Molter Grant Scientific Report

1) Purpose of the visit

This Molter fellowship, held at Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) in Birmensdorf (Switzerland) from 2^{nd} of January to 2^{nd} of April 2012, with the guide of Dr. Frank Hagedorn, was planned for conducting microbial analyses on soil samples taken from Mediterranean ecosystems affected by different land uses (agricultural field, abandoned fields and forest) and different soil type (calcareous and non calcareous soils). These typical Mediterranean plots have been previously studied within at the University of Barcelona, in which carbon content and physical soil parameters among the ecosystems were characterized.

We hypothesize that, in calcareous soils, carbonates have an important role in the physical and chemical protection of organic matter. The protective role of carbonates will be different in soils depleted with organic matter (arable and grassland) than in soils rich in organic matter (forest soils). So, physical soil parameters, as soil type, and different land uses may control microbial community composition and activity.

The final purpose of this stage consisted on my personal traineeship on microbial methodologies (PLFA's analysis and microbial respiration) in order to develop and apply them in the future at the Soil science unit of the University of Barcelona.

2) Description of the work carried out during the visit

The experimental design consisted of a 2 factor model: land-use and soil type. Based on a previous soil survey, we selected soils from 3 different areas close to Barcelona. Areas with carbonated and non carbonated soils were similar in climate (Mediterranean) and different in parent material. In each area, we sampled soils from 3 different land uses: agricultural field (arable), abandoned field (grassland) and forest; with similar texture and similar climatic, geological and topographic conditions. From each soil and land-use type we took samples from the upper 10 cm of the mineral soil from three different sites. Hence, for this study, a total number of 18 samples were taken (2 soil types x 3 land-uses x 3 replicates). The soil samples were size fractionated into four fractions: coarse sand (2000-200 µm), fine sand (200-50 µm), coarse silt (50-20 µm) and fine silt and clay (<20 µm) (Rovira et al. 2010). This part was carried out at University of Barcelona.

Soil analysis performed at WSL within the Exchange Grant consisted of

1) Phosholipid fatty acid (PLFA) analysis of each bulk soil sample to characterize the nature of microbial communities.
2) Study of the stability of the organic matter in bulk soil and each fraction by means of laboratory incubations of soils and measuring CO_2 respirations by using a LICOR-840 infra-red gas analyser.
3) Analysis of organic ^{13}C and ^{15}N in bulk soils and soil fractions.

At the description of the results, we will only show the results about PLFA and microbial respiration analysis because some isotopic analyses are still being processed at WSL laboratory.

As described in the proposed work plan, carbohydrate signature in particle sizes using HPLC were also planned for this exchange grant, as they could asses complementary results for PLFA's analysis. Nevertheless, after a profitable discussion with Dr. Frank Hagedorn, we decided to focus our analyses on the study of microbial functional groups and microbial respiration as this kind of assays are more suitable for reflecting possible soil microbial perturbations and soil organic matter dynamics.

3) Description of the main results obtained

Title:

Microbial communities in calcareous and non calcareous soils of the Mediterranean area across different land uses

Summary:

Microbial community (assessed as abundances of phospholipids fatty acids (PLFAs)) were performed to a set of soil samples obtained from two different Mediterranean soil types, calcareous soil and non calcareous soil and three different land uses: cultivated field, abandoned field and forest. We hypothesized that, in calcareous soils, carbonates have an important role in the physical and chemical protection of organic matter. This different protection caused for the soil type will be translated in differences in microbial communities and activity. At the same time, differences in land use and carbon content will also promote changes in soil microbial community structure.

Soil samples were taken from 0 to10 cm depth using a soil corer. Moist soil samples were sieved (<4mm) and kept frozen until their analysis, which took place at the Unit of Forest soils and Biochemistry, at Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) in Birmensdorf (Switzerland), under the supervision of Dr. Frank Hagedorn. In total, 18 samples were analyzed, 9 samples for each soil type (carbonated and non carbonated soils) distributed in three different land uses (cultivated field, abandoned field and forest). There were 3 replicates for each experimental condition. Water content and total organic C (TOC) in soils were measured. We determined the PLFA abundance of soils, which are biomarkers for specific groups of microorganisms, following the procedures described by Frostegard et al. (2011). After lipid fractionation into neutralipids, glicolipids and phospholipids, phospholipid fatty acids were methylated at 60 °C for 2 h using trimethylchlorosilane and methanol (1:9 v/v) (Thiel et al., 2001). We measured microbial activity by incubating soils and measuring CO_2 production 0, 1, 7, 14, 20, 35, 49, 63, 77, 91, 105 and 119 days after the incubation started.

