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# Changes in mineral nitrogen, soil organic matter fractions and microbial community level physiological profiles after application of digested pig slurry and compost from municipal organic wastes to burned soils

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ABSTRACT

In this study, mineralization of digested pig slurry and compost from municipal organic wastes in burned soils was followed for 60 days. The effects of amendments on organic matter fractions and microbial community level physiological profiles (CLPP) were also investigated at the end of the incubation period. Soil from a forest 10 days after a fire had a greater basal respiration, and more organic matter that a nearby soil that was not affected by fire, presumably as a consequence of black ash addition following the wildfire. Nitrification was inhibited in soils treated at 105 and 250 °C in the laboratory, but amendment application allowed nitrification to take place in the latter soil, and led to significant flushes of mineralization. Slurry amendment resulted in greater increases in mineral N compared with compost. Soil treated at 250 °C had the greatest content of water-extractable compounds (WE) at the expense of acid-extractable compounds (AE), but during the incubation the variations in these two fractions had an opposite trend, i.e. soil gained AE and lost WE fractions. The variation in N-acetyl-glucosamine-induced respiration was different between compost- and slurry-amended soils, with the greater values in the former. The effect of amendments could be further differentiated by principal component (PCA) and cluster analyses based on the variations in organic matter fractions and CLPP between the beginning and the end of the incubation period. Amendment application led to shifts on the PCA maps that depended both on the amendment and soil treatment. In fresh soil and in that treated at 250 °C, the unamended, compost- and slurry-amended treatments remained relatively close on the PCA maps and had linkage distances $<1.0$. In contrast, amendment application to other soils led to large shifts on the PCA maps and to linkage distances $>1.0$. Pig slurry led to the greatest changes in burned soil, while compost induced the greatest shifts in soil treated at 105 °C.

This study suggests that an application of organic amendments after a severe fire event may contribute to a faster recovery of soil functions.



## 1. Introduction

Forest wildfires are a major cause of soil degradation in Mediterranean areas and these events are predicted to increase according to climate change scenarios (Santos et al., 2001). Low-intensity fires can increase temperature at the soil surface to 100 °C, and to 50 °C at a 5-cm depth, while very intense fires may cause temperatures of 700 °C at the soil surface and 250 °C at a 10-cm depth (Neary et al., 1999).

The effects of fire on soil properties have been extensively studied (for reviews see Neary et al., 1999; Certini, 2005). One of the consequences of wildfires can be loss of soil organic matter. Volatilization of organic compounds starts between 100° and 180 °C, and at 200 °C decomposition of resistant compounds such as hemicelullose and lignin takes place (González-Pérez et al., 2004). However, fires also contribute to inputs of organic materials from partially burned materials, and in the long-term may even result in greater organic matter accumulation in soils due to impaired mineralization (Johnson and Curtis, 2001). Forest fires can also result in structural changes in soil organic matter; Fernández et al. (1997) reported that lipids were less affected than the cellulose fraction and Choromanska and DeLuca (2002) reported that the sugar fraction increased due to lysing of cells and distillation of the partially burned litter. There is usually a strong pulse in $N{-}NH_4$ after fire due to enhanced amounts of dead plants and microbial biomass (Diaz-Ravina et al., 1996).

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Another consequence of wildfires is the impact of raised temperature on soil microorganisms, which can be killed between 50° and 120 °C (DeBano et al., 1998). Microbial biomass may even be completely destroyed in the soil surface layer, and take many years to recover to pre-fire levels (Fritze et al., 1993). The composition of soil microbial communities, as evaluated by phospholipid fatty acid analysis, is also affected by fire (Bååth et al., 1995).

Re-vegetation of burned areas occurs spontaneously, but measures should be taken to promote plant growth to protect bare soil from the enhanced risk of erosion after a wildfire. A possible measure is the application of organic amendments to replenish organic matter pools (Guerrero et al., 2001). The amount of organic biodegradable residues generated worldwide is increasing and environmentally-sound strategies for their recycling should be developed. Their use in soils affected by wildfires has been advocated to promote the re-establishment of vegetation (Düring and Gäth, 2002), but the information on the effects of organic amendments in these soils is scarce. It is known that co-composted sewage sludge and green wastes increased the content of organic matter, total N, P and exchangeable cations such as K and Mg when applied to a burned soil in France (Larchevêque et al., 2008). However, no comparison of two differing organic amendments appears to have been carried out, and the effect of these on biological properties of burned soils has received no attention.

Information obtained from field studies is often considered unreliable due to the presence of confounding effects, and it is preferable to heat the soil at known temperatures in the laboratory (Bárcenas-Moreno and Bååth, 2009). In the present study, we used that approach but also included a soil impacted by a wildfire.

The objectives of this study were (1) to assess the potential of digested pig slurry and compost from municipal wastes to provide nitrogen to burned soils, (2) to identify changes in organic matter fractions and microbial community level physiological profiles resulting from the application of these amendments.

