Ecotoxicology and Environmental Safety 129 (2016) 242–249

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Assessing microbial activities in metal contaminated agricultural volcanic soils – An integrative approach

C. Parelho [a,b,*], A.S. Rodrigues [a,c], M.C. Barreto [b,d], N.G.C. Ferreira [e], P. Garcia [a,b]

[a] Department of Biology, University of the Azores, 9501-801 Ponta Delgada, Portugal
[b] cE3c, Centre for Ecology, Evolution and Environmental Changes, and Azorean Biodiversity Group, University of the Azores, 9501-801 Ponta Delgada, Portugal
[c] CVARG, Center of Volcanology and Geological Risks Assessment, University of the Azores, 9501-801 Ponta Delgada, Portugal
[d] Department of Technologic Sciences and Development, University of the Azores, 9501-801 Ponta Delgada, Portugal
[e] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

ARTICLE INFO

Article history:
Received 20 November 2015
Received in revised form
1 March 2016
Accepted 19 March 2016
Available online 6 April 2016

Keywords:
Soil enzyme activities
Soil microbial activities
Andosols
Trace metal
Integrative Biological Response

ABSTRACT

Volcanic soils are unique naturally fertile resources, extensively used for agricultural purposes and with particular physicochemical properties that may result in accumulation of toxic substances, such as trace metals. Trace metal contaminated soils have significant effects on soil microbial activities and hence on soil quality. The aim of this study is to determine the soil microbial responses to metal contamination in volcanic soils under different agricultural land use practices (conventional, traditional and organic), based on a three-tier approach: *Tier* 1 – assess soil microbial activities, *Tier* 2 – link the microbial activity to soil trace metal contamination and, *Tier* 3 – integrate the microbial activity in an effect-based soil index (Integrative Biological Response) to score soil health status in metal contaminated agricultural soils. Our results showed that microbial biomass C levels and soil enzymes activities were decreased in all agricultural soils. Dehydrogenase and β-glucosidase activities, soil basal respiration and microbial biomass C were the most sensitive responses to trace metal soil contamination. The Integrative Biological Response value indicated that soil health was ranked as: organic > traditional > conventional, highlighting the importance of integrative biomarker-based strategies for the development of the trace metal "footprint" in Andosols.

1. Introduction

Soil is a dynamic living resource, vital to ecosystems functioning and represents a unique balance among physical, chemical and biological factors (Shukla and Varma, 2011). Soil microbial activity directly influences the ecosystem stability, fertility and sustainability, being widely accepted that a good level of microbial activity is essential for maintaining soil quality. Natural events, such as volcanism, and anthropogenic activities, such as long-term agricultural practices, continuously affect the quality of soil (Santos et al., 2011; Parelho et al., 2014).

Due to the complexity of soil structure and function, a good soil quality indicator must be integrative, combining a number of measurements into an easily understood and quantitative measure. Since soil enzymes and other biochemical parameters differ in origin, function and location within the soil matrix (Burns, 1982), and respond to different key environmental signals, it would be useful to combine the information they provide into a single numerical value. Complex indexes, such Integrative Biological Response (Beliaeff and Burgeot, 2002) have been developed as effect-based monitoring tools and may be used to integrate soil microbial responses into more simple and realistic stress model, providing a more complete status of soil quality.

The activities of soil enzymes, microbial biomass and soil basal respiration are important indicators of microbial and biochemical processes and functions, because of their role in soil organic matter (SOM) decomposition, carbon sequestration, nutrient cycling and availability (Dick, 1997; Caldwell, 2005). In addition, the efficient soil co-extraction of RNA and DNA makes possible to calculate the RNA:DNA ratio, which can be an important indicator of the metabolic status of soil microbial communities. These microbial activities can be used as indicators of soil quality to monitor soil metal contamination (Niemeyer et al., 2012; Xian et al., 2015) and agricultural soil management practices (Bowles et al., 2014; Pandey et al., 2015). Despite the numerous field

* Corresponding author at: Department of Biology, University of the Azores, Apartado 1422, 9501-801 Ponta Delgada, Azores, Portugal.
E-mail addresses: carolina.pf.parelho@uac.pt (C. Parelho), armindo.s.rodrigues@uac.pt (A.S. Rodrigues), maria.cr.barreto@uac.pt (M.C. Barreto), nuno.ferreira@ciimar.up.pt (N.G.C. Ferreira), patricia.v.garcia@uac.pt (P. Garcia).

http://dx.doi.org/10.1016/j.ecoenv.2016.03.019
0147-6513/

studies demonstrating the adverse effects of metal contamination on soil microbial activities (Kuperman and Carreiro, 1997; Antunes et al., 2011; Niemeyer et al., 2012), there is a lack of knowledge regarding the soil microbial responses to chronic metal contamination when soils are naturally enriched with trace metals and have been extensively used for agricultural purposes, such as in agricultural volcanic soils (Andosols). Due to their volcanic heritage, these soils are naturally enriched with a high range of trace metals (Doelsch et al., 2006; Parelho et al., 2014). In addition, allophanic ash volcanic soils are recognized for their abundance of neoformed amorphous aluminosilicates (such as allophane and imogolite) and organo-mineral compounds (Fontes et al., 2004), that confer unusual properties to soils, such as a very high binding capacity for metals (Sugiyarto, 2013). In the case of volcanic soils intensively exploited for agricultural purposes, the scenario is particularly aggravated, since the use of agrochemicals (pesticides and fertilizers) can contribute to the accumulation of trace metals in soil matrix, causing even higher negative impacts over the soil ecosystem (Parelho et al., 2014).

Within this particular context, the aim of this study is to determine the soil microbial responses and assess soil health status in naturally metal contaminated volcanic soils under different agricultural practices (conventional, traditional and organic farming systems). To achieve this goal, the study was based on a three-tier approach: *Tier* 1 – assess the biological effects of agricultural practices in soil microbial activities (β-glucosidase, acid phosphatase, dehydrogenase, microbial biomass carbon, basal soil respiration, metabolic quotient and RNA:DNA ratio), *Tier* 2 – link the microbial activity to soil trace metal contamination, and *Tier* 3 – integrate the microbial activity in an effect-based soil index (Integrative Biological Response) to score soil health status in trace metal contaminated agricultural soils.

2. Material and methods

2.1. Study area description, geology and soil trace metal loads

The study area corresponds to the Picos Fissural Volcanic System, located in the western half of the island of São Miguel, the largest (744.6 km^2 and 138.551 inhabitants) of the Azores archipelago.

According to Ricardo et al. (1977), two main associations of soil types can be observed in the studied area: (1) thin allophanic soils and thin Andosols, the latter over lava flows, and (2) thin allophanic soils and coarse Regosols, the latter mainly associated to basaltic pyroclastic deposits.

