POST-FIRE DYNAMICS IN ORGANIC MATTER COMPOSITION OF TOPSOIL AND ERODED SEDIMENTS IN RECENTLY BURNT FORESTS IN NORTH-CENTRAL PORTUGAL – CHARACTERIZATION USING ANALYTICAL TECNIQUES

Scientific Report

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1. Aim and objectives of the Molter Grant

Wildfires affect the quality and quantity of soil organic matter (SOM) pools. Specifically, fire-mediated destruction of Mediterranean ecosystems is in part caused by a depth transformation of soil physical, chemical and biological characteristics, associated with qualitative and quantitative changes in the most functional fraction of soil, such as organic matter (González-Pérez et al., 2004). These changes may negatively affect soil health and quality, favoring the occurrence of erosive processes and the loss of soil, which is a non-renewable natural resource (Arias et al., 2005). To this end, the overall aim of this grant was to study the effects (direct and indirect) of wildfire on the quality of *in-situ* soil organic matter (SOM) of eucalypt plantations, one of the prevailing and, at the same time, most fire-prone forest types in north-central Portugal. For this, it was necessary to apply sophisticated analytical techniques such as lipid-biomarkers analysis by gas chromatography-mass spectrometry (GC-MS), SOM characterization by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), and black Carbon determination by the Lim and Cachier method (1996). The application of these analytical techniques was only possible by resorting to the IRNAS-CSIC team and their laboratorial facilities and equipments.

The specific objectives of the grant were to:

(1) determine the direct effects of wildfire on *in-situ* SOM quality, by comparing soil samples collected immediately after a 2010-wildfire at two neighboring burnt/long-unburnt eucalypt plantations;

(2) assess the indirect effects of wildfire on *in-situ* SOM quality, by comparing soil samples collected 6, 12 and 18 months after the 2010-wildfire at the above-mentioned eucalypt plantations;

(3) evaluate the indirect effects of wildfire on the quality of SOM losses by runoff, by comparing sediment samples exported during selected erosion events from the same eucalypt plantations;

(4) assess the "selectivity" of SOM erosion, by comparing the results for the above-mentioned soil and sediment samples.

2. Overview of activities

To attain the proposed objectives, two neighboring eucalypt plantations were selected and sampled at 5 occasions with roughly 6-month intervals, starting immediately after a wildfire in August 2010, and continuing until August 2012. One of the study sites was located within the 2010-burnt area and the other just outside it. During these three months, a set of 30 soil samples were collected, on which it was necessary to carry out different treatments and analyses, including the original program and unprogrammed complementary treatments and analyses: chemical oxidation, water holding capacity, pH and carbon sequestration potential. The performed activities are described below.

2.1. Extraction of the SOM's labile lipid fraction

The air-dried soil samples (sieved to < 2mm; 3-5g) were extracted using a Soxhlet BUCHI B-811 extractor with a dichloromethane-methanol (3:1) mixture during 40 cycles (8h) (Figure 1). This equipment was programmed in three steps: extraction phase, washing phase and drying phase. Extracted lipids were subsequently analyzed by spectrometry (GC-MS).

Figure 1 – Soxhlet extractor.

2.2. Analysis of lipid fraction and interpretation of chromatograms

The total extracted lipids were subsequently analyzed by spectrometry GC-MS, using a Hewlett-Packard 5730A gas chromatograph linked with a Hewlett-Packard GCD mass spectrometer.

The individual compounds were identified by comparing mass fragments with published mass spectra libraries (NIST and Wiley); figure 2 shows an example of GC-MS results for one of the burnt soil samples.
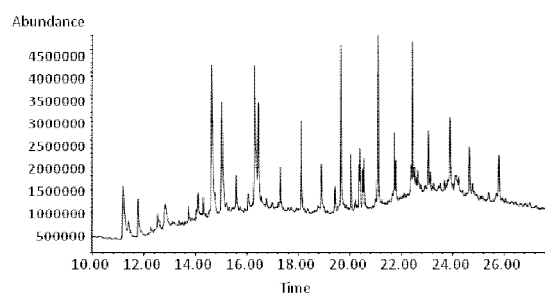

Figure 2- GC-MS total ion chromatogram (untreated).