## 2. Materials and methods

### 2.1. Experimental design

Soil was collected from the A horizon (0–5 cm) from a forest soil (Litosol) in the Sintra mountain (38° 45′ 35.37″ N, 9° 25′ 51.36″ W), 10 days after a natural fire in March 2008, both in an area affected by the fire and in a contiguous area, of the same soil type, that was not reached by fire. The main tree species present was *Pinus pinaster* Aiton, and the shrubs present included *Ulex europaeus* L., *Erica australis* L., *Quercus coccifera* L., *Arbutus unedo* L. and *Davallia canariensis* L. The Sintra Mountain has a climate with some Mediterranean characteristics, but also with a strong influence from the Atlantic Ocean, with a mean annual temperature of 13.2 °C and annual precipitation of 1104 mm.

The two soil samples were air dried for two days, sieved (≤2 mm) and mixed to obtain homogenized samples. Part of the unburned soil was heated for 12 h at three different temperatures (65°, 105° and 250 °C for 12 h) to simulate fires of different intensity.

Three hundred gram of each soil, burned, fresh and treated at three different temperatures, was mixed with two organic residues (Table 1), and aerobically incubated in plastic containers for approximately 60 days in the dark, under controlled room temperature (24 ± 2 °C) at 60% of the maximum water-holding capacity of the soils (as previously determined). Digested pig slurry and municipal solid waste compost were applied at a rate of 50 ml and 6 g $kg^{-1}$ of soil, respectively, corresponding to an application rate of about 170 kg N $ha^{-1}$ (ED, 1991). Treatments of each soil without organic amendments were included as controls. Sufficient containers were prepared to allow for three replicates for each soil, treatment and sampling date (see below).

**Table 1**
Characteristics of the digested pig slurry and composted municipal solid waste (averages ± standard deviations, $n = 3$).

| Characteristic | Pig slurry | Compost |
|---|---|---|
| pH | 8.1 ± 0.1 | 8.0 ± 0.1 |
| Organic matter (% DM) | 71 ± 6 | 48 ± 4 |
| N-Kjeldhal (g $kg^{-1}$ DM) | 23 ± 3 | 18 ± 3 |
| Fraction extracted with methyl chloride (% DM) | 6 ± 1 | 2 ± 1 |
| Water-extractable fraction (% DM) | 15 ± 1 | 10 ± 1 |
| Acid-extractable fraction (% DM) | 45 ± 4 | 31 ± 2 |
| Basal respiration (μg $CO_2$ $h^{-1}$ $g^{-1}$) | 33 ± 5 | 3.1 ± 0.5 |
| SIR-glucose (μg $CO_2$ $h^{-1}$ $g^{-1}$) | 35 ± 5 | 3.9 ± 0.6 |
| SIR-citric acid (μg $CO_2$ $h^{-1}$ $g^{-1}$) | 203 ± 12 | 9.9 ± 1.0 |
| SIR-amino-butyric acid (μg $CO_2$ $h^{-1}$ $g^{-1}$) | 50 ± 2 | 2.3 ± 0.4 |
| SIR-acetyl-glucosamine (μg $CO_2$ $h^{-1}$ $g^{-1}$) | 41 ± 2 | 4.1 ± 1.8 |

SIR – substrate-induced respiration.

### 2.2. Evolution of mineral nitrogen

Destructive samples were taken for analysis at 0, 2, 7, 14, 21, 34 and 60 days after the beginning of the incubation, and analyzed for mineral N ($N-NO_3$ and $N-NH_4$) by segmented flow spectrophotometry after a 2 M KCl extraction (Mulvaney, 1996). The change in mineral N in unamended soil was calculated by subtracting the initial concentrations of $N-NO_3$ and $N-NH_4$ of the original soils from the respective values measured at each sampling date.

The effects of compost and pig slurry on N mineralization were calculated from the difference method, i.e. the amount of additional mineral N at each sampling date was calculated by subtracting the mineral N present in the unamended control from the corresponding compost- or slurry-amended soils.

### 2.3. Fractionation of soil organic matter

At the beginning and end of the experiment an analysis of proximate carbon fractions by sequential extraction was performed according to Ryan et al. (1990). One gram of soil from each replicate was extracted with 25 ml of methyl chloride in an ultra-sound bath at 110 W for 30 min. After filtration, the remaining soil was dried at 105 °C for 4 h and weighed. The weight loss corresponded to fats, oils and waxes (solvent extractable, SE fraction). One hundred milliliter of hot deionized water was added to the soil and the mixture heated in a boiling water bath for 3 h. The extract was filtered and the remaining soil dried at 105 °C for 4 h. The weight loss corresponded to sugars and other water-soluble compounds (water-extractable, WE fraction). Concentrated sulfuric acid was added to the soil (1%, m/m) and the mixture heated in a water bath at 30 °C for 1 h. Deionized water was added (28:1, v/v) and the mixture autoclaved at 120 °C for 1 h. After filtration the remaining soil was dried at 105 °C for 4 h and weighed. The weight loss corresponded to cellulose and related compounds (acid-extractable, AE fraction). The soil was then ashed at 500 °C for 4 h and weighed. The weight loss corresponded to residual organic matter (ROM).

### 2.4. Microbial community level physiological profiles

Microbial community level physiological profiles (CLPP) were determined by the MicroResp™ method (Campbell et al., 2003) that uses a colorimetric detection (with cresol red in the detection plate) to measure soil respiration in the presence of different C sources. Four substrates were prepared at 1% (m/v) in deionized water to determine substrate-induced respiration (SIR): D-glucose, citric