The selected farms (Fig. 1) correspond to the main producers of vegetables in the Island and are located in the same geological complex (Picos Fissural Volcanic System), ensuring the same bedrock and pedological conditions, being only differentiated by the type of agricultural soil management. The selected farms were all evenly distributed across the region of study and located at a similar altitude (50–100 m) to minimize the rainfall variability between sites and rain shadow effects.

Three types of farming systems (conventional, organic and traditional) were selected and compared to a reference soil. In the local context, a conventional system refers to farming practices in which the use of synthetic agrochemicals (both pesticides and fertilizers) is legally framed by European and national guidelines. Organic systems are certified by the European Commission, therefore the use of synthetic agrochemicals is prohibited and soil amendments are confined to organic fertilizers (compost and manure). Traditional practices represent the most common farming system in Azores, where in the past (last 150 years) synthetic agrochemicals were used in an uncontrolled manner and based

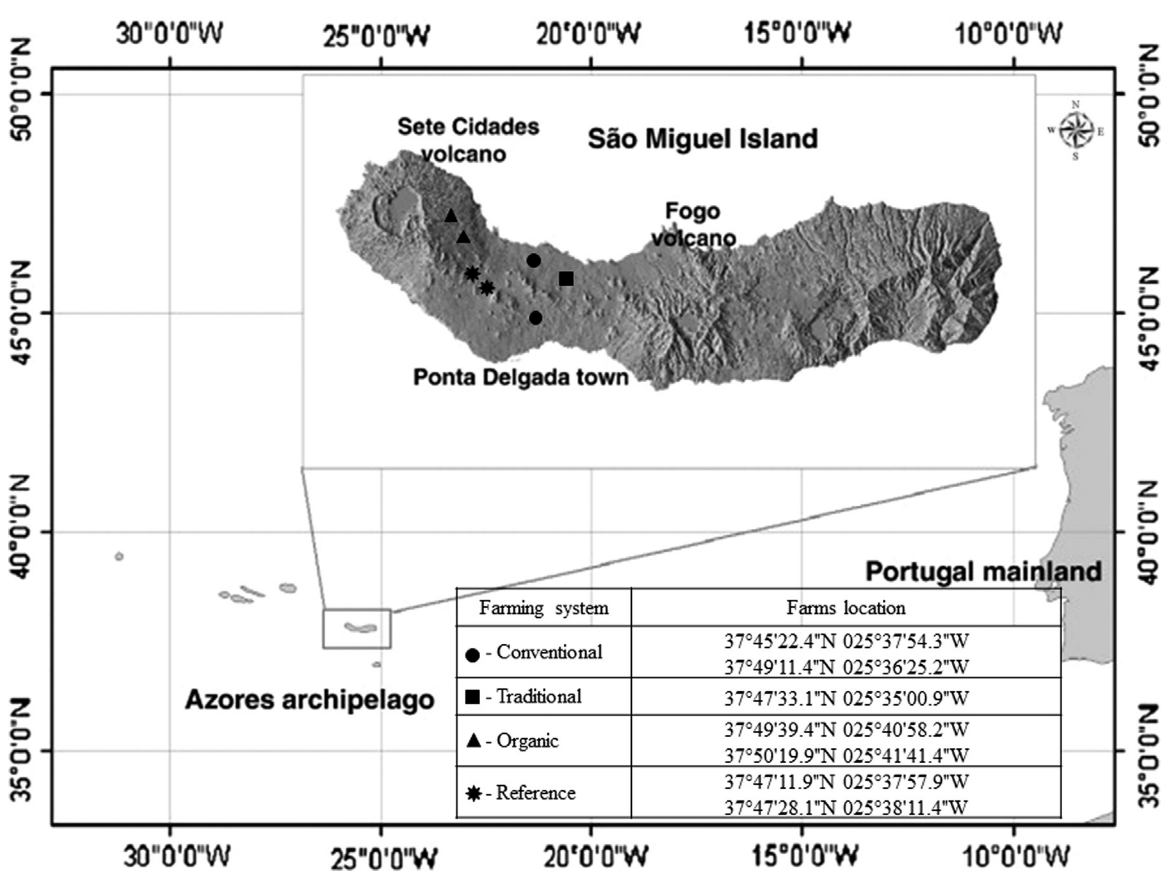

Fig. 1. Location of the Azores archipelago in the North-Atlantic Ocean. Inset: São Miguel Island with farms location (conventional, traditional and organic farming systems) and reference soil. Adapted from Cordeiro et al., 2012.

Table 1
Mean (± SD) concentration of trace metals (mg kg^{-1}, d.w.) in topsoil samples (0–20 cm) from conventional, traditional and organic farming systems and reference soil. Means within each line followed by different letters are significantly different at $p<0.05$ (Tukey test). Mean values (± SD) of soil physicochemical properties are also presented. Adapted from Parelho et al., 2014.

Trace metal (mg kg^{-1})	Conventional soil (n=12)	Traditional soil (n=6)	Organic soil (n=12)	Reference soil (n=6)
Li	13.14 ± 0.26 a	6.82 ± 0.30 b	8.06 ± 0.34 b	3.02 ± 0.14 c
V	61.92 ± 1.38 a	137.83 ± 4.42 b	49.08 ± 4.35 c	80.83 ± 2.00 d
Cr	32.59 ± 0.82 a	59.42 ± 1.26 b	20.13 ± 1.80 c	12.60 ± 0.84 d
Ni	40.23 ± 0.74 a	108.12 ± 3.92 b	27.36 ± 5.19 c	17.87 ± 0.93 c
Cu	81.18 ± 9.08 a	287.17 ± 8.77 b	101.09 ± 7.49 a	13.60 ± 0.96 c
Zn	217.25 ± 12.67 a	187.50 ± 10.16 a	236.83 ± 34.80 a	79.60 ± 4.53 b
As	4.31 ± 0.24 a	7.57 ± 0.50 b	6.31 ± 0.47 b	1.50 ± 0.17 c
Cd	0.44 ± 0.02 a	0.36 ± 0.01 a	0.37 ± 0.03 a	0.16 ± 0.00 b
Pb	34.02 ± 5.40 ab	42.93 ± 1.31 b	43.07 ± 7.30 b	10.70 ± 0.16 a
Electric conductivity (μS cm^{-1})	262.3 ± 162.6	51.5 ± 17.8	65.6 ± 10.2	52.5 ± 9.8
pH (H_2O)	5.8 ± 0.3	6.5 ± 0.l	6.4 ± 0.1	6.4 ± 0.3
Clay-silt content (%)	1.64 ± 0.5	6.2 ± 1.6	7.3 ± 0.8	0.6 ± 0.1

only on local traditional knowledge; however, nowadays the use of agrochemicals is considerably reduced. All selected farms have been explored under the same farming system for at least 10 years. The soil used as reference corresponds to a forest reserve of centennial Japanese cedar (*Cryptomeria japonica*), an area with no historical records or evidence of farming activity. A more detailed farm history with the specific agrochemicals inputs and farming practices is presented in Supplementary Material – Table 1.