The results showed that despite TOC content was higher in forests (especially in carbonated soils) than in fields and abandoned fields, forests showed the lowest values of total PLFA per gram of organic carbon (Fig. 1). TOC content increased with cultivated field < abandoned field < forest.

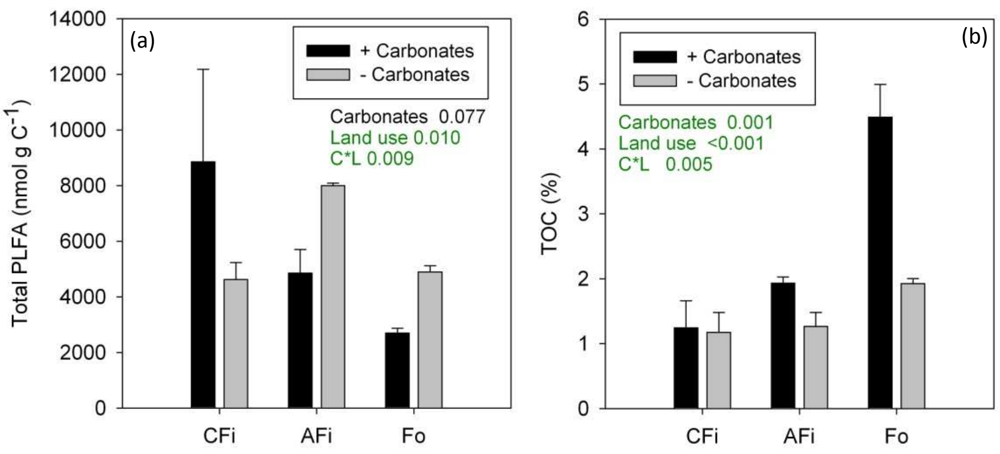

Figure 1. Total PLFA biomass (a) and Total organic C content (b) for all land uses (cultivated field (CFi), abandoned field (AFi) and forest (Fo)) and for different types of soils (soils with carbonates and without carbonates). Error bars represent standard errors (n=3). Only significant differences were shown at the figures (p<0,05).

PCA analysis carried out with all microbial PLFAs, clearly separated carbonated and non carbonated soils along the first principal component (Fig. 2a). Forest soils were separated from cultivated and abandoned fields along the second component PC (Fig. 2b).The soil microbial community composition showed different patterns depending on the soil type and land use. In carbonated soils representative PLFA of gram positive and gram negative bacteria per gram of organic carbon had higher values in fields than in forests (Fig. 3a and 3b). However, in non carbonated soils the highest values for the same PLFA were in abandoned fields. Actynomicetes didn't show significant differences in any case, but fungi were sensitive to different soil type showing higher values in non carbonated soils than in carbonated soils (Fig. 3c,g and 3d,e).

