Factors controlling short-term soil microbial response after laboratory heating.

Preliminary results

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Introduction

Soil microbial response after fire is controlled by numerous variables which conclude with a mosaic of results depending mainly on organic carbon alterations or pH fire-induced changes. This fact has complicated soil post-fire microbial response studies during years compiling high variability of opposite result in the bibliography. The main objective of the present preliminary study was to evaluate the relative importance of different factors controlling short-term microbial response after laboratory heating by mean to the control of pH and nutrient heating-induced changes.

Material and Methods

Soil sampling and heating

Soil used in the experiment was collected in an unaltered area covered by high-mountain vegetation in Sierra Nevada National Park at 2000 m above sea level. Complex soil samples were formed mixing different subsamples from the 5 first cm of soil.

Soil from the unaltered area was submitted to differentafter heating treatment: 300, 450 and 500 ºC during 20 min in a muffle furnace to simulate a rage of fire intensities including soil from unaltered area as control.

Soil:water extract and culture media preparation

Heated (H300, H450 and H500) and unheated (UH) soil samples were used to prepare soil:water extract (1:2, w:w) shaking during 2h. In order to isolate possible nutrient availability or pH heating-induced changes, different culture media were prepared using soil:water extract from different heating treatments and adding different nutrient suplements (glucose, yeast extract and K_2HPO_4) and pH rectification to obtain the total of 11 different culture media described in table 1. In addition Tryptic soy agar (TSA) and agar with nutrient addition (AN+) culture media were inoculated to control soil microbial abundance in the absence of soil extract.

Microbial spreading, incubation and quantification

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Ten-fold serial dilutions were prepared mixing 5 g of **unheated** fresh soil samples with 45 ml of sterile saline solution, and 0.1 ml of 10^{-2}, 10^{-3} , 10^{-4} serial dilution were spread on the soil extract-based culture media (Fig. 1). Colony forming Units (CFU) of viable and cultivable microorganisms were quantified after 2 days of incubation at 25 ºC.

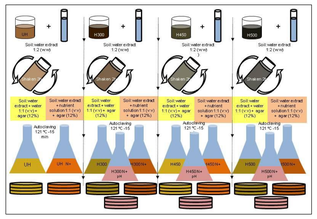

Figure 1. Culture media based on soil:water extract.

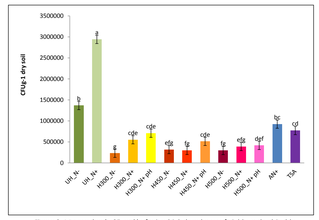

Figure 2. Mean value (± SE, n=3) of microbial abundance of viable and cultivable microorganisms (CFU) isolated in the different soil:water extract-based media. Different letters indicate significant differences among treatments (*Tukey's* post-hoc test, P<0.05*)*

Treatment	Heating Temperature (ºC)	Nutrient addition	pH rectification	Final pH
UH N-	Unheated	NO	NO	6.3
UH N+	Unheated	YES	NO	6.1
H300N-	300	NO	NO	7
H300 N+	300	YES	NO	7
H300 N+ pH	300	YES	YES	6.2
H450 N-	450	NO	NO	7.3
H450 N+	450	YES	NO	7.3
H450 N+ pH	450	YES	YES	6.1
H500 N-	500	NO	NO	7.4
H500 N+	500	YES	NO	7.4
H500 N+ pH	500	YES	YES	6.1

Table 1. Description of different culture media prepared with soil:water extract..

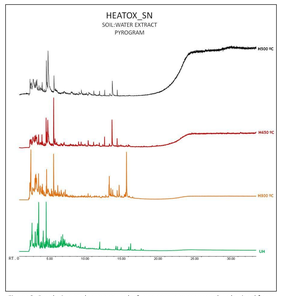

Figure 3. Pyrolysis-gas chromatography/mass spectrometry results obtained from soil:water extract before media preparation.

Results

Heating effect on soil:water extract-media was evident since the number CFU in those media prepared by mean of heated soil was lower than the half of those counted in media prepared with unheated soil inoculated with the same dilution. Nutrient addition appear to promote microbial proliferation in unaltered and 300 ºC treatments, while nutrient and pH compensation appear to attenuate heating effect on samples heated at 300 and 450 ºC. In the other hand, media prepared with soil:water extract form soil heated at 500ºC showed similar CFU abundance in all supplement treatments.(Fig.3).

Microbial abundance counted in UH-N- and UH-N+ media was higher than in the normal soil microbial isolation media as TSA and AN+, probably due to pH and specific organic compounds from the original soil where inoculated microorganism have grown. Nevertheless, microbial abundance in heated soil:extract based media was markedly lower than CFU counted in TSA and AN+ although when nutrient and pH were restored in soil heated at 300 and 450 ºC CFU increased to reach values close to those found in TSA and AN+ . (Fig.2)

In addition, the analysis of heating-induced soil organic matter alteration by mean of pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) technique shows marked modification in the organic matter composition, with important reduction in the number of compounds (peaks) found in the pyrogram (Fig. 3). Data integration allows total peaks quantification of each sample showing a marked diminution with increased temperature (data not shown) follow a linear correlation (R^2=0.892).

Discusion

The lower abundance of microbial proliferation on media prepared with heated soil extract evidences the negative fire impact on soil to microbial proliferation. Fire induced diminution of soil organic compounds necessary for microbial growth has been one of the possible explanation of microbial biomass delay in post-fire recovery (Prieto-Fernandez et al., 1998; Bárcenas-Moreno et al., 2011) which is partially confirmed with the diminution in compounds number revealed by pyrolisis result in our study. The nutrient addition to the culture media improve significantly microbial growth in UH and H300 culture media corroborating results obtained by Bárcenas-Moreno and Bååth (2009) who found low or absence of recovery of microbial activity after the application of high intensities heating treatment (400 and 500 º C) to soil which was inoculate with microorganisms from unaltered and unheated fresh soil, explained by the marked decreased in C and N soil content. Nevertheless, nutrient addition in H450 and H500 barely improve microbial proliferation. On the other hand, pH rectification to original soil pH in media prepared with heated soil appear to have slight but positive effect. In spite of nutrient and pH heating-induced changes mitigation, our study evidences the existence of other factor limiting microbial growth comparing with unheated soil-based media. This factor could be related to the destruction of some "essential" compound with heating or the presence of some new substance with inhibit microbial growth. The presence of inhibitory compounds for microorganisms due to soil heating have been previously evidence for bacterial (Díaz-Raviña et al., 1996) and fungal proliferation (Widden and Parkinson,1975), although the specific compounds or the mechanisms of the inhibition are questions without response nowadays. The studies focus on organic matter alteration due to partial combustion occurred during forest fire have evidenced the formation of organic compounds characterized for high aromaticity and low solubility (González-Pérez et al., 2004; Almendros et al., 1990), denominated pyromorphic compounds which are less accessible for microbial degradation (González-Pérez et al., 2004). In this preliminary study we are trying to obtain a more detailed data about organic matter transformation by heating, but the complexity of the pyrolisis results interpretation delays the conclusions enumeration until all the result will be evaluated.

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