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Master Thesis

Are climate induced changes in alpine vegetation reflected in plant function traits? – A case study from Hochschwab, Austria.

submitted by

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Colophon

This document is a Master Thesis for the completion of the Master of Science at the University of University of Natural Resources and Life Sciences, Vienna, Austria.

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Summary

Climate change is expected to impact the future of biodiversity, especially in mountain areas where plant growth is strongly limited by low temperature. Indeed, long-term monitoring has documented changes in alpine species composition and diversity on mountain summits in Austria and elsewhere. Understanding how and why plants respond to environmental changes will help to predict future changes and potentially design measures to protect biodiversity.

To test the potential to explain changes in species abundance, we measured functional traits related to temperature, water, and nutrients for 29 species from Hochschwab, Styria. We explored which functional traits are related to Landolt environmental indicator values (EIVs), calculated community-weighted mean (CWM) for traits and EIVs per plot and tested if these changed. We used data from a long-term monitoring study of changes in community composition by GLORIA, obtained from the same location between 2001 and 2015, to test if changes in the abundance of species can be explained by either EIVs or functional traits.

While 20 species increased, and 24 species decreased (out of a total of 140 species) in abundance by > 40%, we found that the overall plant community did not change. The CWM of the functional traits also did not change significantly. This is likely because the community is strongly dominated by one species, *Carex firma*, which did not change much in abundance.

Testing the change in abundance of 29 species with trait data we found that the change in abundance was significantly correlated with plant height, leaf shape and also with the second principal component of a principal component analysis that included all traits. This study thereby shows the potential to use functional traits related to environmental adaptations to explain and understand the impact of climate change on community composition.

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1. Introduction

1.1 Climate change in the Alps

Climate change is predicted to have massive impacts on the future of biodiversity, especially in mountain areas (Bellard, et al., 2012; IPCC, 2019). Climate warming in high mountain areas has outpaced the global warming rate, with a likely range of 0.3 ± 0.2 ℃ per decade, vs. the global rate of 0.2 ± 0.1 ℃ (IPCC 2018). Local warming rates also differ between season, with the warming being more profound in summer and spring in the European Alps (Auer et al., 2007; Ceppi et al., 2012, as cited in IPCC, 2019).

The alpine environments of Europe, which are considered to be the zone above the tree line- until the permanent snow line, are hotspots of biodiversity (Körner, 2021; Nagy, et al., 2003). They contain 20% of all native European vascular plant species and 17% of those species appear in the Alps, of which 10% of those species and subspecies are endemic.

Thuiller, et al., (2005) projected distributions for 1350 European plant species under different climate scenarios, and found that many European species could be threatend in the future. They used climate projections from the IPCC and a niche-based framework to project species extinction risk. The most severe climate scenario is called the A1 scenario, which describes rapid economical and population growth and a CO_2 concentration of 800 ppm in 2080. With this scenario, 22% of all the species used in their research become critically endangered. Specifically for the Alps, Dullinger et al., (2020) modeled plant diversity changes related to land-use and climate change, and found that alpine species are predicted to lose species based on climatic, and not land-use scenarios. However, models can only tell us so much, which is why it is important to monitor the actual changes in species abundance.

To understand and assess the extent of climate-driven changes, the “Global Observation Research Initiative in Alpine environments” (GLORIA, https://gloria.ac.at) operates a world-wide long-term monitoring network with permanent plots in various alpine environments. The aim is to provide standardized and quantitative data on the species richness, composition, and abundance, to quantify the changes in species and vegetation patterns in various permanent plots that are resurveyed every five to ten years. In 2001, a long-term study with permanent plots was set up by the GLORIA network on the Hochschwab mountain range in Styria, Austria. These plots were re-surveyed in 2008 and 2015, and this is also the area where our study takes place.

According to the IPCC Special Report on the Ocean and Cryosphere in a Changing Climate, the composition and abundance of species has changed in recent decades in high mountain ecosystems (IPCC 2019). Steinbauer, et al. (2018) found that species richness had increased (87% over 145 years) on mountain summits in Europe, which has accelerated in the most recent years in parallel with increasing temperature. Due to the increase in temperature at high elevations, there has been a general decline in snow and glaciers. This allows previously absent species to grow at higher elevations (H. Pauli, et al. 2012). As a consequence, there may be a high risk that many cold-adapted species, including endemics, will face local extinctions, particularly if stress-tolerant, slow growing alpine species are outcompeted by plants that benefit from the higher temperatures.

By having a model that can predict or explain the species abundance, we aim to better understand why certain species become more common, while others less so. There are tools for predicting and explaining the change in species abundance. One of the most indispensable tools to predict responses of plants to climate change is the use of functional traits (Lavorel and Garniers 2002). They are increasingly applied in plant ecology to investigate the relationships between traits and environment. Another tool is the use of Ecological indicator values (EIVs).

1.2 Functional traits in the alpine environment

Plants that occupy high alpine and nival zones are considered as living in an extreme environment (Lütz 2012). These plants have developed a range of adaptation strategies that allow them to survive the low temperatures, long snow cover and frequent frost risk. These adaption strategies are reflected by functional traits. As described in Violle, et al., (2007), a functional trait is defined as *“any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole plant”.* Various traits are important for determining whether or not a species will survive in a certain environment. As Hoover, et al. (2014) explains: “knowledge of the traits that influence dominant species responses to and recovery from climate extremes will be key for predicting ecosystem dynamics and function in a future with more extreme events’’.

Functional traits can be used to demonstrate stress resistance mechanisms of plants and may thus explain effects of climate change. For instance, leaf area and vegetation height are traits that are related to the the timing of snowmelt, with taller plant species being more likely to establish in inside snowpatches (Venn, et al. 2011). Functional traits can be devided into ‘soft’ and ‘hard’ traits. Hard traits are linked to the functioning of a plant, like physiological traits, and capture a precise function of the plant. These traits are often hard to measure and expensive, which is why soft traits are often measured as proxies or surrogates (Violle, et al. 2007).