The soil trace metal loads of Li, Cr, Ni, Cu, Zn, Cd and Pb considered for this study were previously assessed and described by Parelho et al. (2014) as the priority metals affecting agricultural Andosols in Picos Fissural Volcanic System (Table 1). General soil physicochemical properties and trace metal contents are presented in Table 1 and were characterized previously in a parallel study (Parelho et al., 2014).

2.2. Soil sampling

Surface soil cores (0–20 cm) were collected during June and July 2013, with PVC core barrels. Immediately after collection, soil samples were sieved (< 2 mm) to remove organic debris and larger inorganic fragments, kept in black polyethylene bags and stored at 4 °C, until the next day for analysis. For nucleic acids soil extraction (DNA and RNA), after the sieving, soil samples were directly frozen in liquid nitrogen and kept at −80 °C until further analysis. All of the surfaces that contacted the soil were sterile or had previously been rinsed with a 10% (v/v) bleach solution followed by sterile deionized water to remove any contaminating nucleic acids. A detailed description of the soil sampling scheme in provided in Supplementary material – Fig. 1.

2.3. Soil microbial activities

2.3.1. Enzyme activities

β-glucosidase is an enzyme involved in the C cycle that catalysis the conversion of disaccharides into glucose (Alef and Nannipieri, 1995); it is characteristically useful as a soil quality indicator giving the reflection of past biological activity and capacity of soil to stabilize the SOM (Shukla and Varma, 2011). β-glucosidase activity was determined according to the procedure described by Dick et al. (1996). Results are expressed as μg *p*-nitrofenol h^{-1} g^{-1} dry soil.

Phosphatases are involved in the P cycle and catalyse the hydrolysis of ester–phosphate bonds, leading to the release of phosphate, which can be taken up by plants or microorganisms (Nannipieri et al., 2011). The acid phosphatase activity was determined as described by Dick et al. (1996). Results are expressed as μg *p*-nitrofenol h^{-1} g^{-1} dry soil.

Dehydrogenases are intracellular enzymes participating in the processes of oxidative phosphorylation of microorganisms (Alef and Nannipieri, 1995). The overall dehydrogenase activity of a soil depends on the activities of various dehydrogenases, which are a fundamental part of the enzyme system of all microorganisms (enzymes of the respiratory metabolism, the citrate cycle and N metabolism). Dehydrogenase activity thus serves as an indicator of the microbiological redox systems and may be considered a good measure of microbial oxidative activities in soils (Kumar et al., 2013). The dehydrogenase activity was measured using 2,3,5-triphenyltetrazolium chloride 1% as substrate, according to Rossel et al. (1997) method. Results are expressed as μg triphenyl formazan (TPF) h^{-1} g^{-1} dry soil.

2.3.2. Nucleic acid extraction

RNA and DNA were co-extracted using the RNA PowerSoil Total RNA Isolation Kit and DNA Elution Accessory Kit (MoBio Laboratories, Carlsbad, CA, USA). DNA and RNA were quantified with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The RNA to DNA ratio (RNA:DNA) was calculated as the ratio of mean RNA (μg g^{-1} dry soil) to mean DNA (μg g^{-1} dry soil) of 3 soil samples per farm.

2.3.3. Other biochemical activities

Soil basal respiration (SBR) was determined by quantifying the carbon dioxide (CO_2) released in the process of microbial respiration during 8 days of incubation at 25 °C, according to Jenkinson and Powlson (1976) method. Results are expressed as mg C–CO_2 h^{-1} kg^{-1} dry soil.

To estimate the microbial biomass C (MB-C), the chloroform fumigation–incubation method was applied (Vance et al., 1987). Total organic C content in the soil extracts was measured with a dichromate digestion method (Vance et al., 1987). The MB-C was calculated as 0.33 times the difference in extractable organic C between the fumigated and unfumigated soils (Sparling and West, 1988). Results are expressed as mg MB-C h^{-1} kg^{-1} dry soil.

The metabolic quotient (qCO_2), a measure of the steady-state (basal) energy required to maintain the microbial biomass, which is analogous to maintenance energy requirements (Pirt, 1965), was calculated as the ratio of basal respiration to microbial biomass C (Anderson and Domsch, 1990).

2.4. Integrative biological response

Soil microbial activities described in Section 2.3 were combined into the Integrative Biological Response index (IBR) described by Beliaeff and Burgeot (2002). Briefly for each biomarker (soil microbial activity) the general mean (m) and the standard deviation

(s) was calculated, followed by a standardization to obtain Y, where $Y=(X-m)/s$, and X is the mean value for the biomarker at a given sampling site. Then Z was calculated using $Z=-Y$ or $Z=Y$, in the case of a biological effect corresponding respectively to an inhibition or an activation. Regarding the biological effect, β-glucosidase, acid phosphatase, dehydrogenase, SBR, MB-C and qCO_2 were considered to decrease within adverse conditions, while RNA:DNA ratio was assumed to increase (Miliou et al., 1998).

The score (S) was calculated by $S=Z+|Min|$, where $S \geq 0$ and $|Min|$ is the absolute value for the minimum value for all calculated Y in a given biomarker at all measurements made. Star plots were then used to display score results (S) and to calculate the Integrative Biological Response (IBR) as:

$$IBR=\sum_{i=1}^{n} A_i$$

$$A_i=\frac{S_i}{2}\sin\beta\left(S_i\cos\beta+S_{i+1}\sin\beta\right)$$

$$\beta=\tan^{-1}\left(\frac{S_{i+1}\sin\alpha}{S_i-S_{i+1}\cos\alpha}\right)$$

where S_i and S_{i+1} are two consecutive clockwise scores (radius coordinates) of a given star plot; A_i corresponds to the area the connecting two scores; n the number of biomarkers used for calculations; and $\alpha=2\pi/n$.

2.5. Statistical analysis

Analysis of variance (ANOVA) was used to compare soil microbial activities from all soil matrices. When ANOVA showed significant differences ($p<0.05$) between data sets, paired comparisons of each mean using Tukey HSD test was performed. To associate the soil microbial responses to soil trace metal contamination, non-parametric correlations (Spearman correlation, $p \leq 0.05$) were used. All statistical analyses were conducted using SPSS 21.0 for Windows.

3. Results

3.1. Soil microbial activities

In conventional and traditional farming systems, where the soils are under the most intense agricultural practices, the values of the enzyme activities were always lower than in reference soil: the enzyme activity in conventional farming soils was on average 63% lower, whereas in traditional farming soils the activity was decreased by 40%. The mean values of enzyme activities for organic farming soils were generally similar to those corresponding to the traditional farming, whereas the differences were more acute between conventional farming and the former (Table 2).

The β-glucosidase activity, a C cycle enzyme, in soils under long-term conventional farming practices (mean value 74.034 µg p-nitrofenol h^{-1} g^{-1} dry soil) was clearly the lowest, differing significantly ($p<0.05$) from all the other; in traditional and organic farming soils, β-glucosidase activity was similar and slightly lower than the mean value obtained for the reference soil (Table 2).

