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Heated soil-water extract effect on bacterial growth: pH or toxic compounds?

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Abstract

Fire-induced soil changes influence indirectly on soil microbial response, mainly due to pH increases and organic matter alterations. Nevertheless, field studies include overlapped effects and it is difficult distinguish the real origin of microbial response. In this work we have performed a laboratory experiment focus on the study of heated soil-water extract effect on bacterial growth, trying to isolate pH and soluble organic carbon alterations induced by heating soil at different temperatures. Bacterial growth was estimated by 3H-leucine incorporation technique which allows isolate bacterial activity response to an alteration. Different heated treatments were applied to unaltered forest soil samples, to simulate moderate (heating at 300 ^{o}C) or high (heating at 500 ^{o}C) intensity fire. In order to isolate possible pH changes effect, the experience was repeated adding pH buffers to bring the extract to the unaltered soil pH. Preliminary results show bacterial growth inhibition in both heated treatment compared to bacterial growth of the same bacterial suspension incubated with water. The reestablishment of pH improve the bacterial growth of samples incubated in heated soil-water extract, with a more marked effect on incubation soil-water extract from soil heated at 500 ^{o}C. These results evidence the importance of pH changes on low pH adapted bacterial community and the presence of other factors presents in the soluble fraction that are limiting bacterial proliferation.

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1 INTRODUCTION

Partial carbon combustion produced during a wildfire can originate both, an increase in microbial activity due to dissolved organic carbon increases (Bárcenas-Moreno & Bååth, 2099), 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 & Parkinson, 1975; Diaz-Raviña et al., 1996). Nevertheless, in wildfire studies, usually all the fire-altered factors are overlapped and it is difficult or even impossible determine the real factor controlling microbial response after fire. Several studies have determined pH and organic carbon alteration as the main factors controlling short-term microbial response after fire, although it is difficult isolate to each other, limiting the understanding of microbial dynamic after fire which is need to predict ecosystem post-fire dynamic and design better post-fire human interventions.

The objective of this work was to evaluate effect of fire-induced changes of soluble organic fractions on bacterial growth, trying to distinguish between the influence of pH changes and the formation of some substance that could inhibit or reduce microbial growth.

2 MATERIAL AND METHODS

2.1 SOIL SAMPLES AND HEATING

Soil used in the experiment was collected in an unaltered area in Sierra Nevada National Park at 2000 m above sea level. This area is covered by high-mountain vegetation (*Berberis hispanica*; *Cytisus oromediterraneus* as main

Table 1. Acidity (pH) measured in soil:water extract, bacterial suspension and mix of both with and whitout pH buffer adition.

Sample	Proportion	pH	pH with buffer 5.5
UH	1:5 (soil:water/ w:w)	5.70	
H300 soil:water extract	1:2 (soil:water/ w:w)	7.93	
H500 soil:water extract	1:2 (soil:water/ w:w)	8.20	
Mix UH: H300	1:1 (bacterial suspension: extract)	7.89	5.60
Mix UH: H500	1:1 (bacterial suspension: extract)	7.91	5.66

shrubs species). Complex soil samples were formed mixing different subsamples from the 5 first cm of soil after litter removing, following 4 lineal transects. Samples were sieved (<2mm) and air dried before heating treatment and the remainig soil analyses.

Soil from the unaltered area was submitted to different heating treatment: 300 and 500 ^{o}C during 20 min to simulate moderate and high intensity fire, respectively, in a muffle furnace pre-heated at the corresponding temperature 30 min before soil heating.

2.2 SOIL:WATER EXTRACT PREPARATION

Heated (H300 and H500) soil samples were used to prepare soil:water extract (1:2, w:w), weighting 25 g of each soil and adding 50 ml of distilled water and shaking during 2h in a magnetic shaker. After shaking, the mix was subjected to two different filtrations:

- 1st filtration to separate soil particles from liquid extract
- 2nd filtration to sterilize the extract using sterile syringe filter 0.8 µm pore

After filtration steps soil:water extract was introduced in microtubes with bacterial suspension and ^{3}H-Leuncine mix and incubated during two hour.

2.3 BACTERIAL GROWTH MEASUREMENT

Bacterial growth was measured with the leucine (Leu) incorporation technique, which is a relative estimate of bacterial growth (Bååth et al., 2001). The soil used to prepare bacteria suspension was the same soil from unaltered area (UH) used to heating, but incubated during 4 days before the leucine measurement, at 50% of water holding capacity and with 2mg g^{-1} dry soil of alfalfa:straw (1:1, w:w) mix, added to stimulate the bacterial growth before contact with heated soil:water extract. The day of bacterial growth measurement 1 g of incubated soil was mixed with 20 ml of distilled water, vortexed (3 min) and centrifuged (10 min at 1000 x g) to obtain bacterial suspension (UH).

