Final report MC - PIEF-GA-2013-624849-LEAFISOTRENDS

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1. Cover page

The progress of the work was organized in order to achieve the 4 milestones that were originally planned.

On a first step (milestone 1), target plant species had to be selected from the more than 2500 plant species found in Switzerland and in the herbaria available for the project. Plant species with broad and narrow habitat distribution from low-, mid- and high-altitudes were chosen. In addition, these plant species have been organized in functional groups, defined in terms of plant life forms and ecological requirements. A total of 85 plant species have been chosen. Between 40 and 50 samples have been analysed per plant species, with the collection of a total number of 3334 herbarium specimens.

Collection, preparation and analysis of this large amount of samples (milestone 2) proceeded simultaneously. All samples have been prepared, weighted in an electronic high precision scale and encapsulated for their carbon, oxygen and nitrogen isotope analyses by isotope ratio mass spectrometry. In addition, the specific leaf area of all these samples was measured. We also performed a cellulose extraction test and we found that bulk leaf material can be used for the isotopic analysis of herbarium samples. Avoiding the extraction of cellulose substantially reduced time and cost of sample preparation for isotopic analysis. By the end of the project, the vast majority of the analyses has been performed, with the exception of about 500 hundred samples that still need to be analyzed for their oxygen stable isotope composition. Analyses are currently on their way. This delay was due to past and unexpected interruptions in the stable isotope facilities (shut down of the external facilities used and subsequent laboratories renovation when the analysis where ready to be performed at the host institution).

I received the support of the host institution to prepare the large amount of samples included in this project: Prof. Kahmen provided me with more than 450 student assistant hours and the University of Basel granted me with a total of 480 student assistant hours through the "Stay on Track" funding program.

At the end of the project, a unique database was created, which includes stable-isotope-based physiological information of about 3334 herbarium specimens collected at several locations within Switzerland and from year 1820 until 2015. The size of the database allows stratifying the data according to multiple criteria (i.e. plant species environmental preferences, plant species associations with mycorrhiza, N deposition levels, elevation, plant functional types, climate at region of collection). The assessment of plant physiological changes with time according to these multiple criteria is critical to shed light on how these factors influence the long term physiological responses of plant species from different habitats or with different ecological life history strategies to changes in climate. Understanding the variability in these responses is critical to improve predictions of dynamic global vegetation models in the context of climate and environmental change.

So far, several important findings resulted from the analysis of the data and more analysis will be performed in the future given to the large potential of this database. We found that herbaceous plants have increased their intrinsic water use efficiency in response to increasing atmospheric CO_2 concentration but that this increment was higher in plants from higher elevations. This can be explained by the higher efficiency of CO_2 assimilation of alpine plants compared to plants from lowlands. There were also different changes in water use efficiency among different functional groups, with grasses and forbs showing the higher increments compared to sedges and legumes. The data also show a positive correlation between C and O isotopic values. This is an important finding as it shows a tight coupling of carbon and water cycles at the scale studied in the project. In fact, increasing leaf oxygen isotope composition with time suggests that the above-described changes in water use efficiency are the result of both, increasing carboxylation and decreasing stomatal conductance. Ongoing modelling exercises with the Peclet-modified Craig Gordon Model will allow to quantify the relative contribution of changes in stomatal conductance and carboxylation to the observed changes in water use efficiency. This information in particular will be highly novel and essential for the validation and improvement of dynamic global vegetation models and their ability to predict environmentally induced changes in the carbon cycle. In addition, herbaceous plants showed a decreasing N isotope composition over time, which may indicate progressive tightening of the N cycle due to higher biological activity with increasing atmospheric CO_2 concentration.

A third objective of the project (milestone 3) was the simulation of plant physiological responses to climate change in growth chambers, under controlled conditions. In collaboration with Prof. Jürg Stöcklin (from the University of Basel), we tested how temperature (4°C difference) influenced the physiological performance of two closely related species that can hybridize but have very contrasting altitudinal distribution ranges: *Campanula rotundifolia* and *C. scheuchzeri*. We assessed the effect of temperature on both fertility and leaf gas exchange processes of both species, getting indications about the overall performance of these plants, something that has seldom been evaluated in current ecophysiological studies. Both species increase their respective number of flowers under warmer conditions. Maintaining flowers is costly in terms of transpiration. Only *C. rotundifolia* down-regulated its leaf stomatal conductance under warmer conditions, partially compensating the water lost produced through the flowers. This mechanism could explain why the distribution of *C. scheuchzeri* is restricted to wetter locations from higher elevations compared to *C. rotundifolia*.

Currently, four manuscripts are being prepared (milestone 4) and they are expected to be published in leading peer review journals (e.g. Science, Global Change Biology, Plant Cell & Environment or New Phytologist). An important delay in the achievement of this objective was due to the fact that I went on maternity leave for about 4 months in 2016.

From January to March 2017 I was also on a stay abroad at the University of Lund (Sweden) hosted by Professor Pål Axel Olsson in order to assess if mychorrizal associations in sedge and grass species had changed over time and how this could have influence long term plant responses to global change. The work consisted of an optical analysis of mycorrhizal structures formed and preserved in herbarium roots of 80 specimens. The stay away was completed with total satisfaction. There are indications of variable colonization intensities of roots by mychorriza over time, with the highest colonization intensities around the 1950s and subsequent decline, which may be related to N deposition levels and changes in the N cycle.