Common soft traits are leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC) and adult plant height. The SLA is a ratio between the leaf area and the dry mass, which reflects the relative growth rate of the plant (Mengzhou Liu, et al. 2017). Since the SLA reflects growth, plants with a high SLA are considered fast growing species, and plants with a low SLA are considered slow growing species. Species that live at high elevations are often associated with a low SLA, since SLA decreases as an adjustment to temperature at increasing altitude (Scheepens, Frei and Stöcklin 2010). The LDMC is the ratio between the dry mass and saturated mass of the leaf, which indicates plant resource usage (Díaz, et al. 2016). The adult plant height is an important trait for mechanical strength, and to determine a species ability to compete for light (Moles, et al. 2009). Alpine plant species are shaped by extreme conditions and grow close to the ground. This is due to high winds, snowpack, and low temperatures (Körner 2021). The inflorescence height represents plant reproduction: plants with taller inflorescence stems can have improved pollination chances or an increased dispersal of seeds by the wind (Raven, Evert and Eichhorn 2005). And finally, the chlorophyll content is a measure of the plant’s photosynthetic capacity.

Besides these soft traits, we also looked at hard traits that are more directly related to frost tolerance, drought resistance and nutrient relations.

Frost tolerance

In high elevation environments, one of the main limiting factors is low temperatures. Plants exposed to extreme low temperatures during winter need to can protect themselves by frost hardening, but snow cover strongly limits temperature extremes (Hacker, et al. 2011). However, during the spring and summer months, when plants are no longer hardened, they can still be subjected to freezing temperatures. Due to the increase in temperatures, spring phenology in certain plants has advanced compared to previous years in the Swiss Alps, with the risk of frost exposure and potential damage at higher elevations (Vitasse, et al. 2018). Low temperatures affect all metabolic processes and decrease productivity and yield (Larcher 1981). When the temperature drops below the freezing point, frost damage can occur due to the formation of ice inside their cells (Snyder and Melo-Abreu 2005).

To survive, plants have developed different mechanisms for coping with low temperature extremes include avoiding freezing, resisting, or tolerating frost damage (Snyder and Melo-Abreu 2005). Plants can also avoid being frozen using a process called supercooling. The process is common in woody plants, and it permits the leaf and stem tissues to cool without freezing (Cavender-Bares 2005). It allows the cooling of a liquid below freezing temperatures, depending on the solute concentration. Another mechanism is osmotic adjustment. Supercooling and osmotic adjustment are both of these mechanisms involve the manipulation of the water potential. Osmotic adjustment allows the plant to reduce the amount of ice that is formed inside the plant, through the increase of concentration of solutes in the water (Snyder and Melo-Abreu 2005). With an increased concentration of solutes, water within the plant becomes more viscous, especially at low temperatures. This allows water molecules to diffuse at a slower rate so that ice nuclei are less prone to develop (Wolfe, Bryant and Koster 2002).

Drought resistance

As a consequence of climate change, the intensity and frequency of droughts are expected to increase due to an increased temperature and changing precipitation patterns (Gobiet, et al. 2014). Drought stress occurs when plants have a limited availability of water. Meteorological drought happens when there is a lack of precipitation over a longer period of time, along with an increased evapotranspiration due to high temperatures. However, drought not only depends on rainfall, but also on the water holding capacity of the soil and rhizosphere (Seleiman, et al. 2021). The study area (Hochschwab) is in the Northern Calcareous Alps of Austria and is composed mainly of Wetterstein limestone. Calcareous soils are generally drier, due to high porosity of the rock, while soils derived from siliceous substrates tend to be moister (Michalet, et al. 2002). Because calcareous rocks, which are mainly made up of dolomite, gypsum, and limestone, are highly soluble, these components are easily dissolved by rainwater, which means plants growing on this substrate are more prone to experiencing water deficits (Jiang, et al. 2020).

Earlier studies in alpine grasslands have shown that heat waves have minor effects on plants as long as there is water available (Boeck and Verbeeck 2011, Hoover, Knapp and Smith

2014, Boeck, et al. 2016). However, the effect of heat waves in combination with drought showed clear signs of stress in alpine plants.
Water deficit in plants has an effect on many of the processes and traits for plant growth and survival, and can impair different morphological, physiological, and biochemical processes and traits, like decreasing plant productivity, nutrient uptake, and can affect CO_2 fixation (Farooq, Hussain, et al. 2012). In order to cope with drought, plants have developed different strategies and mechanisms in order to be resistant. Drought resistance, as defined by May and Milthorpe (1962), is "the ability of plants to grow satisfactorily when exposed to water deficits''. Strategies include drought avoidance, tolerance, escape, and recovery.

Drought avoidance, which is the avoidance of stress conditions at the tissue level, is accomplished by promoting the reduction of water loss, closure of the stomata and having a well-developed root system for efficient water uptake, and water storage (Munns & Sharp, 1993 as cited in Meshram, et al., 2022; Farooq, et al., 2009). While all plants close their stomata to avoid water stress, there is a difference in how much water is lost after closing the stomata. This is called the minimum conductance and reflects the conductance to vapor diffusion across the epidermis once the stomata are closed, i.e., through the cuticle and any leaky stomata. This was shown to be an important component of drought tolerance, since a lower g_{min} means leaves will survive longer before experiencing critical water deficit (Duursma, et al. 2018). Some physiological and morphological features that allow the plant to be more drought avoidant are leaf size and thickness, and succulence. Generally, plants that have smaller and thicker leaves are more drought resistant (Esau 1960). Succulence is the ability of plants to store water in leaves, stems and roots and indicates the water storage capacity (Griffiths & Males 2017). A plant having thicker leaves can mean that they are able to store more water for extended periods of time.

Drought tolerance refers to ''the ability of plants to sustain a certain level of physiological activities under severe drought stress''. Osmotic adjustment (Farooq, Wahid, et al. 2009), allows the cell to decrease the osmotic potential, which helps maintain the cell water balance and the turgor pressure. Increasing the concentration of solutes lowers the turgor loss point (TLP), which is the leaf water potential at which the leaf starts to wilt (Bartlett, Scoffoni and Sack 2012). It thus enables a leaf to maintain cell turgor under water stress.

The hydraulic conductivity and xylem cavitation are also traits which are important for drought tolerance (Vilagrosa, et al., 2012). When the sap tension of the xylem of vascular plants becomes high enough, an embolism, which is the formation of vapour-bubbles, is formed. Emboli reduce the xylem hydraulic conductivity and can result in shoot dieback and plant death (Brodribb and Cochard 2009).

Finally, C_4 and CAM photosynthesis are also adaptations to water deficit and are associated with higher water use efficiency (Ward, et al. 1999). This is due to the fact that photo respired CO_2 is re-fixated (Ghannoum 2009).

