by 80 years) in the FYM-treated soils appears to be able to resume normal metabolic activity, at least in terms of its high and typical microbial biomass ATP concentration (Contin et al., 2001).

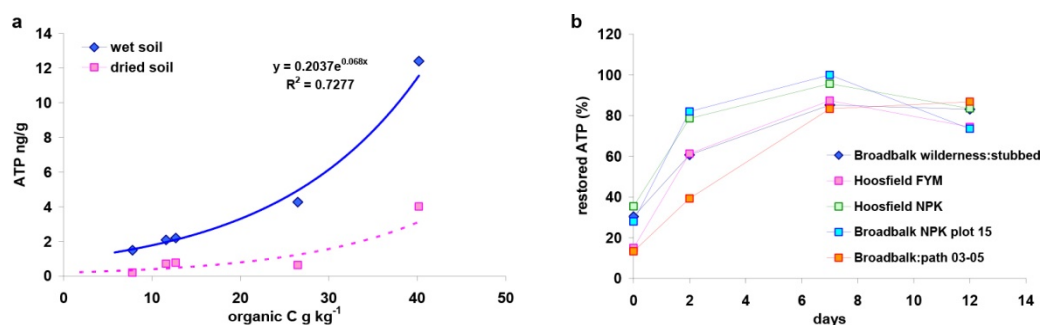


Figure 2. Viable microorganism content evidenced by the soil ATP concentration in soils of various organic C content before and after soil drying (a) and percentage ATP restored (percentage of the amount present in each original wet soil sample) after 12 days from rewetting (b) in Rothamsted long-term experiments.

### QUALITATIVE AND QUANTITATIVE CHANGES IN SOIL ORGANIC MATTER UNDER EXTREME MINERALIZATION CONDITIONS

Qualitative and quantitative changes in humic fractions following long-term soil



incubation may help to assess changes caused by SOM mineralization, leading to loss of soil organic matter. In the experiments described in the previous paragraph, humic acids (HAs) were extracted by a sequential extraction procedure (De Nobili et al., 1999), which allows extraction of biochemically stabilized (free HA) and chemically plus biochemically stabilized SOM (bound HA). Chemical stabilization of SOM occurs as a result of chemical or physico-chemical binding to soil mineral surfaces (Baldock and Skjemstad, 2000). Free and bound HA display distinctively different chemical and spectroscopic features and represent distinct soil C pools with respect to mineralization resistance. De Nobili et al. (1999) found that the bound humic C fraction predominated in arable soils that had reached equilibrium C levels at low C inputs. Free humic substances, which are not bound to soil minerals, decreased substantially during soil incubation at zero C inputs and are therefore preferentially utilized by microorganisms with respect to bound humic substances. Changes in functional groups point to the existence of complex equilibria between the different fractions (De Nobili et al., 1999, 2008). These do not permit a clear understanding of the dynamics of organic C in this compartment; however, this type of fractionation allows us to highlight changes in soil humic fractions, which may be particularly useful to predict SOM modifications as well as effects of measures taken to ameliorate soils or achieve sustainable C levels.

Serramiá et al. (2013) investigated effects of compost addition on soils under conditions that favour intense mineralization. The relative abundance of selected functional groups of free and bound HA extracted from the control and amended soils after 90 and 150 d are given in Figure 3.

The alkyl C chemical-shift region (0-45 ppm) accounted for 30-35% of the total signal intensity. The proportion of N-alkyl + methoxyl C in free HA (15-18%) decreased in the free HA fraction from 90 to 150 d of incubation, coinciding with an increase in the bound fraction, confirming that changes occurred in the structure of free HA before further chemical stabilization in the bound fraction. Aliphatic alcohols in structural polysaccharides resonate between 50 and 110 ppm (Kögel-Knabner, 2002). The signals in this region were much more intense in free HA from amended soils. Signals in the aromatic C region (110-160 ppm) were stronger in HA from the bound C fraction (from 15.4 to 18.5%), than in the free HA (9.2-5.5%), the phenolic group signal of amended soil, being even more clearly distinguishable after 150 d. The Ph-C resonance in amended soils indicated that free HA and to a lesser degree bound HA incorporated altered lignin structures derived from the composts applied (90 d) or released during humification of composting mixtures in the soil (150 d). The region between 160 and 210 ppm, attributed to the resonance of carboxyl-, carbonyl- and amide- C, was the only one that showed a net increase during incubation of soil without compost addition and reflects the intensity of oxidation processes. No significant variations were found between control and amended soils for bound HA. All this highlights the qualitative changes that result from exploitation of SOM as an energy source by soil microorganisms. Addition of compost can counteract the effects of extreme mineralization and maintain substrate qualities of SOM which can be beneficial for soil microorganisms.

