Microbial response to the effect of quantity and quality soil organic matter alteration after laboratory heating

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1. Introduction

Fire-induced soil changes influence indirectly on soil microbial response, mainly due to pH increases and organic matter alterations. Partial carbon combustion can originate both, an increase in microbial activity due to dissolved organic carbon increases (Bárcenas-Moreno and Bååth, 2099, Bárcenas-Moreno et al., 2011), as well as limitation of microbial growth, either due to diminution of some fractions of organic matter (Fernández et al., 1997) or due to the formation of toxic compounds (Widden and Parkinson, 1975; Diaz-Raviña et al., 1996). The magnitude or direction of these changes is conditioned mainly by fire intensity and plant species, so forest with different vegetation could promote different quantity and quality alterations of soil organic matter after fire which leads to different soil microbial response.

The objective of this work was to differentiate between the fire-induced quantitative and qualitative changes of soil organic matter effects on microbial response, distinguishing between the possible reduction of carbon content and the formation of substances with inhibitory effect on soil microorganisms, inoculating microorganisms from an unaltered forest area on heated soil extract-based culture media.

2. Material and Methods

2.1. Experimental design

To differentiate between quantitative and qualitative soil organic matter alteration effect on soil microorganisms we collected soil from an unaltered forest area, under the influence of pine tree *Pinus silvestris* (PIN) and *Quercus ilex. subsp. rotundifoliae* (OAK). Two different species samples were subjected to two different laboratory heating treatments: unheated-control (UH) and heated at 450ºC during 20 min. Then, different soil samples were used to prepare soil-extract based culture media, with and without nutrient supplements which were inoculated with the original soil ten-fold serial dilutions (Fig. 1).

2.1. Sampling area

Soil samples used to prepare culture media were collected in a pine and an oak forest in a surrounding area to the National Park of Sierra Nevada (South of Spain) at 1700 m of altitude. Both forest areas present the same soil and climatic characteristics, and were sampled follow the same methodology. Five soil subsamples were collected under the litter influence of each dominant tree species, *Pinus silvestris* (PIN) and *Quercus ilex. subsp. rotundifoliae* (OAK) , from the first 5 cm after litter removing, forming two different complex soil samples, under pine and under oak. Soil complex samples were divided into air-dried samples to prepare heating treatments, culture media and soil characterization, and fresh soil samples were stored at 4º to inoculate culture media.

2.2. Laboratory heating

Two different heating treatment were selected to this preliminary test, unheating and heating at 450 ºC during 20 min in a pre-heated muffle furnace.

2.3. Soil extraction

Soil water extract was obtained by mixing 1:2 (w:w) soil and water and shaking the slurry for 2 h (Diaz-Raviña et al., 1996) followed by filtration and centrifugation at 5000 g during 10 min (to minimize fine particles content).

2.4. Culture media

Soil extract culture media were prepared by mixing 1:1 (v:v) soil extract and water (N-) or nutrient solution prepared with glucose, yeast extract and K_2HPO_4 (N+) to make organic carbon culture media content equal between heated and unheated soil treatments, obtaining 8 different culture media (Fig. 1).

2.5. Microbial spreading, incubation and quantification.

Ten-fold serial dilutions were prepared mixing 10 g of unheated fresh soil samples with 90 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, taking into account the plant species origin of the culture media (Fig. 1). Colony forming Units (CFU) of viable and cultivable microorganisms were quantified after 5 days of incubation at 25 ºC.

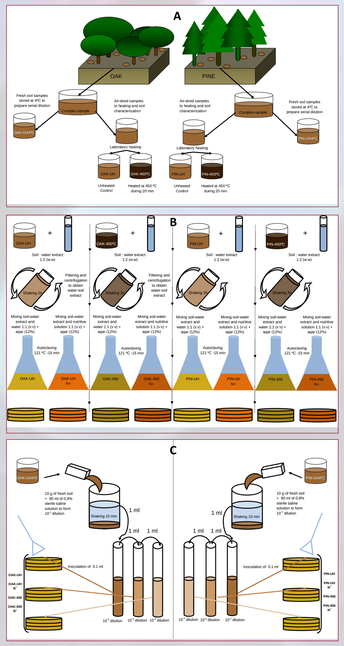

Figure 1. Experimental design. A) soil sampling; B) Soil extract-based culture media; C) Serial dilution and spreading.

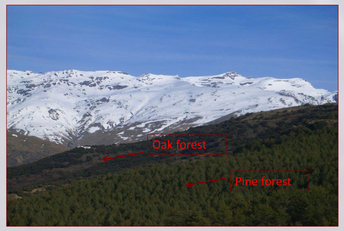

Figure 2. Sierra Nevada National Park surrounding area where soil samples were collected under oak and pine forest.

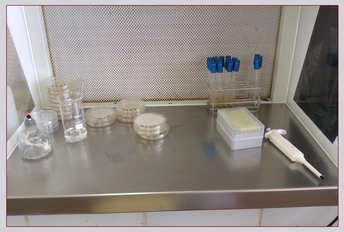

Figure 3. Detail of microbial spreading on different soil extrac-based culture media.

3. Results

3.1 Soil characteristics:

Total and soluble organic carbon, nitrogen soil content and C:N ratio are showed in the figure 4. Heated soil showed lower values than unheated control from the corresponding plant species influence for all the parameters determined. Unheated soil samples taken under oak (OAK-UH) influence showed lower total organic carbon content compared with samples taken under pine influence (PIN-UH) although carbon availability was higher in OAK-UH than in PIN-UH (Fig. 4 B). Nitrogen content in OAK-UH and PIN –UH was similar (Fig. 4C), resulting in a marked difference in the C.N ratio with PIN-UH showing the highest value (Fig. 4D). Heated samples from different species did not show marked differences to each other.