Soil chemistry and nutrient availability
Plant growth at high elevations is not only limited by extreme temperatures and unpredicted precipitation, but also by the nutrient availability and soil chemistry (Körner 2021). In many areas, the species richness and species composition are highly influenced by

the soil pH and other pH-related chemical properties (Romeo, et al. 2015). As previously explained in the drought resistance chapter, Hochschwab is comprised of calcareous soils dominated by calcium carbonate ($CaCO_3$). The main soil type in Hochschwab is Cambisol, which has a high pH (>7) and affects nutrient availability (Romeo, et al. 2015, Poggio, et al. 2021).

Soil acidification is expected to increase due to the deposition of anthropogenic nitrogen (WallisDeVries and Bobbink 2017). The amount of nitrogen deposition has increased over the years, which has negatively impacted the species composition and diversity in many areas, including the Alps. The increased nitrogen content favours certain species, who can easier take up the excess nitrogen (WallisDeVries and Bobbink 2017). It can cause differences in the availability of nitrogen in the form of NH_4^+ or NO_3^-.

1.3 Ecological indicator values

Ecological indicator values (EIVs) represent estimates for individual species about their ecological optimum for selected environmental factors, expressed as ordinal numbers. The first EIVs for vascular plants in Western and Central Europe were developed by Ellenberg and co-workers, who published a list of indicator values in 1974 (Ellenberg 1974). More recently, Landolt (2010) created a similar list of EIVs for plants in the Swiss Alps.

One main advantages is that, at least in Central Europe, EIVs are available for most species in databases, without the need for time-consuming additional measurements in the field. They also represent semi-quantitative values of environmental variables, which could not be obtained by a single measurement. EIVs are commonly used as proxies for environmental variables, which may often be difficult or expensive to measure in the field. However, the use of EIVs has also been criticized as the values are derived from the field experience of plant ecologists and are thus subjective. They also do not represent the species in a specific habitat, and the same species may have a different response depending on their geographical location.

Bartelheimer and Poschlod (2016) reviewed existing literature which contained comparisons between species from controlled experiments and combined them with EIVs to test if it is possible to identify properties of species composition, in relation to environmental factors. They found that different plant properties(like SLA, or the total N-uptake) are related to EIVs for soil reaction, soil moisture and nutrients, but less for temperature. They conclude that the use of EIVs are an indispensable tool for predicting plant properties and species distributions (Bartelheimer and Poschlod 2016).

Earlier research has used EIVs to detect relationships between EIVs and plant functional traits (Steinbauer, et al. 2022, Paetzolt 2022). Paetzolt (2022) used linear models to predict the relation between Landolt R and a set of functional traits, and found that g_{min}, tCWC, height and SLA best explained the variation in the Landolt R value. While Steinbauer, et al. (2022) used a different EIVs, a proxy for temperature called TI. They calculated community-weighted means for this EIVs to show if the overall community shifted in a certain direction, and found that over time, the plant community composition shifted to one with a higher temperature preference. In this study, we use EIVs (Landolt, 2010) to show the spread of habitat preferences and to check whether or not EIVs can be used for explaining changes in species abundance.

1.4 Objectives

Climate change is affecting the composition, abundance, and diversity of alpine plant species and is creating winners and losers. By understanding why species respond differently to the changing climate, we hope to be able to predict which species are in danger of becoming extinct and possibly design strategies to help vulnerable species. For the present thesis this was done by investigating the relationship of species functional traits and ecological indicator values and the environment.

We measured different plant functional traits, some of which are specifically related to drought and frost resistance, and nutrient availability of 42 different species to explore the relationship between those traits and the change in species abundance between the years 2001-2015 in Hochschwab, Styria.

The objectives for this study were to:

- Distinguish alpine plants by functional traits, specifically traits related to drought resistance, frost resistance and nutrient relations.
- Identify relationships between functional traits and the environment.
- Explore whether EIVs or functional traits can help explain observed changes in species abundance.

2. Methods and materials

2.1 Study area and sampling

The study was carried out at Hochschwab (N◦ 47.375, E° 15.832), a mountain range in the North-Eastern Alps in Styria, Austria, in August 2022. GLORIA has been monitoring species abundance in plots on four different summits in 2001, 2008 and 2015 (Table 1). The plots were also surveyed in 2022, but these data were not yet available.

The GLORIA Field Manual (Pauli, et al. 2015) explains in detail how the vegetation data was acquired. To summarize, at the summits in Hochschwab, four 1-m^2 quadrates were established in 2001, each located at one side of the summit (N, E, S, W). First, the cover (%) of all vascular plant species within the plot is estimated. Then, a point intercept method is applied using a 1m x 1m frequency grid frame. This is done to detect changes in the cover of common species. At each intersection of the grid, a sampling pin is inserted into the grid perpendicular and the name of the species that is hit is written down. Finally, the cover of all species within the frequency grid frame is estimated, and a final cover is calculated using these methods.

***Table 1: Summary of the sampled plots**. Plot_ID, elevation in meters, the name and abbreviation of the peak and the coordinates.*

Plot ID	Elevation	Summit	Summit	N°	E°
1	2214	G´hacktkogel	GHK	47.6138916	15.13152027
2	2065	Weihbrunnkogel	WEK	47.6247406	15.16285038
3	2255	Zagelkogel-NW-summit	ZAK	47.6107597	15.12331963
4	1910	Zinken-NW-summit	ZIK	47.5982589	15.09152031

Out of the 201 species recorded on these plots by GLORIA, 64 were on our list with potential target species. These species were chosen from the GLORIA summits Hochschwab list they make up 70% of all the population at Hochschwab, which we took to make sure that our samples represent the actual situation. Plants were sampled >20 m from GLORIA plots and for 42 species on the target list, five mature individuals were collected with >= 5 m distance between individuals. Due to time constraints, 42 out of the 64 target species were sampled. Appendix A shows which species were collected at which summit. The vegetative height (from the ground to the top of foliage leaves) and inflorescence height were measured in the field. Plants were collected including part of the subterranean organs and transported back in moist Ziplock bags to Schiestlhaus where a makeshift laboratory had been set up.

