## ESF exchange grant 3893 – Scientific report

Title

# Isotopic investigation to reveal priming potentials under future elevated carbon dioxide concentrations.

Applicant

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Host

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The cooperation between the two institutes worked out very well. The work was carried out according to the plans and we were able to obtain interesting and promising results about the process of soil organic matter priming under future climatic conditions. Furthermore, the study improved during the external stay due to very inspiring and open minded discussions.

### **Purpose of the visit**

The priming of soil organic matter (SOM) is a major issue when investigating the impact of future climatic conditions on ecosystem behavior (Billings et al. 2010). SOM priming is known as an increased SOM mineralization, which is triggered by labile carbon (C) input like root exudates or plant litter. Dependent on the C quality the decomposition of the recalcitrant soil C pool can be speed up or slowed down, which results into either SOM mineralization or stabilization. Especially in terms of future climatic conditions we want to know how predicted elevated carbon dioxide ( $eCO_2$ ) concentrations will influence ecosystem C pools in the future.

The further described project was conducted to test the hypotheses that 1) "Elevated carbon dioxide concentrations induce soil organic matter priming." and, 2) "Priming is caused by a shift towards a fungal and Actinomycete dominated microbial community under elevated  $CO_2$  concentration." With the following lab incubation study we tested the long term effect of  $eCO_2$  on potential SOM priming.

#### Description of the work carried out

Soil was collected from the multi-factorial climate manipulation experiment Climaite (Mikkelsen et al. 2008) in March 2012. Shortly, the experiment consists of a full combination of the three climate relevant factors elevated  $CO_2$  (FACE,  $eCO_2$ ), extended drought periods (D) and increased temperature (T). Samples were taken from five treatments: ambient,  $eCO_2$ ,  $DCO_2$ ,  $TCO_2$ ,  $TDCO_2$ ). Soils were transported to the Scotland (The James Hutton Institute, Aberdeen, UK) and stored at 4 °C until further processing. Soil was sieved (2 mm Ø) before microcosm preparation. All samples were packed to a bulk density of 1 g cm<sup>-3</sup> of dry soil. Samples were adjusted to the same soil water content (SWC) which was maintained throughout the experiment. Microcosms were stored and handled at 8 °C.

An acclimatisation period of 18 days was allowed to achieve stable  $CO_2$  efflux rates. Soil harvests took place at days 0, 6 and 14. Soil microbial biomass was determined using chloroform fumigation. Isotopic values of the soil microbial biomass C (MBC) were determined from the  $K_2SO_4$  extracts as described in Garcia-Pausas and Paterson (2011) using wet oxidation.

An amount of 0.2 mg glucose per g dry soil (3 atom% <sup>13</sup>C-glucose) was added to half of the microcosms (glucose treatments). Remaining microcosms received dH<sub>2</sub>O only (controls). Glucose was added 6 days in a row. Measurements of soil CO<sub>2</sub> efflux rates were conducted at days 1, 2, 3, 4, 6, 7, 9, 11 and 14. The microcosms were closed and incubated for 6 hourse. CO<sub>2</sub> concentrations (EGM-4, PP-Systems, Amesbury, USA) and  $\delta^{13}$ C values (GasBenchII, Delta<sup>PLUS</sup> Advantage IRMS, Thermo Finnigan, Bremen, Germany) of the head space gas were measured.

The proportion of glucose- and SOM-derived C in gas samples were calculated using a two end-member-mixing model as described in Garcia-Pausas and Paterson (2011). Glucose- and SOM-derived portions of CO<sub>2</sub> efflux rates were calculated from the proportional contribution of the two sources and multiplied by the total soil CO<sub>2</sub> efflux. Proportions of C sources (glucose or SOM) in MBC were calculated using the isotopic values of the MBC. Total amounts of C in the microbial biomass were calculated using the  $k_{EC}$  factor (Vance et al., 1987; Garcia-Pausas and Paterson, 2011). The amount of primed SOM was calculated by subtraction of SOM-derived CO<sub>2</sub> in the control treatments from the SOM-derived CO<sub>2</sub> in glucose treated microcosms. The amount of primed SOM in MBC was calculated the same way.

It is planned to extract microbial phospholipid fatty acids (PLFAs) from soil samples at all time points to gain insights into the soil microbial community and its behaviour under different climatic treatments. Furthermore we want to gain knowledge of C sources contributing to the MBC of different microbial functional groups.

