RECOVERY OF BURNED FOREST SOIL BY ORGANIC RESIDUE APPLICATION – SUBSTRATE INDUCED RESPIRATION IN SOIL

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1 INTRODUCTION

The Mediterranean is one of the most vulnerable regions to climatic changes. One of the impacts of these changes is a substantial increase in the meteorological risk of fire. The forest fire regime instantaneously responds to climatic changes and may become the dominant factor of alteration in forest communities (Santos & Miranda, 2006). Therefore, understanding the impact of fire on natural ecosystems may be important in the recovery of post-fire ecosystems, hence allowing a better forest restoration.

A fire impacted soil can recover, namely through soil organic matter (OM) correction by the application of organic residues. Applying agricultural (e.g. pig slurry) and municipal solid wastes as organic matter sources to soil may represent a good way to recycle these wastes.

Fire leads to important changes in the physical, chemical and biological properties of soils, which are relevant for the future productivity and sustainability of ecosystems (Neary et al., 2005). The extent and duration of these effects on soil properties depend on the intensity and residence time, ergo fire severity (Certini, 2005).

Soil microorganisms perform an important and essential role in soil biological processes. Biological properties however, are extremely sensitive to soil warming, where lethal temperature thresholds for most organisms are below 100 ^{o}C. Biological diversity represents a sensitive means to determine soil quality. This is related to functional diversity, which comprises the ability of organisms to use a vast range of carbon substrates and be able to perform several biochemical reactions. Soil health and quality can be assessed by microbial community level physiological profiles (CLPP), using different carbon substrates. MicroResp^{TM} can be applied to a vast range of soils and has good sensibility to detect changes in microbial communities, offering a rapid and sensitive method to determine CLPP (Chapman et al., 2007).

Soil (Litosol) was collected in Sintra Mountain (Portugal), on a *Pinus Pinaster* forest, 11 days after a forest fire. The impact on recovery of burned soil by organic residue application and its influence on soil microorganisms was studied using the MicroResp^{TM} method, concerning fire effects on soil microorganisms and the need to recycle wastes.

2 MATERIAL AND METHODS

2.1 Soil and treatments

Soil (Litosol) was collected in Sintra – Cascais Natural Park (W-Portugal), after a forest fire on March 2008 in the south hillside of Sintra Mountain (Latitude 38^{o} 45’ 35.37’’ N, Longitude 9^{o} 25’ 51.36’’ W). The Sintra Mountain has a Mediterranean Pluviseasonal – Oceanic, low Mesomediterranean, low humid climate, with average annual temperature of 13.2 ^{o}C and average annual precipitation of 1104 mm. Pre-fire vegetation was dense and green brushwood, with sparse *Pinus pinaster A.* trees, altogether providing 100% soil cover. The brushwood was composed by *Ulex europaeus L.*, *Erica australis L.*, *Quercus coccifera L.*, *Arbutus unedo L.* and *Davallia canariensis (L.) Sm*. The most representative species were *U. europaeus*, *E. australis* and *Q. coccifera*, which vary from moderate to high inflammability. Unburned soil (fresh soil) was collected from a nearby site. This area had leaf deposition and was surrounded by *Acacia* sp. and *P. pinaster* trees. The brushwood was less dense and the prevailing species were *U. europaeus* and *D. canariensis*.

Representative soil samples were collected 11 days after the forest fire, to a depth of 5 cm. Dry leaves and twigs were removed from the surface layer before collection. Several sub-samples were collected from both burned and unburned sites of approximately 40 kg and 120 kg of burned soil and fresh soil respectively. The sub-samples collected in each site were mixed to form representative samples of the burned and unburned areas. Soil samples were air dried for two days and sieved (< 2.0 mm). Part of the fresh soil was heated for 12 hours in a muffle furnace at temperatures of 65 °C, 105 °C and 250 °C. The treatments studied were burned soil, fresh soil, soil treated at 65 °C, 105 °C and 250 °C.