2.3. Sample analysis using solid-state 13C NMR spectroscopy, infrared spectroscopy and py-GC/MS

2.3.1. Pre-treatment: demineralization of soil samples with hydrofluoric acid (HF)

This process was performed in two steps. In the first phase, the soil samples (6-10g) were centrifuged with 50ml of deionized water for 10 min at 3000rpm. The supernatant was filtered, dried and weighted (Figure 3). The second step consisted in adding 50 ml of 10% (w/w) HF and shaking for 2 h at 250 rpm. The samples were subsequently centrifuged during 10 min at 3000rpm. The supernatant was removed and
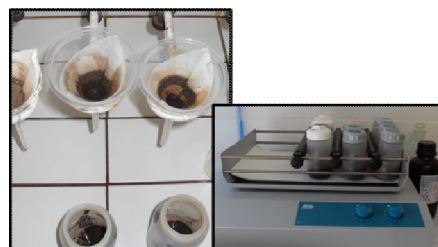

Figure 3 - Demineralization treatment.

discarded and the residue was again submitted to HF treatment. The second step was repeated five times. After that, the remaining fraction was washed five times with 50ml deionized water and freeze-dried. The application of this process is important to eliminate the mineral fraction and the paramagnetic compounds.

2.3.2. Pre-treatment: chemical oxidation with acid dichromate

After the demineralization of soil samples, 1g of HF treated soil was oxidized with 20ml of 0.1 M $K_2Cr_2O_7$/2 M H_2SO_4 solution at 60 °C in an ultrasonic bath for 6h (Figure 4). Afterwards, the oxidation residues were washed four times with deionized water and freeze-dried. With this pre-treatment it is possible to isolate the most refractorysoil organic matter fraction.

Figure 4 - Ultrasonic bath.

2.3.3. Analysis

The original and treated samples were analyzed by infrared spectroscopy. The originally foreseen analyses using the solid-state ^{13}C NMR and py-GC/MS were not performed, in the first case due to insufficient time, and in the second case due to unexpected equipment failures. However, the time-consuming pre-treatments were completed and therefore the samples are ready to be analyzed using these methods during a future visit to the IRNAS laboratories (already programmed, as described below). Furthermore, the time not used in

these analyses was used to perform complementary procedures not originally included in the workplan.

2.4. Determination of pH and water holding capacity (WHC)

The pH of the original samples was measured in soil suspension obtained after mixing 10g of soil with 50 ml of deionized water, with duplicate experiments per sample. Dry soil samples were first saturated with water in excess, after which the excess of water was removed (2h). WHC was determined as the absorbed humidity during this period (Figure 5).

Figure 5 – WHC measurement.

2.5. Carbon sequestration potential by IRNAS' Respicond Apparatus IV

The relative carbon sequestration potential is being estimated by means of the IRNAS' Respicond Apparatus IV, which monitors the potential CO_2 release from soil samples during a laboratory incubation experiment of 3 months (Figure 6). The Respicond Apparatus IV is a sophisticated, computerized respirometer that is based on the principle of using the change in conductivity of an electrolyte (KOH) to detect CO_2. Soil samples were prepared for the apparatus during the visit, and the experiment was initiated in the beginning of December; it is still ongoing, with the CO_2-release being monitored at 2h-intervals for the 3-month duration of the experiment.

Figure 6- Respicond Apparatus IV.

3. Preliminary results

The results analyzed so far indicate that the soil lipid fraction of the upper soil layer (0-2 cm depth) decreased sharply due to the wildfire, and that this effect lasted for more than 24 months. The lipid fraction in the different sampling periods varied between 0.9 and 1.1 % at the burnt site, as compared to a range between 2.4 and 5.7 % at the long-unburnt site. The main differences between the burnt and unburnt soil samples were found in the distribution

patterns of alkyl compounds. An accumulation of low molecular weight homologues was observed in all the burnt samples, suggesting that fire led to thermal breakdown and cracking of long-chain molecules (Figure 7).
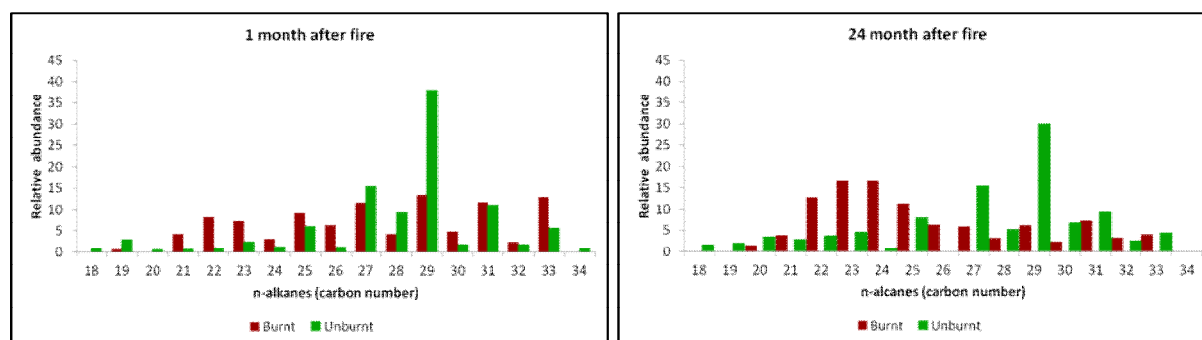

Figure 7- Relative abundance of *n*-alkanes in burnt and unburnt soil samples, 1 and 24 months after the fire.