### **Description of preliminary results**

MBC derived from SOM didn't change significantly from day 0 to day 6. However, glucose amendment increased the MBC compared to controls. There was a significant effect of time on MBC derived from SOM and glucose from day 6 to day 14: MBC in control treatments (SOM-derived) dropped by ~80 %. SOM-derived MBC in glucose treatments dropped by ~70-90 % whereas the glucose-derived MBC dropped by ~20-80 % with the lowest drop in the future and the highest drop in the ambient treatments.

Total CO<sub>2</sub> production was lowest in eCO<sub>2</sub> plots and was similar for ambient and future treatments in control and glucose amended microcosms. In glucose treated microcosms the total amount of accumulated SOM-derived CO<sub>2</sub> was ~28 % of total glucose-derived CO<sub>2</sub> for all climate treatments. Comparing the total amount of accumulated SOM-derived CO<sub>2</sub> from controls and glucose treatments there was a significant difference (and thus priming) in ambient and eCO<sub>2</sub> plots but not in the future combination. Glucose-derived CO<sub>2</sub> showed an exponential increase during the time of glucose addition during days 1 to 4 (Figure 1). The amount of glucose-derived CO<sub>2</sub> decreased gradually after stopping glucose amendment. There were no significant differences between climate treatments.

The ratios of SOM- and glucose-derived  $CO_2$  effluxes show a sharp decrease in SOM-derived  $CO_2$  from day 1 to day 2 (Figure 1) followed by a constant ratio of both C sources until day 10 and an increased SOM-derived  $CO_2$  efflux at the last two measured time points. Negative values derived from respiration rates that became negative due to less SOM-derived respiration in glucose treatments than controls. Negative values of primed SOM-derived  $CO_2$  mean SOM stabilization (also known as negative priming).

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Interpretation of preliminary results

It was shown that eCO<sub>2</sub> has the potential to induce mineralization of recalcitrant SOM pools (Carney et al., 2007). In this respect the investigated climate manipulation experiment runs since seven years and processes of SOM priming could be going on or might have happened in the past. However, in the current lab incubation study climate treatments had no significant effect on SOM priming. All climate treatments showed the same soil CO<sub>2</sub> efflux patterns and similar MBC development when labile C was added. Thus, this study could not verify that eCO<sub>2</sub> induced SOM priming according to hypothesis 1. However, the future climate combination didn't show a sharp increase in the MBC development as the other treatments and consequently the effect of labile glucose addition didn't trigger the microbial community to the same extent.

Microbial community responses reflected in total MBC values could be a result of adaptation to the new soil carbon pools manipulated with the future climate combination. The slightly different behavior of the MBC and the fact that no significant difference in SOM-derived soil CO<sub>2</sub> efflux was found suggests a development towards a new ecosystem equilibrium. On the other hand the other tested climate treatments didn't go through the same soil processes within the last years and seem to be closer to the ambient treatment in terms of MBC development when labile C was added. Microbial PLFA profiles will help to identify possible differences in the microbial community that caused the different behavior of MBC development in ambient and eCO<sub>2</sub> plots compared to the future simulation.

Our assumption that differences between climatic manipulated treatments were caused by a change in microbial community composition needs to be proven with PLFA profiles. Differences among treatments are supposed to be a result of different microbial activities and a complete change in microbial community composition. The PLFA approach is only an indication of community change because it's only displaying the functional group level but not species. Redundancy of soil microbial species over time can occur but cannot be proven with PLFA profiles. Thus, the slightly different behavior of MBC in future plots can be a result of species change that was facilitated by the full combinational climate treatment but their individual response in e.g. response to labile C input can be different. For example their ability of producing enzymes that break down easily available C can be limited because of higher amounts of plant litter inputs.

Even though we were not able to show differences in SOM mineralization triggered by simulated future climatic conditions we were able to induce the process of SOM priming under ambient and eCO<sub>2</sub> conditions. Furthermore, all treatments followed the same response pattern to labile C additions (Figure 1). The resulting patterns can be explained with a process focused approach that proposes labile C as activator of the soil microbial community (Cheng and Kuzyakov, 2005). The same results can be explained with an ecological concept of r- and K strategist that form the soil microbial community (Fontaine et al., 2003)

#### References

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### Future collaboration

On the basis of the presented experiment a study on mRNA genes involved in SOM priming is carried out by Thomas Freitag at "The James Hutton Institute". Furthermore, the experiment established a good basis for further experiments in cooperation between the two institutes.

#### Projected publications/articles resulting from the grant

The presented work will be completed with the microbial PLFA analyses and submitted to Global Change Biology or Soil Biology and Biogeochemistry.