In all soils under long-term agricultural practices, the activity of the acid phosphatase enzyme was significantly lower than in reference soil ($p<0.05$, Table 2), accomplishing a significant reduction of about 34%.

The oxidative degradation of SOM (dehydrogenase activity) was lower in conventional farming soils when compared to the remaining soils, although not differing significantly from traditional farming soils (Table 2). Soil basal respiration and MB-C presented significantly lower values in agricultural soils when compared to the reference soil (Table 2). Contrary to expected, in agricultural managed soils the qCO_2 and RNA:DNA ratio presented no significant differences when compared to the reference soil (Table 2). Despite not being statistically different, the RNA:DNA ratio in conventional farming was higher ($2.7\times$) than in reference, traditional ($2.3\times$) and organic ($1.4\times$) soils.

3.2. Microbial activities and soil trace metal contamination

Among the studied enzyme activities, dehydrogenase and β-glucosidase were the most sensitive to soil trace metal contamination. Strong negative correlations were found between dehydrogenase activity and Li ($r_s(36)=-0.540$, $p=0.001$) and Zn ($r_s(36)=-0.668$, $p<0.001$), whereas moderate negative correlations were obtained for Cr ($r_s(36)=-0.355$, $p=0.0331$) and Cd ($r_s(36)=-0.330$, $p=0.05$). Strong negative correlations were found between β-glucosidase activity and Li ($r_s(36)=-0.845$, $p<0.001$) and Cd ($r_s(36)=-0.514$, $p<0.001$), while moderate correlations were observed with Cr ($r_s(36)=-0.402$, $p=0.015$) and Ni ($r_s(36)=-0.417$, $p=0.012$). No significant correlations were obtained between acid phosphatase and the studied trace metals.

Results showed that soil basal respiration was highly sensitive to the presence of most of the studied soil trace metals. Indeed, strong negative correlations were found between SBR and Li ($r_s(36)=-0.777$, $p<0.001$), Cr ($r_s(36)=-0.518$, $p=0.001$) and Cd ($r_s(36)=-0.594$, $p<0.001$), while moderate correlations were observed with Ni ($r_s(36)=-0.385$, $p=0.02$) and Zn ($r_s(36)=-0.385$, $p=0.02$). The MB-C also revealed to be sensitive to soil trace metals, being observed moderate negative correlations with Li ($r_s(36)=-0.459$, $p=0.005$), Cr ($r_s(36)=-0.400$, $p=0.016$), Cu ($r_s(36)=-0.448$, $p=0.006$) and Zn ($r_s(36)=-0.359$, $p=0.031$). A positive correlation was observed between the RNA:DNA ratio and Cd ($r_s(20)=0.519$, $p=0.019$). No significant correlations were obtained between qCO_2 and the studied trace metals.

Table 2
Mean values ($\pm$SD) of soil microbial activities in topsoil samples (0–20 cm) from conventional, traditional and organic farming systems and reference soil. Means within each line followed by different letters are significantly different at $p<0.05$ (Tukey test).

Soil microbial activities	Conventional soil ($n=12$)	Traditional soil ($n=6$)	Organic soil ($n=12$)	Reference soil ($n=6$)
β-glucosidase (µg p-nitrofenol h^{-1} g^{-1})	74.034 ± 26.900 a	205.049 ± 43.527 b	192.542 ± 84.330 b	249.344 ± 53.362 b
Acid phosphatase (µg p-nitrofenol h^{-1} g^{1})	209.063 ± 9.928 a	243.169 ± 8.156 a	228.457 ± 63.474 a	345.700 ± 81.866 b
Dehydrogenase (µg TPF h^{-1} g^{-1})	0.156 ± 0.073 a	0.204 ± 0.028 ab	0.342 ± 0.183 b	0.701 ± 0.131 c
RNA:DNA ratio	0.460 ± 0.244	0.397 ± 0.075	0.243 ± 0.160	0.173 ± 0.015
SBR (mg C-CO_2 h^{-1} kg^{-1})	0.529 ± 0.247 a	0.773 ± 0.416 a	0.846 ± 0.294 a	3.033 ± 0.768 b
MB-C (mg MB-C h^{-1} kg^{-1})	28.633 ± 17.746 a	20.275 ± 5.525 a	51.862 ± 46.167 ab	86.063 ± 41.013 b
qCO_2	0.023 ± 0.030	0.018 ± 0.023	0.029 ± 0.032	0.025 ± 0.006

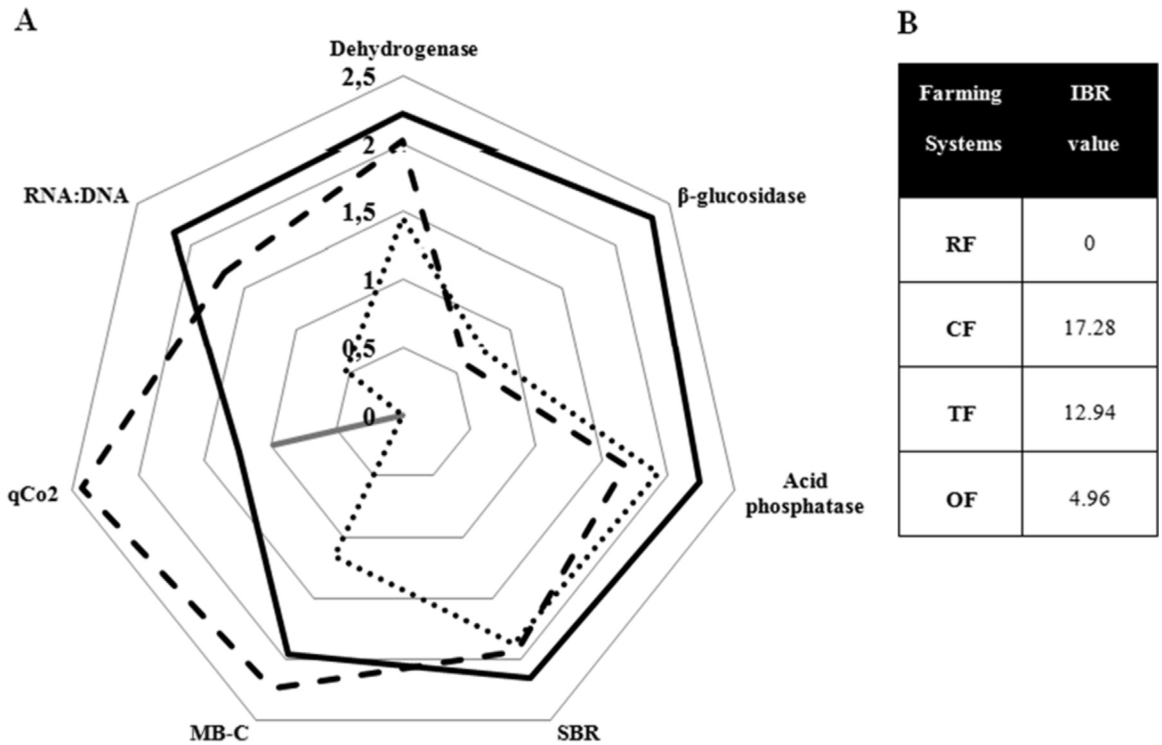

Fig. 2. Biological response score star plot (A) and Integrative Biological Response (IBR) (B) in conventional (black line, CF), traditional (dashed line, TF) and organic (dotted line, OF) farming systems and reference soil (gray line, RF).