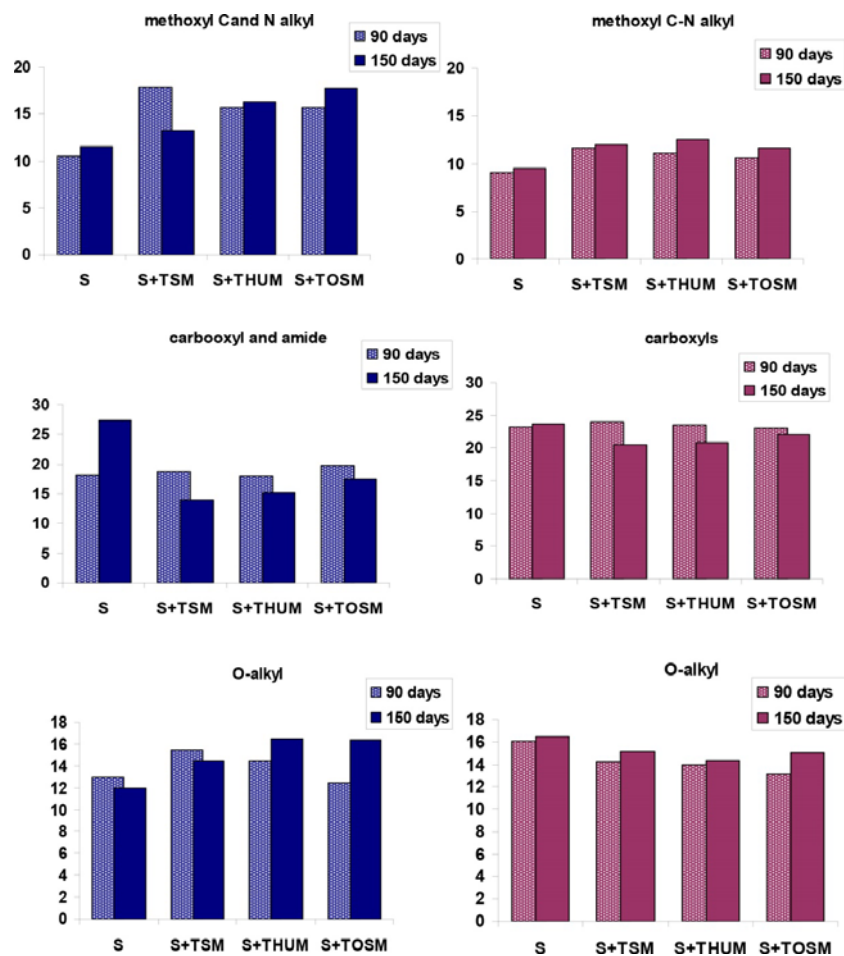


Figure 3. Changes in functional groups of free (left) and bound (right) humic acids during incubation of soil (S) and soil amended with three different types of compost: S+TSM (two-phase olive mill waste (T) + sheep manure); S+THUM (T + horse manure + urea); S+TOSM (T + olive tree prunings + sheep manure).

## CONCLUSIONS

Biological soil properties are those most closely related to issues of soil quality and sustainability. However, soil microbial biomass, albeit being a highly diversified community, displays some strongly conserved characteristics that probably derive from selection and adaptation to common severe and shifting limiting conditions, such as substrate limitation and soil drying, which must be considered in the individuation of indicators related to soil biological properties. A better knowledge of these characteristics would allow us a deeper insight into mechanisms involved in the survival, functioning and resilience of the soil microbial biomass and therefore ultimately on sustainability of biological soil functions. The nutritional status of soil microbial biomass in terms of C inputs is reflected by changes in quantity and quality of humic fractions, particularly those corresponding to materials not bound to mineral surfaces (free fulvic acids and HA). These could therefore represent good descriptors of the intrinsic state of soil organic matter which, together with a combination of biological parameters, can help to identify operational thresholds for the calibration of intensities of practices that may allow implementation of a sustainable soil management.

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