All treatments were analyzed for pH in a 1:2.5 (w/v) aqueous solution (Póvoas and Barral, 1992), for N-NH_4^+ and N-NO_3^- by spectrophotometry with a Skalar segmented flow autoanaliser, after extraction with 30 ml of 2 M KCl (1:10 soil/solution ratio) (Mulvaney, 1996), and for total organic matter by burning in a muffle furnace, at 450 °C for 7-8 hours (NFU 44160, 1985).

2.2. MicroResp™ physiological profiles

Composted municipal solid waste (MSW), digested pig slurry (PS) and a mixture of both organic residues were mixed manually with batches of 300 g of each soil, in amounts corresponding to an application of 170 kg N ha^{-1} (ED, 1991), and replicated three times. Mixtures of soils and residues, as well as a control soil without residues, were aerobically incubated at 25 °C in plastic bags, at 60% of their maximum water-holding capacity. Soil samples were collected after two months of incubation.

Physiological profiles were determined by MicroResp™ method (Campbell et al., 2003), which can distinguish differencies in CLPP between soil samples submitted to different temperatures, substrates and residues, using a colorimetric detection system (cresol red) that allows the measurement of soil respiration. The detection plate contained a gel-based bicarbonate buffer with indicator dye that turns from red-violet to yellow due to changes in pH (acidification) caused by CO_2 absorption in the alkali (Campbell et al., 2008; Chapman et al., 2007).

Microtiter deep-well plates were prepared, three per residue (control, MSW, PS, mixture), each plate with two replicates per treatment (burned soil, fresh soil, soil treated at 65 °C, 105 °C and 250 °C) with a total of 6 replicates per sample. 4 – amino - butyric acid (5 g/50 ml/substrate) was prepared to determine substrate induced respiration (SIR) (200 µL per well). Basal respiration (BR) was determined by using two hundred µL of water as substrate. Each detection plate contained purified agar at 1% (150 µL per well), with cresol red indicator dye (12.5 µg ml^{-1}), potassium chloride (150 mM) and sodium bicarbonate (2.5 mM). Detection plates were read at 600 nm, immediately before and after a 6 hours incubation period at 25 °C, in a microplate reader. Data were normalized for time zero, to eliminate colour differences between wells due to eventual differences in gel density.

Data were interpreted using analysis of variance (2-way ANOVA) performed with Statistica® (Neter et al., 1990). Fishers LSD tests were conducted to show the significant differences, at a significance level of 0.05 (Zar, 1996).

3 RESULTS

3.1 Soil properties

Table 1 shows that burned soil had the highest pH, followed by soil treated at 250 °C. However, at 105 °C pH decreased. Soils from other treatments had similar pH values. Burned soil had the highest OM content. Burned soil and soil treated at 250 °C had greater N-NH_4^+ contents than soils from other thermal treatments. Although significant, other N-NH_4^+ contents were at similar range. Nitrate content was higher in burned soil, followed by fresh soil. The treatment that led to lower nitrate content was soil treated at 250 °C.

TABLE 1 **Selected physical and chemical properties of unamended soils before incubation**

Parameter / **Treatment**	**pH** **(H_2O)**	**OM** **(%)**	**NH_4^+** (mg kg^{-1})	**NO_3^-**
Burned soil	6.41 a	15.62 a	60.85 b	7.46 a
Fresh soil	5.41 c	5.25 c	14.37 d	6.48 b
Soil treated at 65 °C	5.42 c	6.86 b	16.86 c	4.62 d
Soil treated at 105 °C	5.14 d	6.03 bc	16.01 cd	5.63 c
Soil treated at 250 °C	5.54 b	3.41 d	78.57 a	0.71 e

Values followed by the same letter are not significantly different at $p<0.05$

3.2 Respiration rate after 6 hours

Average BR ranged from 0.089 to 1.188 µg CO_2 – C g^{-1} h^{-1} in the control soil; from 0.096 to 0.765 µg CO_2 – C g^{-1} h^{-1} for treatments with MSW; from 0.053 to 1.732 µg CO_2 – C g^{-1} h^{-1} for treatments with PS and from 0.052 to 0.971

µg CO_2 – C g^{-1} h^{-1} for treatments with mixture of residues (Figure 1a). Only PS improved BR in burned soil. In the control soil treatment, burned soil had the highest BR. PS and mixture application also led to a higher BR in burned soil than in the other thermal treatments. When MSW was added to the soils, burned soil and soils treated at 65 °C and 105 °C had greater BR than fresh soil and 250 °C.