3.3. Integrative biological response and soil health scoring

Seven microbial activities (β-glucosidase, acid phosphatase, dehydrogenase, SBR, MB-C, qCO_2 and RNA:DNA ratio) are represented in the star plot (Fig. 2A). According to the Integrative Biological Response index (better and worse scores are respectively represented by lower or higher values) the soil microbial activity is gradually impacted by farming practices (reference < organic < traditional < conventional). Overall, conventional farming soils were the most affected (higher IBR values; Fig. 2B), with the highest IBR scores for β-glucosidase, acid phosphatase, dehydrogenase, SBR and RNA:DNA ratio (Fig. 2A). Traditional farming soils had the highest IBR scores for MB-C and qCO_2. The reference soil always presented a zero score ($A=0$) for all the biological responses except for the qCO_2, thus showing an overall IBR value of zero (Fig. 2A,B).

4. Discussion

Long-term agricultural soil management, such as crop rotation, tillage, fertilizers, compost, manure or pesticides applications, greatly affects soil microbial parameters (Reeve et al., 2010). In spite of the vast information related to a better understanding soil microbial communities shifts to metal contaminated soils, the link between soil microbial activities and soils naturally enriched with trace metals needs to be untangled, particularly in long term managed agricultural Andosols. Although our results represent a single time point study (and thus temporal variations in soil microbial activities cannot be considered), previous studies have shown that long-term patterns within soil microbial communities are expected to remain generally intact (e.g. Suleiman et al., 2013).

Soil enzymes have been recommended as standard biochemical indicators to assess the quality of metal-polluted soils; however, it is important to differentiate between extracellular and intracellular enzymes (Zhang et al., 2013). The dehydrogenase activity, which is only present in viable cells and essentially depends on the metabolic status of the soil biota, may be considered a direct measure of soil overall microbial activity, while β-glucosidase and phosphatase activity can also occur extracellularly. In our study, the β-glucosidase and dehydrogenase activities were more sensitive to conventional agricultural practices and trace metal contamination, than the acid phosphatase activity. Other studies have also reported a similar pattern of enzyme activities in metal contaminated soils and related it to the higher sensitivity of intracellular enzymes and C cycle involved enzymes to metal stress (for example, Zhang et al. (2013) and Burges et al. (2015).

According to our results, conventional and traditional farming systems, where lesser inputs of organic materials are undertaken, have lower amounts of MB-C and decreased SBR. Soil microbial biomass is an important labile pool of SOM that can be negatively affected by land use changes (Haghighi et al., 2010), SOM quantity and quality (Ludwig et al., 2015) and, intensity of agricultural practices (Panettieri et al., 2014). In addition, the lower MB-C in agricultural soils may not only be related to a lower carbon content or substrate in these agroecosystems, but also to the low soil aggregation and aggregate stability due to soil management practices, such as intensive tillage. This relation was previously demonstrated by Wei et al. (2013) that associated the loss of soil organic carbon stocks to the decrease of macro aggregate fractions during the conversion of natural forest to cropland soils. The lower values of MB-C observed in agricultural soils was also reflected in lower SBR values, although similar soil microbial maintenance energy requirements (qCO_2) were observed in all farming systems. This suggests that a change in the soil microbial community size (given by MB-C), structure and composition might have occurred in the soils under long-term agricultural practices and, that these microbial communities have similar maintenance energy requirements than those from the reference soil. Several other studies (Barrios, 2007; Rejeb et al., 2014) have also reported the resilience of soil microbial communities to the chronic stress imposed by agricultural practices and agrochemicals use, as a key process to support long-term agricultural sustainability.

Among all the microbial activities tested, the dehydrogenase and β-glucosidase activities, SBR and MB-C were the most sensitive parameters to soil trace metal loads. The high applicability of dehydrogenase and β-glucosidase activities for ecotoxicological testing soil trace metal effects on soil microbial communities was also suggested by previous authors (i.e., Rossel et al., 1997; Burges et al., 2015).

The significant negative correlations between SBR and MB-C and several trace metals, showed that long-term soil microbial communities exposure to metal stress (even at low levels) is associated with microbial biomass decrease, and therefore, tolerance and shifts in the community structure are expected to compensate the loss of more sensitive populations, as reported in previous studies (Kızılkaya et al., 2004; Azarbad et al., 2015). There is considerable amount of evidence that a decrease in soil microbial biomass and soil respiration can occur as a result of long-term exposure to trace metal contamination from past application of sewage sludge, continuous use of animal manures highly enriched with Cu, Zn and Cd (e.g. Kätterer et al., 2014) and chronic application of Cu-containing fungicides (e.g. Wakelin et al., 2014). In fact, the use of organic fertilizers as soil amendments is a cross practice to all the studied farming systems and probably is the main source of Cu, Zn and Cd entering these agricultural Andosols. Burges et al. (2015), in a study that evaluated the impact of multimetal (Cd, Pb, Cu, Zn) pollution events in a variety of soil microbial activity parameters, also found that those metals were associated to a decrease in soil enzyme activities and biomass and, that such contamination resulted in a dramatic increase in Cu and Pb relative bioavailability.

The Integrative Biological Response values showed that soil microbial activities were severely compromised in soils under conventional practices, where an increased stress was observed and, to a lesser extent, in traditional followed by organic farming practices. In the local context, conventional practices differ from the remaining by the use of synthetic fertilizers and pesticides. The observed high loads of Li in these soils, results from the continuing application of carbamate insecticides, with lithium pefluorooctane as pesticides-polymer systems (Parelho et al., 2014). The soil microbial activities that presented a higher IBR score under conventional practices were dehydrogenase, β-glucosidase, acid phosphatase, SBR and RNA:DNA ratio. All of them, except RNA: DNA ratio and acid phosphatase, are negatively correlated with Li (dehydrogenase, β-glucosidase, SBR and MB-C) and Zn (dehydrogenase, SBR and MB-C). Soil microbial communities are key organisms for providing "supporting" ecosystem services. Through their activity they contribute to soil-based delivery process (nutrient capture and cycling; organic matter input decomposition; soil organic matter dynamics and soil structure maintenance), which are necessary to the production of the other ecosystem services (Kibblewhite et al., 2008). Our results revealed that, under conventional practices, the local soil microbial communities activities are depressed (IBR value = 17.28) probably due to the higher concentration of Li in the soil (13.14 mg kg^{-1}), suggesting that the ecosystem derived services can be compromised in these farming systems.