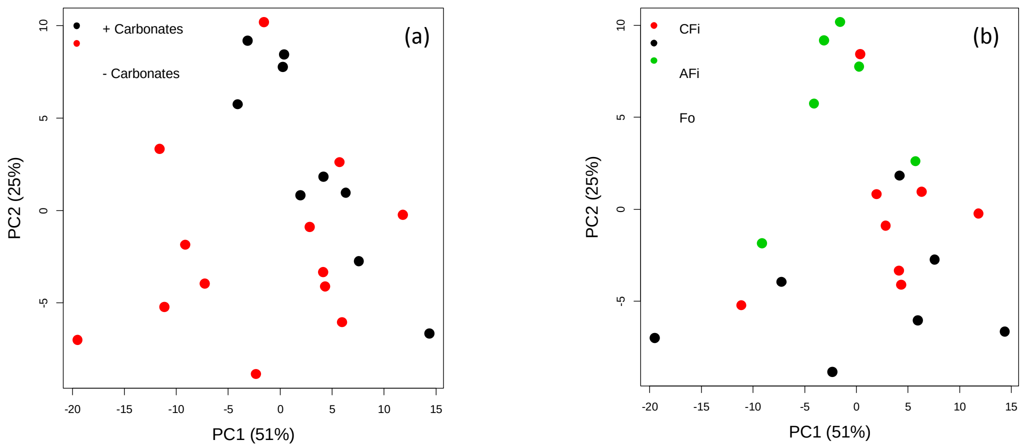

Figure 2. Principal component analysis of the PLFA data set performed for soil type (a): calcareous (black circles) and no calcareous soils (red circles); and for land use (b): Cultivated fields (CFi)(red circles), Abandoned fields (AFi)(black circles) and Forest (Fo) (green circles).

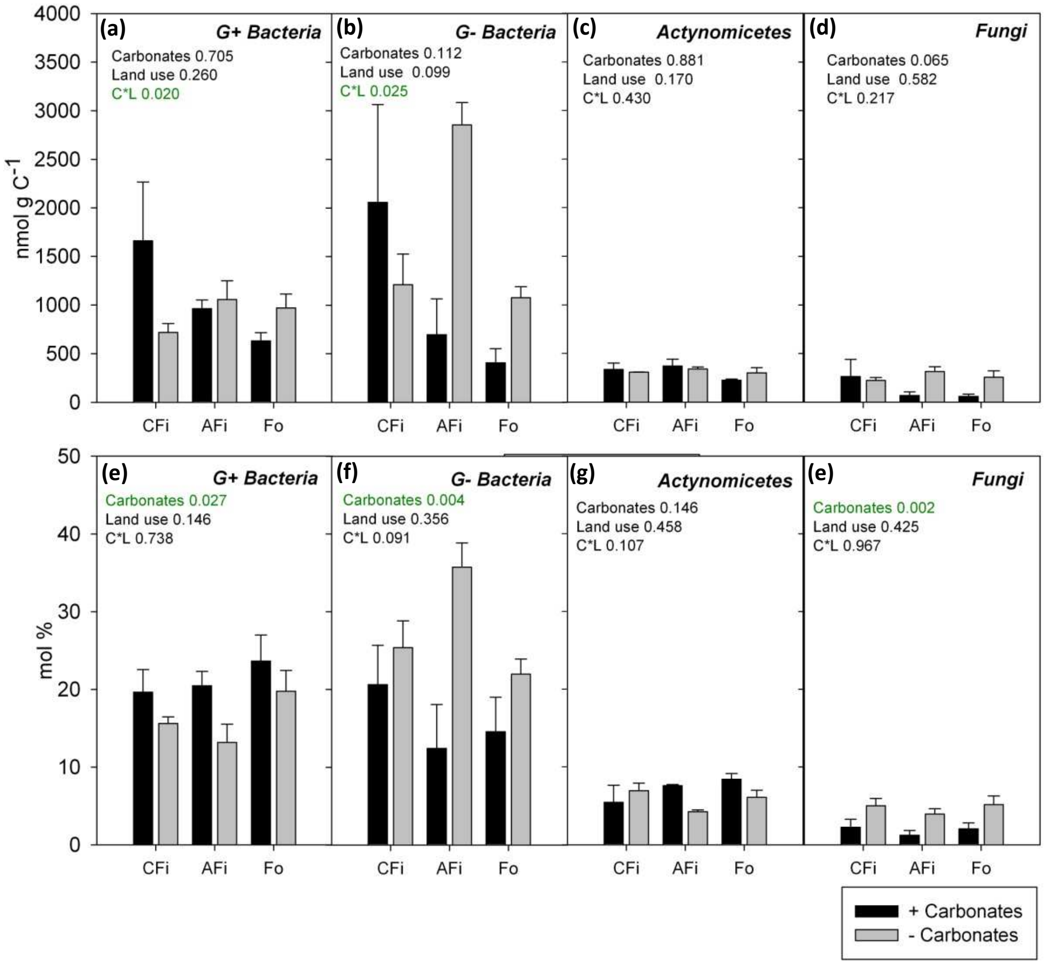

Figure 3. Values of various PLFA biomarkers for all land uses (cultivated field (CFi), abandoned field (AFi) and forest (Fo)) and for two types of soils (soils with carbonates and without carbonates). Error bars represent standard errors (n=3).