The average SIR using 4 – amino – butyric acid as substrate, ranged from 0.128 to 1.730 µg CO_2 – C g^{-1} h^{-1} in the control; from 0.184 to 1.304 µg CO_2 – C g^{-1} h^{-1} for treatments with MSW; from 0.161 to 1.578 µg CO_2 – C g^{-1} h^{-1} for treatments with PS and from 0.135 to 1.802 µg CO_2 – C g^{-1} h^{-1} for treatments with mixture (Figure 1b). Regarding the effect of residue amendments on thermal treatments, only MSW enhanced SIR in soil treated at 105 °C. Burned soil had greater SIR than other thermal treatments in the control soil and with PS and mixture amendments. In the control soil, fresh soil and treated at 65 °C had significantly higher respiration than soil burned 250 °C. MSW amendment promoted significantly higher respiration in burned soil and soils treated at 65 °C and 105 °C, when compared to fresh and 250 °C soils. With PS, 65 °C and 105 °C soils were significantly higher from soil treated at 250 °C. With mixture amendment, 65 °C and 105 °C soils had greater SIR than fresh soil and soil treated at 250 °C. Fresh soil was also greater than 250 °C soil.

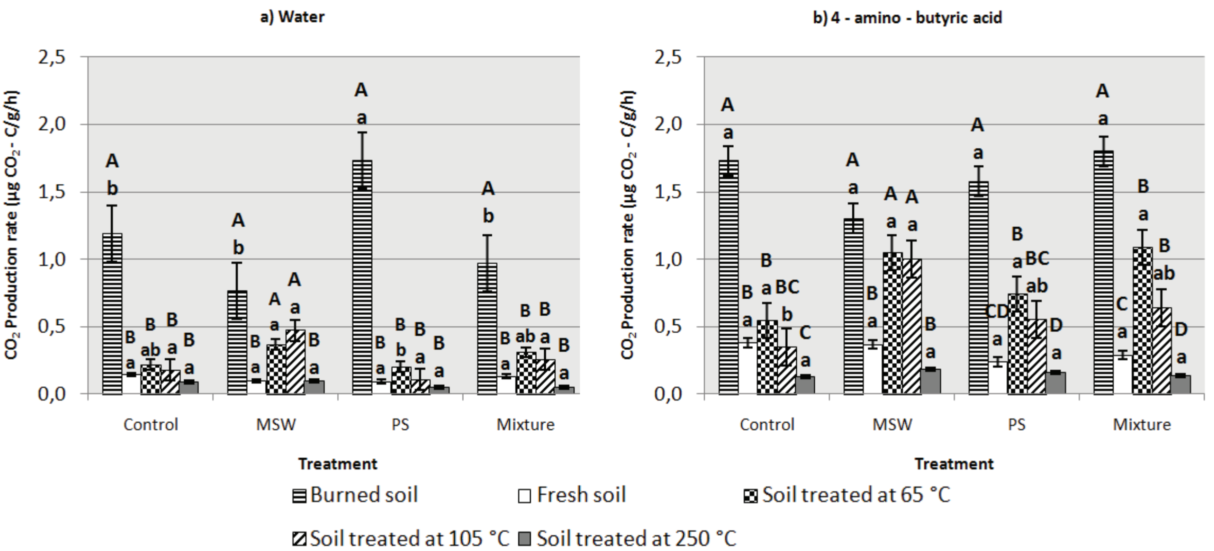

FIGURE 1 **Respirometric evolution of carbon dioxide (µg CO_2-C/g/h) after 6 hours, for each treatment. Error bars indicate the standard error.**

Different small letters show significant differences between amendments for each thermal treatment (burned soil, fresh soil, soil treated at 65, 105 and 250 °C; read equal bars). Capital letters show significant differences between thermal treatments for each amendment (control, MSW, PS and mixture; read bars grouped by residue).