The studied traditional farming soils have been handled by traditional methods since the 19th century, where agrochemicals (both fertilizers and pesticides) were frequently used without official restrictions. According to Parelho et al. (2014), the observed high trace metal loads in these farming systems (Cr and Ni) is mainly associated with a past and uncontrolled exposure to agrochemicals. The most contributing biomarkers for traditional farming were dehydrogenase, MB-C and qCO_2. Although qCO_2 was not correlated with local loads of Cr and Ni, MB-C and dehydrogenase were moderately associated with Cr soil loads. Our results are in accordance with Gonçalves et al. (2014) whom concluded that long-term and intensive soil amendment with sludge (40 t ha^{-1}) increases Cr soil loads up to critical levels (30 mg kg^{-1}), causing a significant decrease in the soil microbial biomass. In fact, for the studied traditional farming soils were observed the highest Cr loads (59.42 mg kg^{-1}) and the lowest soil microbial biomass (20.275 mg MB-C h^{-1} kg^{-1}). Due to the high persistence of Cr and Ni in soils (Kabata-Pendias and Mukherjee, 2007), their present-day concentrations in the studied traditional farming soils may be associated to the observed soil microbial effects. Although in a much lesser extent, soil microbial activity was also depressed in organic farming systems. The acid phosphatase activity and SBR were the parameters that mostly contributed to the IBR value in organic farming soils, although only the SBR response pattern was associated with soil trace metal contents. The multi-metallic contamination of agricultural Andosols with Cu, Zn, Cd and Pb is of particular concern in organic farming soils, where soil amendments are confined to organic fertilizers (compost and manure), since according to several studies (e.g. Kätterer et al., 2014) chronic organic soils amendments are associated with the high loads of these trace metals in soils.

According to recent studies, a higher diversity of soil microbes does not necessarily increase the functional stability of the ecosystem, since ecological functions of different species may overlap (functional redundancy) and therefore influence the stability-diversity relationship (Lupatini et al., 2013; Orr et al., 2015). These studies highlight that coupling between functioning and composition of bacterial communities is not necessarily correlated, reinforcing the greater sensitivity of the soil ecosystem functionality than of the community structure. Therefore, we can assume that the observed soil microbial activities represent the "real" response of microbial communities to metal contamination in volcanic soils under different agricultural land use practices.

The application of the biomarker-based procedures has been widely used within environmental risk assessment procedures. However, the incorporation of biomarkers into regulatory legislation for soil quality programs has generally been lacking. The regulatory frameworks for soil quality still rely on biological, chemical and physical indicators as stand-alone decision criteria, like the approach adopted by the European Commission in 2006 for the Thematic Strategy for Soil Protection (TSSP). The objective of TSSP is to protect the soil while using it sustainably and assumes the erosion, declining organic matter, contamination, compaction, salinization, loss in biodiversity, soil sealing, landslides and flooding as the main threats to soil. Therefore, despite TSSP contribution to the knowledge of the physical, chemical and biological status of soil in Europe, an integrative conceptual framework is still lacking to assess soil health globally. The use of integrated biomarker-based models provides comprehensive information about the biological effects of pollution in organisms (Beliaeff and Burgeot, 2002) and may therefore serve as useful tools for environmental managers. Among such, the IBR index has mainly been applied as an effective monitoring tool to assess quality of aquatic ecosystems. Nevertheless, more recently, it has also been applied in earthworms exposed *ex situ* to soils from abandoned mines exhibiting high levels of metals and allowed the discrimination of metalliferous soils with different health status (Asensio et al., 2013). To our knowledge, this study is the first to use IBR index to assess soil health status based on soil microbial activities.

In this study, the IBR revealed to be a suitable index to condense the whole set of soil microbial activities into a single numeric value (IBR score), enabling an integrative measurement of the combined effect of soil management practices and trace metal soil contamination on soil microbial functionality in the studied agricultural volcanic soils. Besides the regional suitability, to globally apply the IBR index to score soil health status, scientific community should be able to define reference values for soil microbial activities, to express the value as a percentage of variation from this reference value. Thereby, an absolute scale of IBR variation could be established and each IBR calculation would be independent of the set of site considered. Thus, studies focusing on the natural variations of soil microbial activities and aiming to understand, besides contaminants, the environmental variables and physiological status that influence soil microbial responses need to be developed, as previously suggested by several authors (Hagger et al., 2006; Xuereb et al., 2009).

5. Conclusion

This study showed that different agricultural practices in Andosols affected the soil microbial activities by decreasing the abundance of microbial biomass (decreased MB-C) and enzyme activity of microorganisms involved in organic matter decomposition and nutrient cycling, regardless of soil microbial maintenance energy requirements (qCO_2) being similar to that of reference soils.

The observed pattern of soil microbial responses reflects the disturbance and stress that agricultural practices cause, as a result of the progressive accumulation of trace metals in soil matrix and the decrease of SOM quantity and quality in the studied farming systems. In this study trace metal soil loads were used as an indirect measure of agrochemicals application on the studied volcanic farming soils, under the assumed premise that greater exposure to agrochemicals is reflected in a higher trace metal soil accumulation. In this sense, global differences in soil microbial responses observed between farming systems can be assumed as the "real" catabolic and biomass measure of soils, a net result of the joint-action of geogenic (volcanism) and anthropogenic stressors (long-term agricultural practices), rather than the effect of a single factor.

The obtained results support the soil microbial microbiological toolbox as a simple, cost effective and science based biological indicator of trace metal contamination in agricultural volcanic soils, highlighting the importance of using integrative biomarker-based strategies for the development of the trace metal "footprint" in Andosols.

Taking in account the ecological role of soil microbial communities, the soil health status seems to be increasingly compromised in the studied farming systems (organic < traditional < conventional), raising an underlying question that remains to untangle: what is the stress limit that agricultural practices and trace metal soil contamination can pose to soil microbial communities before seriously damaging the soil sustainability? With this purpose, the next challenge embraced by the authors includes cutting-edge multi-omic approaches to characterize the soil microbiome and give a more complete picture of soil as a biological system.

Acknowledgments

The authors are grateful for the financial support of Fundo Regional da Ciência e Tecnologia (ref. M3.1.2/F/048/2011), Fundação Luso Americana para o Desenvolvimento (ref. 59/12), and for the collaboration of Direção Regional da Agricultura e Desenvolvimento Rural. The authors also thank Paulo Melo for soil sampling assistance.

This research was partially supported by the Strategic Funding UID/Multi/04423/2013 through national funds provided by FCT – Foundation for Science and Technology and European Regional Development Fund (ERDF), in the framework of the program PT2020.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2016.03.019.