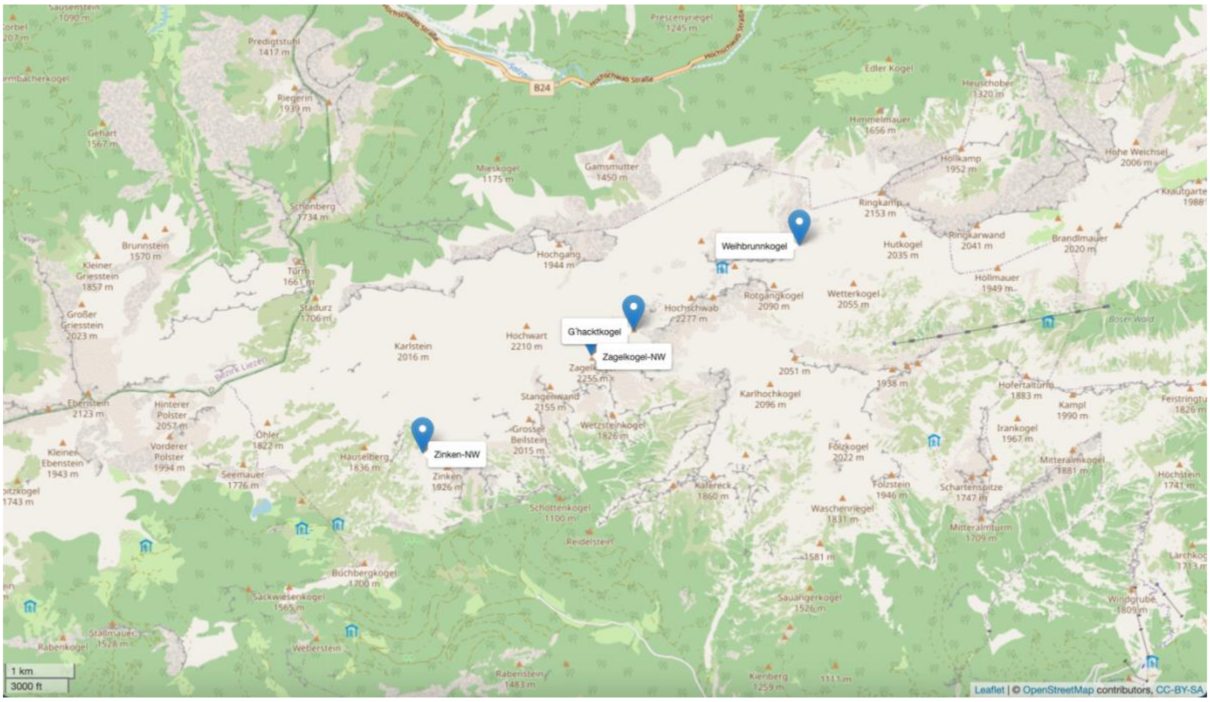

Figure 1: Map of summits Hochschwab*, made using the RStudio package `leaflet` (Cheng, Karambelkar and Xie 2023)*

2.2 Trait measurements

We measured a total of 29 functional traits (Table 2). We measured commonly assessed traits that are important for the mechanical stability, reproduction, and photosynthetic capacity, as well as traits with a relation to drought resistance, frost tolerance and nutrient availability.

Table 2: Summary of measured traits. *It shows the trait measured, the abbreviation used for the analysis and the unit that the measurement is in.*

Trait	Abbreviation used	Unit
Specific leaf area	SLA	mm^2 mg^{-1} dry weight
Leaf area	LA	cm^2
Leaf dry matter content	LDMC	g dry weight g^{-1} fresh weight
Leaf thickness	LT	mm
Succulence	Suc	g cm^{-2}
Aspect ratio	AR	leaf length/leaf width
Solidity	Sol	area of particle divided by its convex hull
Stem specific density	SSD	mg mm^{-3}
Adult plant height	height	cm
Inflorescence height	inflo	cm

Chlorophyll content	Chl	relative SPAD units
Osmotic concentration	Osm	mmol kg^{-1}
Minimal conductance	g_{min}	g cm^{-2} h^{-1}
Critical water content	CWC	RWC
Time at which critical water content is reached	tCWC	hrs.
Temperature at which Fv/Fm declines by 50%	LT_{50}	°C
	Nutrients	
Aluminium	Al	mg/g
Barium	B	μg/g
Calcium	Ca	mg/g
Copper	Cu	μg/g
Iron	Fe	mg/g
Potassium	K	mg/g
Magnesium	Mg	mg/g
Manganese	Mn	μg/g
Molybdenum	Mo	μg/g
Sodium	Na	μg/g
Phosphorus	P	μg/g
Sulfur	S	μg/g
Zinc	Zn	μg/g

The same day as the samples were collected, one leaf per individual plant (five leaves per species) was used for all following analyses. First, those leaves (without the petiole) were scanned with a portable scanner (HP Scanjet G31110 Photo Scanner) with a resolution of 300 dpi. With these scans, the leaf area, aspect ratio (leaf length divided by the leaf width) and leaf solidity (area of particle divided by its convex hull) were calculated with the ImageJ software (imagej.nih.gov). Afterwards, chlorophyll content was measured at three different positions on the leaf using a Chlorophyll Meter (SPAD-502Plus) and the average was taken per sample. Chlorophyll is measured in relative SPAD units, which represent "values that are proportional to the amount of chlorophyll present in the leaf" (Ling, Huang and Jarvis 2011). Leaf thickness was then measured using a Helios thickness gauge (0.01mm accuracy). The leaves were saturated overnight by wrapping in wet tissue paper and sealing in Ziplock bags. The next day, the leaves were first dark adapted for 20 min, blotted dry to remove surface water and then weighed to 0.1 mg. The chlorophyll fluorescence was measured with a Mini-PAM Photosynthesis Yield Analyser (Walz GmbH Effeltrich, DE) and the F_v/F_m (quantum yield of photosynthesis) was written down. The F_v/F_m ratio is the variable fluorescence (F_v) divided by the maximum florescence (F_m). The lower the F_v/F_m ratio, the more stressed the plant is, with 0.83 being the PSII efficiency of a non-stressed leaf (Jägerbrand and Kudo 2016).

Weight loss curves

The initial weight (mg) and chlorophyll fluorescence (F_v/F_m) were recorded before the leaves were placed onto a wire mesh in front of two small fans under low light conditions (< 2 µmol m-2 s-1). For easier identification, a colour coded clip with numbers one to five was weighed and clipped onto the leaf. Chlorophyll fluorescence and weight were measured over 2 - 3 days, depending on the speed of the water loss per species. Initially, the intervals for measuring were short (c. 15 min) and increased as measurements proceeded.

During these measurements, two data loggers recorded the temperature and relative humidity of the air around the wire mesh, which was used to calculate the vapor pressure deficit (vpd) as:

$$Vapor\ pressure\ deficit = \frac{\left(\frac{1 - rH}{100}\right) * 0.6107 * 10^{7.5*T}}{(237.3 + T)}$$

Where rH is the relative humidity (%) and T is the temperature in °C.