4 DISCUSSION

Burned soil had greater OM and N-NH_4^+ contents than other thermal treatments (except for N-NH_4^+ content, higher in 250 °C soil) (Table 1), in part due to deposition of ash and small pieces of partially burned materials. In the burn treatments carried out in the laboratory, ash deposition didn't occur in the same way, since the dry leaves and twigs were removed from the surface layer before sample collection. Nutrient availability normally increases due to OM oxidation during combustion, and in fact soil burned at 250 °C had less OM and N-NO_3^- and more N-NH_4^+ than fresh soil or soil burned at 65 and 105 °C. At 250 °C, the destructive distillation of OM is already known to occur (DeBano, 1998).

The differences in OM content between fresh soil and soils treated at 65 °C and 105 °C can be explained by an enrichment in microorganisms in these soils during the time between the burn treatments and the chemical analysis, due to higher nutrient availability caused by the higher temperatures achieved in the burn treatments and also by the release of soluble sugars and N-NH_4^+ from dead microorganisms (Choromanska and DeLuca, 2001).

Burned soil had the greatest pH, due to the alkaline ions released in the ash (Hernandéz et al., 1997; Wüthrich et al., 2002). The pH also increased in soil treated at 250 °C. However, at 105 °C pH decreased.

Burned soil showed greater respiration values than other thermal treatments, suggesting that the ability of soil microorganisms to use carbon substrates was not reduced in the burned soil, indicating that the fire was most likely of low intensity. This can in part be explained by its higher OM content. Wüthrich et al. (2002) obtained higher respiration values in burned soil in his study, and suggested that the soil respiration was strongly enhanced only a few hours after the burning, and that decomposition of dead cells above- and below-ground (decomposing plant roots) started immediately after the fire.

In this study, microbial activity in burned soil may also have been stimulated due to ash deposition and therefore enhanced nutrient availability after the fire, and by a rise in soil pH. Enhanced nutrient availability after the fire and organic carbon solubilization from dead or damaged vegetable and microbial cells, may have contributed to a significant increase in soil respiration were carbon was easily mineralized and respired.

From the organic amendments made to soils, PS seemed to be more effective improving microbial activity in the burned soil, while in soils treated at 65 °C and 105 °C MSW was more successful. PS application must have greater advantage when microbial communities are already present, since it has components more readily available for microbial uptake, assuming that microorganisms had time to recover before soil collection, 11 days after the fire. Burned soil is likely to have been rapidly re-inoculated from deeper layers and from microorganisms present in air, remaining debris and unburned soil and may have easily recovered during the incubation period due to increased nutrient availability. Also the high OM content and pH value of the burned soil may have favoured microbial activity. MSW has more recalcitrant components than PS but seems to be a source of microorganisms, thus being more important in the inoculation or primary colonization of soils treated at 65 °C and 105 °C. Differences found between fresh soil and the 65 °C and 105 °C treated soils suggest a change in microbial communities after treatment, eventually with destruction of less tolerant microorganisms.

The fire probably was of low intensity. Thin branches remained on trees and shrubs, corroborating this assumption (Moreno et al., 1989). The well aired brushwood allowed rapid passage of the fire and the small residence time would not have caused lethal temperatures in sufficient depth to kill all the microorganisms.

5 CONCLUSIONS

Results suggest that organic amendments should be applied when attempting to restore burned soils, to promote the re-activation of soil microorganisms. The use of organic residues in regeneration programs may be a useful way to promote the recycling of these organic wastes converting them into valuable resources.

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