References

Alef, K., Nannipieri, P., 1995. Methods in Applied Soil Microbiology & Biochemistry. Academic Press, London.
Anderson, T.H., Domsch, K.H., 1990. Application of eco-physiological quotients (qCO_2 and Dq) on microbial biomasses from soils of different cropping histories. Soil Biol. Biochem. 22 (2), 251–255.
Antunes, S.C., Pereira, R., Marques, S.M., Castro, B.B., Gonçalves, F., 2011. Impaired microbial activity caused by metal pollution: a field study in a deactivated uranium mining area. Sci. Total Environ. 410–411, 87–95. http://dx.doi.org/10.1016/j.scitotenv.2011.09.003.
Asensio, V., Rodríguez-Ruiz, A., Garmendia, L., Andre, J., Kille, P., Morgan, A.J., Soto, M., Marigómez, I., 2013. Towards an integrative soil health assessment strategy: a three tier (integrative biomarker response) approach with *Eisenia fetida* applied to soils subjected to chronic metal pollution. Sci. Total Environ. 442, 344–365. http://dx.doi.org/10.1016/j.scitotenv.2012.09.048.
Azarbad, H., Niklińska, M., Laskowski, R., van Straalen, N.M., van Gestel, C.A., Zhou, J., He, Z., Wen, C., Röling, W.F.M., 2015. Microbial community composition and functions are resilient to metal pollution along two forest soil gradients. FEMS Microbiol. Ecol. 91 (1), 1–11. http://dx.doi.org/10.1093/femsec/fiu003.
Barrios, E., 2007. Soil biota, ecosystem services and land productivity. Ecol. Econ. 64 (2), 269–285. http://dx.doi.org/10.1016/j.ecolecon.2007.03.004.
Beliaeff, B., Burgeot, T., 2002. Integrated biomarker response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem. 21 (6), 1316–1322. http://dx.doi.org/10.1002/etc.5620210629.
Bowles, T.M., Acosta-Martínez, V., Calderón, F., Jackson, L.E., 2014. Soil enzyme activities, microbial communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-managed agricultural landscape. Soil Biol. Biochem. 68, 252–262. http://dx.doi.org/10.1016/j.soilbio.2013.10.004.
Burges, A., Epelde, L., Garbisu, C., 2015. Impact of repeated single-metal and multi-metal pollution events on soil quality. Chemosphere 120, 8–15. http://dx.doi.org/10.1016/j.chemosphere.2014.05.037.
Burns, R.G., 1982. Enzyme activity in soil: location and a possible role in microbial ecology. Soil Biol. Biochem. 14 (5), 423–427.
Caldwell, B.A., 2005. Enzyme activities as a component of soil biodiversity: a review. Pedobiologia 49, 637–644. http://dx.doi.org/10.1016/j.pedobi.2005.06.003.
Cordeiro, S., Coutinho, R., Cruz, J.V., 2012. Fluoride content in drinking water supply in São Miguel volcanic island (Azores, Portugal). Sci. Total Environ. 432, 23–36. http://dx.doi.org/10.1016/j.scitotenv.2012.05.070.
Dick, R.P., 1997. Soil enzyme activities as integrative indicators of soil health. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. (Eds.), Biological Indicators of Soil Health. CAB International, New York, pp. 121–156.
Dick, R.P., Breakwill, D., Turc, R., 1996. Soil enzyme activities and biodiversity measurements as integrating biological indicators. In: Doran, J.W., Jones, A.J. (Eds.), Handbook of Methods for Assessment of Soil Quality. Soil Science Society America, Madison, USA, pp. 247–272.
Doelsch, E., Kerchov, V.V., Macary, H.S., 2006. Heavy metal content in soils of Réunion (Indian Ocean). Geoderma. 134, 119–134. http://dx.doi.org/10.1016/j.geoderma.2005.09.003.
Fontes, J., Gonçalves, M.C., Pereira, L.S., 2004. Andosols of Terceira, Azores: measurement and significance of soil hydraulic properties. Catena 56 (1–3), 145–154. http://dx.doi.org/10.1016/j.catena.2003.10.008.
Gonçalves, I.C.R., Araújo, A.S.F., Leal, L.A.P., Melo, W.J., 2014. Soil microbial biomass after two years of the consecutive application of composted tannery sludge. Acta Sci. Agron. 36 (1), 35–41. http://dx.doi.org/10.4025/actasciagron.v36i1.17160.
Hagger, J.A., Jones, M.B., LeonardD.R., M.B., Owen, R., Galloway, T.S., 2006. Biomarkers and integrated environmental risk assessment: are there more questions than answers? Integr. Environ. Assess. Manag. 2 (4), 312–329. http://dx.doi.org/10.1002/ieam.5630020403.
Haghighi, F., Gorji, M., Shorafa, M.A., 2010. Study of the effects of land use changes on soil physical properties and organic matter. Land Degrad. Dev. 21, 496–502. http://dx.doi.org/10.1002/Ldr.999.
Jenkinson, D.S., Powlson, D.S., 1976. The effects of biocidal treatments on metabolism in soil–V: a method for measuring soil biomass. Soil Biol. Biochem. 8 (3), 209–213. http://dx.doi.org/10.1016/0038-0717(76)90005-5.
Kabata-Pendias, A., Mukherjee, A., 2007. Trace Elements From Soil to Human. Springer, Berlinhttp://dx.doi.org/10.1007/978-3-540-32714-1.
Kätterer, T., Börjesson, G., Kirchmann, H., 2014. Changes in organic carbon in topsoil and subsoil and microbial community composition caused by repeated

additions of organic amendments and N fertilisation in a long-term field experiment in Sweden. Agric. Ecosyst. Environ. 189, 110–118. http://dx.doi.org/10.1016/j.agee.2014.03.025.

Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363 (1492), 685–701. http://dx.doi.org/10.1098/rstb.2007.2178.

Kızılkaya, R., Aşkın, T., Bayraklı, B., Sağlam, M., 2004. Microbiological characteristics of soils contaminated with heavy metals. Eur. J. Soil Biol. 40, 95–102. http://dx.doi.org/10.1016/j.ejsobi.2004.10.002.

Kumar, S., Chaudhuri, S., Maiti, S.K., 2013. Soil dehydrogenase enzyme activity in natural and mine soil – a review. Middle-East J. Sci. Res. 13 (7), 898–906. http://dx.doi.org/10.5829/idosi.mejsr.2013.13.7.2801.

Kuperman, R., Carreiro, M., 1997. Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. Soil Biol. Biochem. 29 (2), 179–190. http://dx.doi.org/10.1016/S0038-0717(96)00297-0.

Ludwig, M., Achtenhagen, J., Miltner, A., Eckhardt, K., Leinweber, P., 2015. Microbial contribution to SOM quantity and quality in density fractions of temperate arable soils. Soil Biol. Biochem. 81, 311–322. http://dx.doi.org/10.1016/j.soilbio.2014.12.002.