Using the weight loss data, the relative water content (RWC) was then calculated as:

$$RWC = \frac{fresh\ weight - dry\ weight}{saturated\ weight - dry\ weight}$$

The RWC was then used to fit the following function from Cape and Percy (1996) to the data from the leaf drying curves:

$$RWC_t \sim R_e + (R_0 - R_e) * exp^{-kt'}$$

Where t is the time in hours, R_e is the remaining RWC in equilibrium with the atmosphere, R_0 is the RWC after an initial fast loss, and k is a constant that expresses the exponential water loss after a first fast initial decline in weight. To assess the effect water deficit on leaf physiology, we fitted an empirical function to the relationship between RWC and Fv/Fm:

$$\frac{F_v}{F_m} = a * \ exp^{(-b*exp^{(-c*RWC)})}$$

where a, b, and c are fitted coefficients. From this, the critical water deficit (CWC) at which Fv/Fm had declined to 0.6 was estimated as:

$$CWC = \frac{\log\left(-\left(\frac{\log\left(\frac{0.6}{a}\right)}{b}\right)\right)}{c}$$

The time it takes for a leaf losing water to reach CWC (tCWC), was calculated using the weight-loss data. We plotted the RWC against the time (*hrs*) to create weight loss curves (Appendix C). Using these, we fitted the following function to the curves to calculate tCWC:

$$tCWC = \frac{\log\left(\frac{CWC - R_e}{R_0 - R_e}\right)}{-k}$$

where tCWC is the time (*hrs*) it took a water-saturated leaf to reach a water content where photosynthesis (Photosystem II) was notably affected. In our case, we calculated the tCWC by using the CWC at $F_v/F_m = 0.6$).

After the weight-loss measurements, the leaves were dried in a drying oven at 80 °C for at least 72 hrs and then weighed to measure the dry weight. The saturated fresh weight was measured as the max. fresh weight. Using those measurements, the leaf dry matter content (LDMC) was calculated as:

$$LDMC = \frac{dry\ weight}{sat.\ fresh\ weight}$$

Using the LA and the dry weight, the SLA (ratio leaf area to leaf weight) and Suc (water per leaf area) were calculated:

$$SLA = \frac{LA}{dry\ weight}$$

$$Suc = \frac{(sat\ fresh\ weight\ - dry\ weight\ )}{LA}$$

The constant k was also used to calculate $g_{min,}$ which is the minimal epidermal conductance. When stomata are closed, this is water loss through leaky stomata and the cuticle, the boundary layer being strongly reduced by the fan. It was calculated using Suc, with the following equation:

$$g_{min} = Succulence * k$$

Osmotic concentration

At the Schiestlhaus, leaves from 5 individuals of each species were cut into small pieces and put into an Eppendorf vial (0.8 ml) including a filter inlet (0.2 ml). Samples were initially frozen at the Schiestlhaus (at -20 °C) until transported to Vienna. There, the samples were frozen again at -80 °C in the laboratory in Vienna, thawed, and frozen again. These freeze-thaw cycles serve to break the membranes. The vials were then centrifuged at 25 °C at 14,000 rpm (relative centrifugal force 20160 g) for 10 minutes. Liquid from the leaves was collected and the osmotic concentration was measured with a vapor pressure osmometer (VAPRO ®, model 5520). If the first measurement for the osmotic concentration was < 400 mmol/kg, 3 measurements of the same sample were done, and the third value was the value written down. If the first measurement for the osmotic concentration was > 400 mmol/kg, 2 measurements of the same sample were done, and the second value was the value written down.

Stem specific density

Stem specific density was measured on stem sections several cm long of the main shoot or rhizome as shown in (Figure 2). A beaker was filled with water and put on top of a scale set to zero. The stem/rhizome was then submerged in the water without it touching the glass. This can be done by pricking the stem onto a long needle that is being held by a clamp stand. The weight measured in mg is the volume of the sample (or of water displaced) in mm^3. After measurement, the stem/rhizome was added to a paper bag and labelled and put into the drying oven at 80 °C for at least 48 h.

Stem specific density (SSD) was calculated as

$$SSD = \frac{dry\ weight\ (mg)}{volume\ (mm^3)}$$

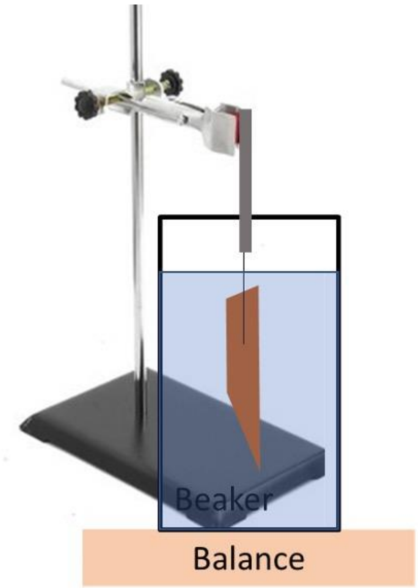

Figure 2: *Set-up stem density measurement*

Nutrient content

The leftover dried leaves were used to measure nutrient content (aluminium, barium, calcium, copper, iron, potassium, phosphorus, magnesium, manganese, molybdenum, sodium, sulphur, and zinc). They were placed into a 2-ml vial with two small steel balls and ground to a fine powder using a ball mill (TissueLyser II). Powder from the five replicates per species was pooled to create one sample per species. The samples were then measured at the Institute of Forest Ecology (BOKU) with an inductively coupled plasma - optical emission spectrometer (ICP-OES, Perkin Elmer Optima 8300 ICP-OES) after digesting 150 – 200 mg finely ground leaves with 68% HNO3 in a microwave oven (CEM MARS6).

Frost tolerance

For the frost resistance measurements more leaves were required, so we collected an additional 40 leaves from several individuals per target species. The leaves were mixed and, depending on the size of the leaf (if the leaves were < c 5 mm, we put several leaves within one circle), one or more were placed onto a transparent plastic sheet with circles numbered 1 to 10. This was done for 8 different temperatures: +4, -4, -6, -9.5, -11.5, -14, -16.5 and -17 °C. The leaves were attached to the plastic sheet using Transpore^{TM} and were wrapped in moist tissue paper. Ice nucleation agent (a drop of Snomax® suspension) was added onto the tissue paper to avoid supercooling and the sheets were placed into water-tight small plastic bags. The plastic bags containing the plastic sheets was placed onto a metal rack that kept the plastic bags in place and was submerged in a 50:50 water/ ethylene glycol. The temperature of the liquid was first cooled to 4 °C, then cooled to a target temperature with a rate of 4 °C/h, kept at target T for 2 hrs and again warmed with a rate of 4 °C/h to 4 °C. There were different experiment times per target temperature (Figure 3). Samples kept at +4 °C were used as control. After the measurements, the samples were placed under low light conditions for 12 hrs. To measure potential damage, chlorophyll fluorescence (F_v/F_m) of the leaves were measured with a GFS-3000 (Walz, Effeltrich). Before being measured, the samples were dark adapted for 20 minutes.

