STReESS: Studying Tree Responses to extreme Events: a SynthesiS

Effects of Experimental Drought on the Carbon Allocation of *Vaccinium* species (CAllVac)

STSM Report

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Purpose of the STSM

The aim of this project was to gain insight into the effects of an extreme summer drought event on the carbon allocation of alpine dwarf shrubs. We studied two dwarf shrub species dominating the encroachment process of a subalpine grassland in the Tyrolean Alps: *Vaccinium myrtillus* and *Vaccinium gaultherioides*. Our main hypotheses were:

(i) Drought will slow down belowground carbon allocation.

(ii) Drought will increase the relative allocation of recently fixed carbon to belowground organs at the expense of aboveground plant parts.

(iii) Drought will decrease the NSC concentration in the studied plants, since C use will exceed C assimilation.

(iv) Drought will increase relative carbon allocation to sugars at the expense of starch.

(v) Leaf and rhizome/root respiration rates and the proportion of recently assimilated C as respiratory substrate will be decreased under drought.

Description of the work carried out during the STSM

Experimental set-up

The experimental area is a subalpine grassland near Kaserstattalm, Stubai Valley (Austria), colonized by dwarf shrub patches. The experiment was set up on the 3rd July 2014 and consisted of 3 blocks, each one with a rain-out shelter and a control plot (Fig. 1), all of them placed on mixed patches of *Vaccinium myrtillus* and *Vaccinium gaultherioides*, together with other plant species (*Agrostis capillaris*, *Chaerophyllum hirsutum*, *Luzula multiflora*, *Festuca ovina*, *Deschampsia flexuosa*, *Briza media*, *Potentilla erecta*, *Hypericum maculatum*, *Crepis conyzifolia* and *Campanula scheuzeri* amongst others).

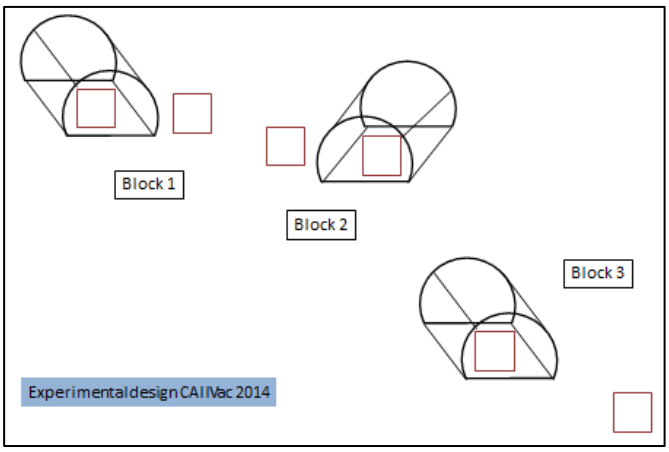

Fig 1. Experimental design CAllVac

We installed two sets of light (PAR), air temperature and air humidity sensors, one outside the rain-out shelters and one inside one of the rain-out shelters. In addition, we installed one soil temperature sensor and one soil moisture sensor in the main rooting horizon of each plot. We trenched the drought-treated plots up to 20 cm to avoid runoff water getting inside the plots. The core plots consisted of $1m^2$, which were delimited by a frame that was used to set the chamber for NEE measurements and for the CO_2- labelling.

Pre-labelling measurements

From the 19th July onwards, we took stomatal conductance measurements at midday on three non-consecutive sunny days with a leaf porometer to check whether, when and to what degree the stomatal conductance was reduced under drought. Measurements were taken on the same leaves, which were marked on the first days. If leaves were damaged, measurements were taken in other leaves on the same twig. On two sunny days we also took measurements

of NEE (net ecosystem exchange) and DR (dark respiration) using manually operated ecosystem chambers equipped with a CO_2 sensor.

Labelling

After 7.5 weeks of the installation of the rain-out shelters we started the labelling, which took place in three days, one for each block. We labelled every plot for 70 minutes after the first pulse.

Plant sampling and respiration measurements

Each sampling consisted of the harvest of one ramet of *V. myrtillus* and one ramet of *V. gaultheriodes*, including both above-ground and below-ground material. The sampling times were the following: 0h (pre-labelling), 2h, 4h, 24h, 48h, 96h (4 days) and 192h (8 days) after the start of labelling, respectively. We detached some leaves and part of the rhizome for respiration measurements and we microwaved the rest of the ramet for subsequent analysis of non-structural carbohydrates (NSC) and nitrogen.

We took leaf and rhizome/root respiration measurements at the following sampling times: 2h, 24h, 48h (2d), 96h (4d). Moreover, for Block 1 we also took respiration measurements at 0h (pre-labelling), which will allow us to establish a background isotopic signature for estimating the ^{13}C excess in respiration for all the plots. We placed the material for incubation in 150 mL Erlenmeyer flasks in a polystyrene box filled with water at 15°C for 40 minutes. We took 5 gas samples for each incubation sample for subsequent isotope and Keeling plot analysis. Once in the lab we placed the samples in the drying oven at 60°C for 72 h and weighed them.

Water potential measurements and leaf traits

Once we had collected all the samples for the isotope analyses, we collected some twigs for water potential measurements. We collected 3 twigs of each species per plot before sunrise and 3 twigs of each species per plot at midday. We cut the twigs with a sharp razor blade to obtain a well-defined cross-section. We kept the twigs in plastic bags completely sealed and once in the lab, we proceeded to measure the water potential using a pressure chamber (Scholander bomb). This part of the experiment was supervised by Prof. Stefan Mayr.

Moreover, we collected leaf samples for specific leaf area (SLA), leaf dry matter content (LDMC) and leaf relative water content (LRWC) measurements.

Ongoing lab work: carbon isotope composition and NSC analyses

At the lab at the University of Barcelona, all the plant material (leaves, new shoots, rhizomes and roots) will be grounded for analyses of NSC and C isotope composition. In addition, the carbon isotope composition of the respiration gas samples will be analyzed.

3. First results

We are preparing our samples for the analyses of the carbon isotope composition and the NSC, therefore we cannot present these results yet.

On the 18th August, *V. gaultherioides* stomatal conductance was reduced under drought ($F_{1,2}$ = 28.12, P = 0.034) but that of *V. myrtillus* ($F_{1,2}$ = 3.25, P = 0.213). On the same date, Gross Primary Production (GPP, calculated as the sum of NEE and DR) was clearly reduced under drought ($F_{1,2}$ = 51.96, P = 0.019).

Pre-dawn xylem water potential was significantly lower in drought plots than in control plots in both species ($F_{1,12}$ = 15.99, P = 0.009 and $F_{1,12}$ = 51.73, P = 0.019 respectively for *V. myrtillus* and *V. gaultherioides*). We did not find significant differences between treatments at midday.

4. Contribution of the results to the Action aims

Our study focuses on the effects of an extreme summer drought on alpine shrubs, which are a very important woody component of the treeline ecotone. Our results will bring essential knowledge on the impacts of drought stress on the carbon allocation of non-tree woody plant species at the treeline, which is especially important because so far studies on carbon allocation in alpine plants under environmental constraints have mainly focused on grasslands, but not on shrubs.

This report may be posted on the Action website.

Confirmation by the host institution of the successful execution of the STSM can be found in the attachment.