All burnt and unburnt samples contained a homologous series of C_{18} – C_{35} *n*-alkanes, peaking at C_{27} and C_{29}. The results further suggest an accumulation, in the burnt samples, of homologues of low molecular weight (as evidenced by a decrease in average chain length) and of even-numbered homologues (as evidenced by a decrease in the Carbon Preference Index-CPI), which has been previously reported (Almendros et al., 1988).

Saturated *n*-fatty acid bimodal series were detected in the range C_{14}–C_{30}, with a sharp first maximum at C_{16} and a second maximum at C_{24}, indicating a strong contribution by epicuticular waxes from vascular plants. The n-fatty acid series distribution seemed a particularly good indicator of soil quality status and post-fire recovery, indicating a partial recovery of soils 24 months after the wildfire (Figure 8), as well as significant seasonal variations in the lipid composition.
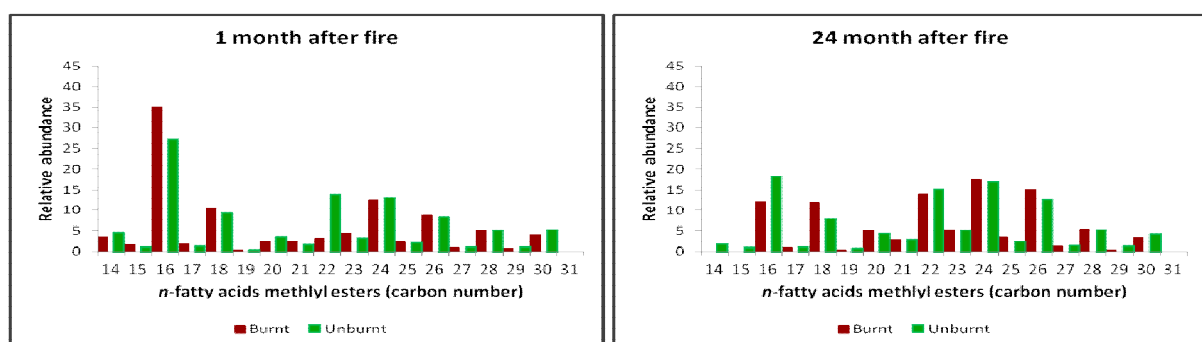

Figure 8- The relative abundance of *n*-fatty acids in burnt and unburnt soil samples, 1 and 24 months after the fire.

4. Future collaboration with the host institution

As mentioned above, the Respicond analysis is still ongoing, while the ^{13}C NMR and py-GC/MS analyses were prepared but could not be completed during the 3 months visit. A new visit to IRNAS is programmed for early 2013 to complete this work. Furthermore, the analysis of the obtained results will be done in collaboration with IRNAS researchers in order to prepare a joint publication. Finally, the framework for the mid-term collaboration of IRNAS with my PhD workplan was agreed, especially by officially including an IRNAS researcher (José Maria de la Rosa) as PhD co-supervisor.

5. Projected publications / articles resulting or to result from the grant

Faria, S. R, De la Rosa, J. M., Knicker, H, González-Pérez, J. A., González-Vila, F.J., Keizer, J.J. Wildfire effects on soil lipid composition in burnt eucalypt stands, in north-central Portugal. Geophysical Research Abstracts, vol. 15, EGU2013-13317, 2013 (submitted).

Faria S. R., De la Rosa J. M., González-Pérez J. A., Villaverde-Capellán, J., Keizer, J.J. The effect of fire in the lipid composition of forest soils under eucalypt stands from north-central Portugal. (In preparation for submission to IMOG 2013)

Faria, S. R, De la Rosa, J. M., Knicker, H, González-Pérez, J. A., González-Vila, F.J., Keizer, J.J. Wildfire effects on soil lipid composition in Mediterranean eucalypt stands. (In preparation for submission to SCI journal)

6. Acknowledgements

7. References

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