The temperature at which the chlorophyll florescence declined by 50% (LT_{50}) was then calculated by fitting a sigmoidal function to the frost resistance curves (Appendix C).

start T	4°C
cooling rate	4°C/h
time at target T	2h

target T (°C)	time needed (h)
4	2
-4	6
-6.5	7.25
-9	8.5
-11.5	9.75
-14	11
-16.5	12.25
-17	12.5

***Table 3**: Cooling rates and target times frost tolerance experiment*

2.3 Data analysis

For the data analysis, we first cleaned the data were by looking for outliers. Outliers can be either mistakes or can show extreme values and is a value that is relatively large or small compared to the rest of the observations. A boxplot was used to visualize the spread of the data and to observe potential outliers. If an outlier was identified, we double-checked the raw data to see if the data point was an extreme or a potential mistake.

A species-wise (SW) dataset was created by taking the mean value of the five individuals per species for each trait. This resulted in a matrix with the plant species as rows and the functional traits as columns. Because some of the target species lacked many data, 29 species with mostly complete data were selected for further analyses and missing data in those species were imputed.

For imputation the R function `phylopars` (library RPhylopars) was used and imputation was based on the correlation between traits, but not the phylogenetic relationship among species. Including phylogeny in some cases produced impossible (negative) values.
This resulted in a dataset with 28 functional traits for 29 species. We did this to ensure all analyses that required a complete dataset can also be performed.

Besides the dataset containing all of the functional traits, species abundance data from Hochschwab from the years 2001, 2008 and 2015 was obtained from GLORIA. This contains the name of the summit, the plot ID, coordinates, the full species name, and the cover of the species in percentage.

Then, the relative change in species abundance between 2001 and 2015 ($change_{01\_15}$) was calculated using the sum of the cover (%) over all plots per year per species as:

$$change_{01\_15} = \frac{(sum\ cover\ \%\ per\ species\ in\ 2015 - sum\ cover\ \%\ per\ species\ in\ 2001)}{(sum\ cover\ \%\ per\ species\ in\ 2015 + sum\ cover\ \%\ per\ species\ in\ 2001)} * 100$$

Where we used the sum of the cover (%) per species in 2001 and 2015. The average cover per species was calculated as:

$$cov_{avg} = \frac{(Sum\ cover\ \%\ per\ species\ in\ 2001 + sum\ cover\ \%\ per\ species\ in\ 2015)}{2}$$

This calculation was done for all species recorded (n=135) including the 29 target species.

Ecological indicator values

Landolt (2010) ecological Indicator Values (EIVs) were used to describe the habitat and environmental preferences of the species (Table 4).

Table 4: Landolt ecological indicator values. *This table contains an explanation of the different Landolt values that were used for this research. Table is used from (Paetzolt 2022) with information from (Landolt 2010).*

Indicator value	Abbr. used	Unit	Explanation
Temperature	T	Number between 1 (alpine & nival) & 5 (warm-colline) in steps of 0.5	Characterizes the average air temperature during the growth season.
Moisture	F	Number between 1 (very dry) & 5 (under water) in steps of 0.5	Signifies the average soil moisture during the growing season.
Soil reaction	R	Number between 1 (strongly acid) & 5 (alkaline) in steps of 1	Characterizes the content of free H-ions in the soil.
Nutrients	Nu	Number between 1 (very nutrient poor) & 5 (very nutrient rich) in steps of 1	Indicates content of available nutrients. Primarily nitrogen, but also phosphorus.

Community weighted means

Community-weighted means were used as they are an approach that is widely used in functional ecology, for it allows testing the effects of environmental variables on changes in species communities. This can demonstrate the effect of environmental filtering on community assemblies (Díaz, Cabido and Casanoves 1998). CWM have the assumption that they reflect the ‘optimal’ local trait strategy, with “species trait values nearest to the CWM values in a particular location, are predicted to have relatively high fitness, because they presumably occur at relatively high abundance, and this contributes most strongly to the CWM” (Muscarella & Uriarte, 2016).To calculate community weighted means for the functional traits, species abundance (cover %) from the GLORIA ecological survey was used. For the EIVs CWM were calculated using the Landolt (2010) values using the following equation:

$$CWM = \frac{\sum(F_i * x_i)}{\sum x_i}$$

where F_i is the mean value of a species functional trait/ EIVs and x_i is the sum of all plot cover values of species i. CWM of EIVs (T, F, Nu, and R) were calculated from all species present in plots, not just the target species. In total, there were 126 GLORIA plots at Hochschwab of which the community-weighted means were calculated.

Transformations

Shapiro-Wilko tests and Q-Q plots were used to determine the normality of the functional traits data. This test was used because it is good for small sample sizes (< 50). Consequently, height, tCWC, CWC, LA, AR, LT, Sol, Suc, Al, Ca, Cu, Fe, Mn, Mo, Na, Zn and LT_{50} were log-transformed to improve normality, other data were not transformed.

For the CWM data, Q-Q plots were used to determine normality. None of the data was normally distributed, thus all CWM traits except LT_{50} were log-transformed. Because LT_{50} contains negative values, a power transformation (LT_{50}^2) was used.

Trait correlations

To visualize the spread of the data, the un-transformed raw data was used to create a correlation plot and tested using the Spearman's rank correlation coefficient, which does not require a linear correlation and is a nonparametric measure of rank: it ranks the data based on its position in the dataset (Gauthier 2001). The correlation plot was visualised using the `corrplot` function from the corrplot package (Wei and Simko 2021).

The relationships among traits was analysed by hierarchal clustering using Euclidian distance in the function `dist`. The ward D method was used for the clustering using the `hclust` function, also from the base R package, and the distance was log transformed to improve scaling.