Soil microbial respiration showed differences between carbonated and non carbonated soils depending on the land use (Fig. 4). In cultivated fields, C mineralization was higher in carbonated soils than in non carbonated soils. No differences were found between the two types of soils in abandoned fields and, in forests, lower C mineralization was found in carbonated soils compared with non carbonated soils.

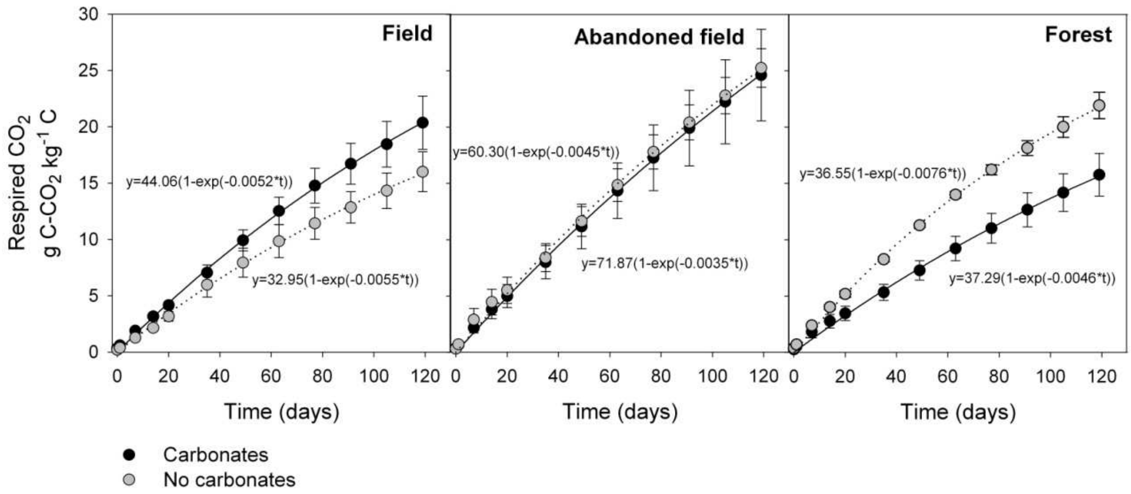

Figure 4. Cumulative carbon (C) mineralization of carbonated and non carbonated soils at three different land uses (Cultivate field, Abandoned field and Forest). Means and standard errors of three plots. The lines are fitted with a second-order exponential function.

In summary, as seen in the data obtained, changes in soil type and land use management can induce changes on the soil microbial communities and microbial activity. The results showed that the presence/absence of carbonates have different influence on the different land uses. Higher values for total PLFA and bacterial PLFA per gram of C have been found in cultivated fields in soils with carbonates. These high values of bacterial communities could compensate the low carbon content in cultivated fields and they would explain the higher C mineralization rates compared with forests. The difference in the abundance of fungal PLFA marker in non carbonated soils could be caused by differences in pH, as slight acid soils commonly showed higher fungal PLFA marker (Rousk et al. 2009). This high fungal PLFA would partially explain the higher mineralization curve in forest soils without carbonates.

4) Future collaboration with host institution *(if applicable)* and projected publications/articles resulting or to result from your grant

Owing to the interest of the results obtained in this profitable collaboration with Dr. Frank Hagedorn, our aim is to release these results in a future article dealing about the relationships between microbial activity in soil and selected microbial biomarkers in soils. Thanks to this first contact with Dr. Frank Hagedorn and the interesting research projects he is leading now I am really interested to keep contact with him and his team for future collaborations when I finish my phD.

References

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Rousk J, Brookes PC, Baath E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl.Environ.Microbiol. 75(6): 1589-1596

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