Bacterial suspension was incubated with milli_Q water or heated soil:water extract (H300 and H500) with and without buffer in 1:1 (v:v) and incubated during 2h before measurement of ^{3}H-leucine incorporated by bacteria.

2.4 PH CONTROL

After the measurement of bacterial growth the pH of incubation mix was measured (Table 1) and the experiment was repeated in order to isolate possible influence of pH changes induced by heating. Buffer pH 5.5 was added to bacterial suspension and soil:water extract to form the incubation mix keep a proportion 9:1 (v:v), allowing bacterial growth at the same pH of UH original pH before heating.

3 RESULTS

3.1 HEATING EFFECT ON PH SOIL:WATER EXTRACT

Soil:water extract and bacterial suspension pH was mesured after the first measurtement of bacterial growht (Table 1). Soil heating increase markedly pH from 5.20 in the original bacterial suspension to 7.91 in the mix of bacterial suspension and 500 ^{o}C heated soil:water extract. After application of 5.5 pH buffer mix the pH to bacterial growht will be 5.6-5.7.

3.2 HEATING TREATMENT EFFECT ON BACTERIAL GROWTH

The first bacterial growth measurement, without the addition of pH buffer show us the effect of heating and pH changes. In general, bacteria incubated with heated soil:water extrac show significant lower growth than bacteria incubated with water (Figure 1).

After repeat the experiment adding 5.5 pH buffer, bacteria incubated with heated soil:water extrac increase their

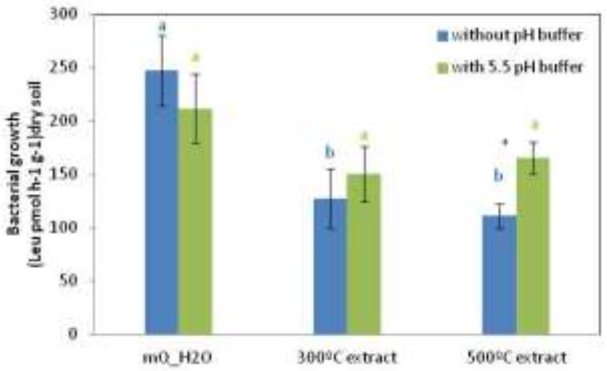

Figure 1. Mean values (± SE, n=4) of bacterial growth estimated by leucine incorporation technique, under different incubation conditions (see section 2.3). Different letter with the same colour show sinificantly different mean values (Tukey's test, P < 0.05). "*" means significant differences in bacterial growth due to the addition of pH buffer (t-test, P < 0.05).

growth compared with unbuffered ones, although this differences were significant only in 500 ^{o}C heted soil:water extract where soil heating pH-induced change was more marked. When compare bacterial growth between different heating tratment in buffered samples, we observe mean values of bacterial growth incubated with heated soil:water extract continue below the one incubated with water although statistical differences were not found in this case (Figure 1).

4 DISCUSSION

4.1 EFFECT OF PH INCREASE IN BACTERIAL GROWTH

Fire-induced pH increases are mainly attributed to the degradation of organic acids (Certini et al., 2005) and the increase of Al and Fe oxides (Fernádez et al., 1997). In our experiment original unheated soil pH was about 5.7 while pH of heated soil-water extract was always higher than 7.5, reaching the highest values with the highest temperature of heating. The pH increase usually favored bacterial proliferation (Rousk et al., 2009) if compare with fungi. Nevertheless in our experiment, bacterial growth after heating showed a significant decrease, which was attenuated when pH was reestablished by buffer addition. It is possible that bacterial community of the acid soil was adapted to the low pH conditions, and thus was not impaired by them, as described in other soils (Bååth, 1996). In addition, the reestablishment of pH was no enough to recover the same bacterial growth in the heated soil:water extract indicating the presence of some other factor affecting microbial growth overlapped with pH alteration. Other studies have manifested bacterial (Díaz-Raviña et al., 1996) and fungal growth (Widden & Parkinson, 1975) inhibition by heated soil extract, and previous studies in the same area have shown the existence of some inhibitory factor of viable and cultivable microorganisms in heated soil:water extract based-culture media (Bárcenas-Moreno et al., 2012). Nevertheless, the isolation and characterization of this factor is an future aim to study.

5 CONCLUSIONS

In this short preliminary experiment we have isolate the effect of pH increase and possible microbial inhibitory factor with an independent of culture methods, evaluating bacterial growth response when natural microbial community is incubated in heated soil:water extract. The results obtained highlight the importance of pH alteration induced by soil heating. Nevertheless there are some traces indicating that other factors could be limiting bacterial growth. More complete studies are need to identify possible inhibitory compounds and different plant communities associated.

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