Ordination methods

Ordination is a multivariate statistical tool that allows complex multivariate data to be visualised with a reduced number of dimensions. This facilitate interpretation of patterns in a community matrix, with typically 2-3 dimensions (Dexter, Rollwagen-Bollens and Bollens 2018). In ecology, ordination allows to summarize community data and shows species that are similar placed close together and species that are very different placed further apart.

Ordination methods can be used in different ways, but in this study, they are used to describe relationships between patterns in species composition and environmental gradients. For this research, we are using unconstrained techniques: principal component analysis (PCA) and non-metric multidimensional scaling (NMDS). We used an unconstrained PCA to identify pattens and relationships between our plant species and functional traits.

A PCA was performed on species-wise & CWM data, log-transformed when needed, using the function `prcomp` from the factoextra package (Kassambara and Mundt, 2020) in R to show the distribution of the functional traits among the species, and how the different species resemble each other. For visualization, the function `fviz_pca_biplot` and `fviz_pca_ind` from FactoMineR (Lê et al., 2008) were used. To check for significant differences between the three sample years in the CWM PCA data, a MANOVA was performed using the `adonis` function from the vegan package (Oksanen, et al. 2022)

For the NMDS, the GLORIA survey data was turned into a community matrix (plot_ID & year x species) with the function `matrify` from the R package labdsv (Roberts., 2019). The NMDS was performed using the `metaMDS` function from the vegan package (Oksanen, et al. 2022). The NMDS scores where then extracted and plotted using the `ggplot` function from the ggplot2 package (Wickham 2016). After performing the NMDS, the stress and R^2 are calculated to show how well the NMDS fits the data, using the function `stressplot` from the vegan package. Plots located closer to each other on the NMDS plot are more similar than the plots that are further apart from each other. To test if there was also a change in the functional trait community, a PCA of the CWM of the functional traits were calculated, with different colored polygons for the three sampling years. To check for significant changes between the years of the community matrix, and ANOSIM test was performed using the `anosim` function from the vegan package. Dissimilarity matrix was created using the Bray-Curtis method.

Linear models

The `lm` function was used to predict which traits have a significant effect, weighing by and the $cov_{avg}^{0.5}$ to give more importance to species that are dominant in the plots. A linear model was run with a reduced number of functional traits to reduce over-parameterization. These are traits that tend to be important (correlating with others) in the corrplot and PCA. `stepAIC` from the R package MASS (Venables and Ripley 2002) was used to determine the best linear model for predicting the change in species abundance ($change_{01\_15}$).

The multicollinearity of the model was checked using the variance inflation factor, which quantifies correlation between the predictors in a model. The higher the VIF, the more difficult it is to assess the contribution of the predictors in the model. A VIF < 3 is considered to indicate that multicollinearity is not a problem for the model. We used the function `vif` from the car package (Fox, et al. 2023).

All analysis were done using RStudio, R version 4.2.2 (2022-10-31) (R Core Team 2022).

3. Results

3.1 Variation in the EIVs Landolt values

Using all species recorded in the GLORIA plots, T values range from 1 (alpine & nival) to 4.5 (collin), F from 1 (very dry) to 4 (very moist), Nu from 1 (very nutrient poor) to 5 (very nutrient rich, which refers mostly to nitrogen and phosphorus) and R from 1 (extremely acid) to 5 (alkaline, high pH) (Figure 3).

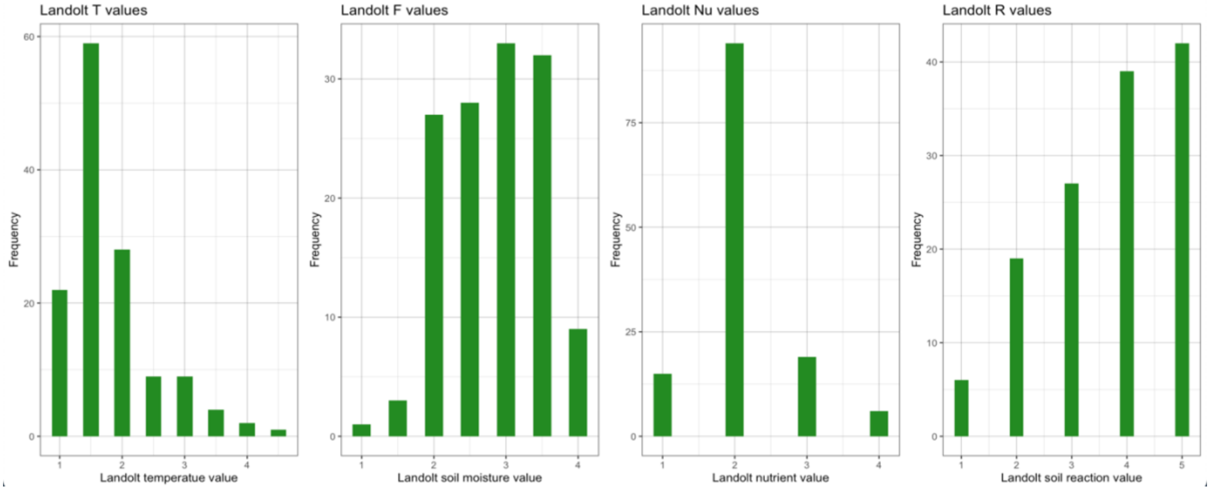

Figure 3: Histogram *of the frequency of the Landolt values for all species (n=135) found in the plots.*

We calculated the community-weighted means for the Landolt values for temperature (T), soil moisture (F), nutrients (Nu) and soil reaction (R) for the 29 target species and all species recorded in plots (Table 6). This shows that the target species are a good representation for the total species pool present, with the mean CWM values for the EIVs being very similar to the CWM for the EIVs of all of the species recorded. The target species have a Landolt T value of 1.64, and all species have a value of 1.50, which is characterized as the alpine zone of grassland vegetation above the treeline) and supra subalpine (zone of arolla-larch forests) zones. Moisture indicators (F) has a mean value of 2.26 for the target species, and 2.17 for all species, which signifies a mesic soil. Nutrient indicators (Nu) has a mean value of 1.48 for the target species, and 1.29 for all species, which are both defined as nutrient-poor soils. Finally, the soil reaction indicator (R) has a mean value of 4.41 for the target species, and 4.81 for all species, which indicates a soil rich in bases (high pH).