Lupatini, M., Suleiman, A.K.A., Jacques, R.J.S., Antoniolli, Z.I., Kuramae, E.E., de Oliveira Camargo, F.A., Roesch, L.F., 2013. Soil-borne bacterial structure and diversity does not reflect community activity in Pampa biome. PLoS One 8 (10), 1–9. http://dx.doi.org/10.1371/journal.pone.0076465.

Miliou, H., Zaboukas, N., Moraitou-Apostolopoulou, M., 1998. Biochemical composition, growth, and survival of the guppy, Poecilia reticulata, during chronic sublethal exposure to cadmium. Arch. Environ. Contan. Toxicol. 35, 58–63. http://dx.doi.org/10.1007/s002449900349.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of phosphatase enzymes in soil. In: Bunemann, E.K., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action. Soil Biology 26. Springer, Berlin, pp. 215–243. http://dx.doi.org/10.1007/978-3-642-15271-9_9.

Niemeyer, J.C., Lolata, G.B., de Carvalho, G.M., Da Silva, E.M., Sousa, J.P., Nogueira, M. A., 2012. Microbial indicators of soil health as tools for ecological risk assessment of a metal contaminated site in Brazil. Appl. Soil Ecol. 59, 96–105. http://dx.doi.org/10.1016/j.apsoil.2012.03.019.

Orr, C.H., Stewart, C.J., Leifert, C., Cooper, J.M., Cummings, S.P., 2015. Effect of crop management and sample year on abundance of soil bacterial communities in organic and conventional cropping systems. J. Appl. Microbiol. 119, 208–210. http://dx.doi.org/10.1111/jam.12822.

Pandey, D., Agrawal, M., Bohra, J.S., 2015. Assessment of soil quality under different tillage practices during wheat cultivation: soil enzymes and microbial biomass. Chem. Ecol. 31 (6), 510–523. http://dx.doi.org/10.1080/02757540.2015.1029462.

Panettieri, M., Knicker, H., Murillo, J.M., Madejón, E., Hatcher, P.G., 2014. Soil organic matter degradation in an agricultural chronosequence under different tillage regimes evaluated by organic matter pools, enzymatic activities and CPMAS ^{13}C NMR. Soil Biol. Biochem. 78, 170–181. http://dx.doi.org/10.1016/j.soilbio.2014.07.021.

Parelho, C., Rodrigues, A.S., Cruz, J.V., Garcia, P., 2014. Linking trace metals and agricultural land use in volcanic soils – a multivariate approach. Sci. Total Environ. 496, 241–247. http://dx.doi.org/10.1016/j.scitotenv.2014.07.053.

Pirt, S.J., 1965. The maintenance energy of bacteria in growing cultures. Proc. R. Soc. Lond. B Biol. 163 (991), 224–231. http://dx.doi.org/10.1098/rspb.1965.0069.

Reeve, J.R., Schadt, C.W., Carpenter-Boggs, L., Kang, S., Zhou, J., Reganold, J.P., 2010. Effects of soil type and farm management on soil ecological functional genes and microbial activities. ISME J. 4 (9), 1099–1107. http://dx.doi.org/10.1038/ismej.2010.42.

Rejeb, I., Pastor, V., Mauch-Mani, B., 2014. Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. Plants 3 (4), 458–475. http://dx.doi.org/10.3390/plants3040458.

Ricardo, R.P., Madeira, M.A.V., Medina, J.M.B., Marques, M.M., Shanches, A.F.A., 1977. Esboço pedológico da ilha de S.Miguel (Açores). Anais do Instituto Superior de Agronomia, 37, 1977, pp. 275–385.

Rossel, D., Tarradellas, J., Bitton, G., Morel, J., 1997. Use of enzymes in soil ecotoxicology: a case for dehydrogenase and hydrolytic enzymes. In: Tarradellas, J., Bitton, D., Rossel, D. (Eds.), Soil Ecotoxicology. CRC Press, Boca Raton, pp. 179–206.

Santos, M.J.G., Morgado, R., Ferreira, N.G.C., Soares, A.M.V.M., Loureiro, S., 2011. Evaluation of the joint effect of glyphosate and dimethoate using a small-scale terrestrial ecosystem. Ecotoxicol. Environ. Safe 74, 1994–2001. http://dx.doi.org/10.1016/j.ecoenv.2011.06.003.

Shukla, G., Varma, A., 2011. Soil Enzimology. 22. Springer, Londonhttp://dx.doi.org/10.1007/978-3-642-14225-3.

Sparling, G.P., West, A.W., 1988. A direct extraction method to estimate soil microbial C: calibration in situ using microbial respiration and 14C labeled cells. Soil Biol. Biochem. 20 (3), 337–343.

Sugiyarto, K.H., 2013. Javanese volcanic allophane adsorbent as heavy metal adsorber to improve the quality of drinking water in Surakarta. J. Environ. Earth Sci. 3, 48–56.

Suleiman, A.K.A., Manoeli, L., Boldo, J.T., Pereira, M.G., Roesch, L.F.W., 2013. Shifts in soil bacterial community after eight years of land-use change. Syst. Appl. Microbiol. 36 (2), 137–144. http://dx.doi.org/10.1016/j.syapm.2012.10.007.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil. Biol. Biochem. 19 (6), 703–707.

Wakelin, S., Gerard, E., Black, A., Hamonts, K., Condron, L., Yuan, Nostrand, T., Zhou, J., O'Callaghan, M., 2014. Mechanisms of pollution induced community tolerance in a soil microbial community exposed to Cu. Environ. Pollut. 190, 1–9. http://dx.doi.org/10.1016/j.envpol.2014.03.008.

Wei, X., Shao, S., Gale, W.J., Zhang, X., Li, L., 2013. Dynamics of aggregate-associated organic carbon following conversion of forest to cropland. Soil Biol. Biochem. 57, 876–883. http://dx.doi.org/10.1016/j.soilbio.2012.10.020.

Xian, Y., Wang, M., Chen, W., 2015. Quantitative assessment on soil enzyme activities of heavy metal contaminated soils with various soil properties. Chemosphere 139, 604–608. http://dx.doi.org/10.1016/j.chemosphere.2014.12.060.

Xuereb, B., Chaumot, A., Mons, R., Garric, J., Geffard, O., 2009. Acetylcholinesterase activity in Gammarus fossarum (Crustacea Amphipoda): intrinsic variability, reference levels, and a reliable tool for field surveys. Aquat. Toxicol. 93, 225–233. http://dx.doi.org/10.1016/j.aquatox.2009.05.006.

Zhang, X., Li, F., Liu, T., Xu, C., Duan, D., Peng, C., Zhu, S., Shi, J., 2013. The variations in the soil enzyme activity, protein expression, microbial biomass, and community structure of soil contaminated by heavy metals. Soil Sci., 1–12. http://dx.doi.org/10.1155/2013/803150.