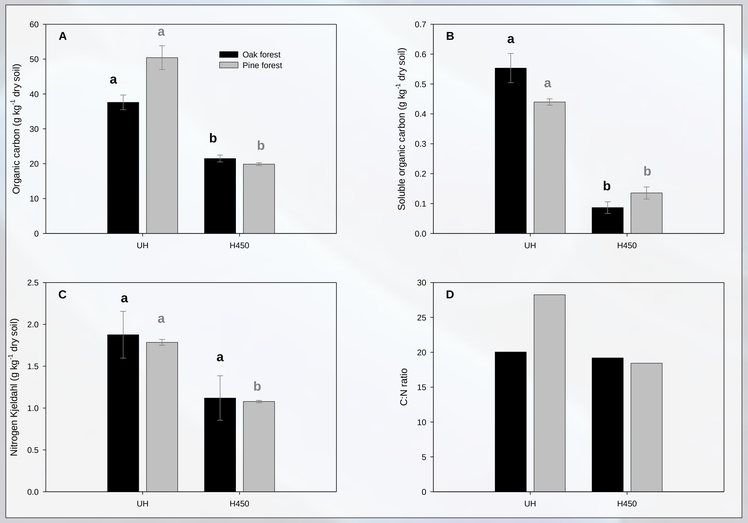

Figure 4. Soil characterization data. A) Soil organic carbon content; B) Soluble organic carbon content; C) Kjeldahl nitrogen content; D) C:N ratio. Different letters with the same color indicate that the results are statistically significant (P<0.05) according to T-test. Error bars ± SE (n=3).

3.2 Microbial abundance response:

Microbial abundance on unheated soil-based culture media showed significant differences according to plant species soil influence, since CFU abundance was significantly higher in culture media prepared with unheated pine forest soil than with unheated oak forest soil extract. The addition of nutrient to unheated soil extract did not show marked effect on microbial proliferation on oak forest culture media while CFU in pine forest culture media amended with nutrient was markedly higher than in the same media without nutrients addition. In general, microbial proliferation on culture media prepared with heated soil extract was lower than those found on unheated soil extract-based culture media, independently of plant species influence. The addition of nutrients to media prepared with heated soil extract induced an slight but not significant increase in microbial abundance regarding the same media without nutrients.(Fig. 5).

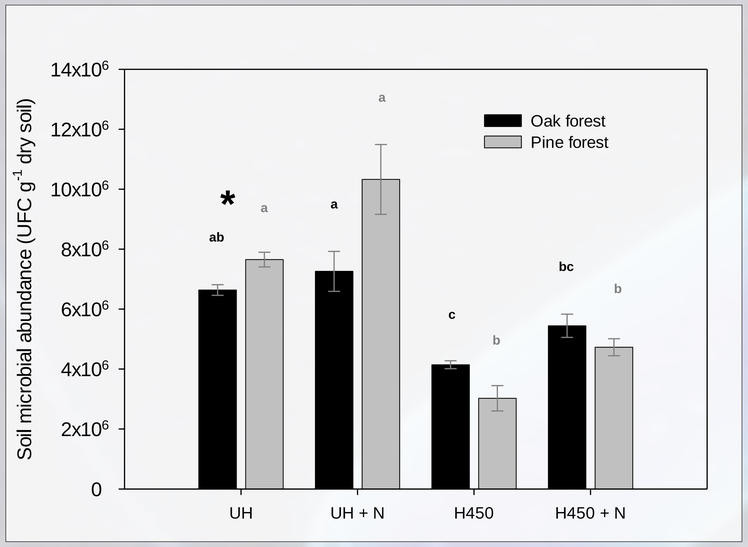

Figure 5. Microbial abundance of viable and cultivable microorgansims on different soil-based culture media. Different letters with the same color indicate that the results are statistically significant (P<0.05) according to Tukey's post hoc test. * means significant differences in the same heating treatment according to plant species soil origin. Error bars ± SE (n=4).

4. Discussion

Microbial abundance of viable and cultivable microorganisms quantified on unaltered soil-based culture media are similar to those obtained in unaltered forest soil from the same area measured in other studies using TSA agar by the authors (data not show) . The lower abundance of microbial proliferation on media prepared with heated soil extract evidences the negative impact of fire on soil condition 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). Bárcenas-Moreno and Bååth (2009) 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. In that study, the low recovery of microbial activity was explained by the marked decreased in C and N soil content which could be limiting microbial proliferation, observing differences between fungal and bacterial growth response. Nevertheless, our study evidence the existence of other factor that appears to be related to the presence of some substance which could be limiting microbial proliferation in spite of the nutrient availability, since the H-450 N^+ treatment showed lower microbial CFU than the unheated control treatment independently of plant species influence. 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) although recent studies demonstrate that pyrogenic organic material can be microbially attacked and mineralized at rate that is comparable to those for soil organic mater (Hilscher et al., 2009) . So, more multidisciplinary studies are need to demonstrate if this fire rearranged compounds can inhibit directly microbial proliferation of some specific microbial populations.

Figure 6. Detail of soil used to prepare culture media. Darker soil samples correspond to soil heated at 450 º C during 20 min. and brown soil correspond to unheated-control soil treatment.

5. Conclusions

This preliminary study has shown the existence of limitation of viable and cultivable microorganisms proliferation on culture media prepared with water:soil extract from heated soils independently of nutrient addition. This results evidence the existence of some compounds related to heating process which could be inhibiting some microbial groups. This finding lead us to start a new research line where more temperatures, soils and plant species will be evaluated, with the possibility to include in the future the isolation and identification of the most sensitive microorganisms.

6. References

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