Table 5: Mean values *of the Landolt values of the CWM of the target species (n=29) and CWM of all of the species (n=135)*

Landolt value	Abbr.	Target	All
Temperature	T	1.64	1.50
Moisture	F	2.26	2.17
Nutrients	Nu	1.48	1.29
Soil reaction	R	4.41	4.81

3.2 Variation of the species-wise data

Variation within and between species can show how organisms interact with each other and their surrounding environment. Functional traits between the species, e.g., plant height and leaf area, can explain adaptations to their environment. We calculated the mean, standard deviation, range, and coefficient of variation for all of the measured functional traits of the 29 different species (Table 5). The CV for Mn and AR are the highest (>100%), which indicates a lot of variation between species. The Osm, Chl, LDMC, Sol, B, Mo, and S are the lowest (<30%), which indicates that there is not a lot of variation between species.

Table 6: Summary of the statistical parameters *of the functional traits of the target species means. It shows the trait, the mean, standard deviation (SD), min and max and the coefficient of variation (CV = SD/mean*100, %) of the target species (n=29).*

Abbr.	Unit	Mean	SD	Max	Min	CV [%]
height	cm	5.57	5.04	22.20	0.58	90
SSD	mg mm^{-3}	0.45	0.15	0.83	0.17	33
g_{min}	g cm^{-2} h^{-1}	4.66e-03	3.59e-03	0.02	7.4e-04	77
CWC	RWC	0.26	0.12	0.62	0.10	44
tCWC	hrs.	13.17	9.28	32.99	1.56	70
Osm	mmol kg^{-1}	538.85	160.99	840.80	262.20	30
LT	mm	0.51	0.19	1.14	0.25	38
Chl	relative SPAD numbers	38.86	10.32	69.88	19.70	27
SLA	mm^{2} mg^{-1} dry weight	14.03	5.13	25.49	5.98	37
LA	cm^{2}	2.94	2.89	14.44	0.08	98
LDMC	g dry weight g^{-1} fresh weight	0.32	0.09	0.47	0.18	28
AR	$\frac{leaf\ length}{leaf\ width}$	6.60	7.79	24.92	1.13	118
Sol	area of particle divided by its convex hull	0.82	0.20	0.98	0.05	24
Suc	g cm^{-1}	0.021	0.013	0.061	8.3e-03	61
LT_50	°C	-8.38	3.16	-4.90	-13.44	38
Al	mg/g	0.12	0.09	0.53	0.04	74
B	µg/g	43.67	11.96	68.62	23.18	27
Ca	mg/g	27.17	25.47	120.97	5.58	94
Cu	µg/g	13.00	8.66	47.47	4.26	67
Fe	mg/g	0.14	0.09	0.57	0.08	67
K	mg/g	9.97	3.71	18.06	3.69	37
Mg	mg/g	4.11	1.88	9.55	1.42	46

Mn	µg/g	91.48	124.80	625.55	6.28	136
Mo	µg/g	11.50	2.80	20.54	8.43	24
Na	µg/g	413.91	166.87	876.03	173.73	40
P	µg/g	1113.45	361.44	2270.32	486.29	32
S	µg/g	2122.11	623.23	3319.13	1091.22	29
Zn	µg/g	125.41	98.44	476.38	52.00	79

In the PCA (Table 7) the first axis was best explained by B, LDMC, S, Ca, P and Mg, and explained 19.1% of the total variation. The second PCA axis was best explained by the LT, Al, chlorophyll, Fe, and SSD and explained 14.8% of the total variation**.** The PCA shows the distribution of the traits among the different species, and how species are similar to each other (Figure 4). The majority of the variance in explained on the first three axis, with the other axis providing <10% of the total variation (Figure 5).

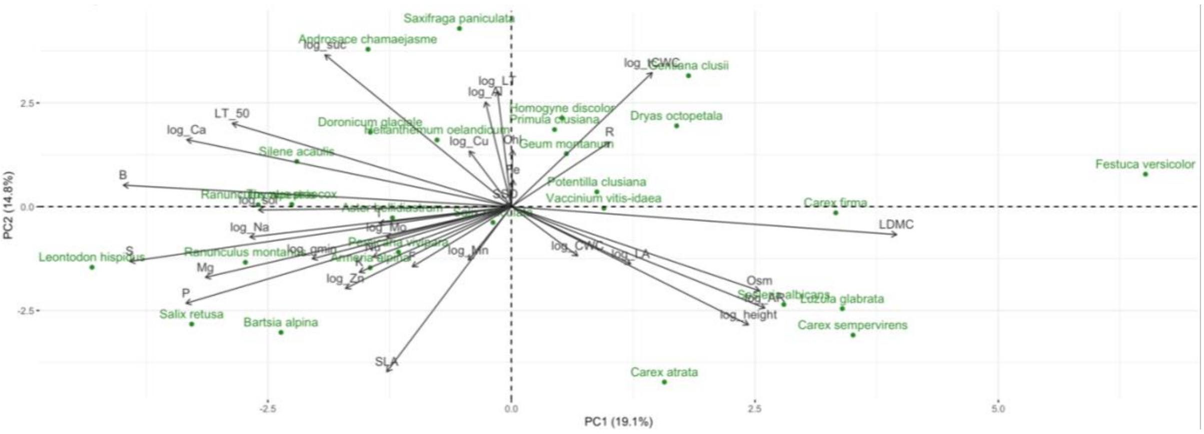

Figure 4: PCA ordination of 29 plant species from Hochschwab, Austria, on the basis of 29 functional traits. *Labels display plant species with the highest eigenvector scores on PC1 and PC2. Arrows display the functional traits.*

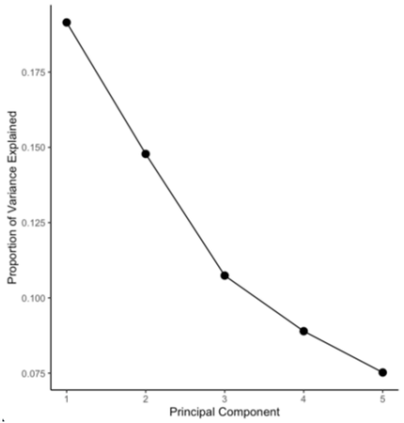

Figure 5: Proportion of the variance *that is explained on the first five PC axes in the PCA of the species mean data*

To assess the species' adaptations to their alpine environment, we specifically measured frost tolerance (LT$_{50}$) and traits related to water relations (Osm, tCWC, CWC, g_{min} and succulence). Species that appear less frost tolerant (high LT$_{50}$ values) are *S. acaulis, D. glaciale*, *H. oelandicum,* and *S. albicans*. *S. acaulis* has the lowest frost resistance (LT$_{50}$) of -4.9 °C, which means that when the air temperature reaches that value*,* 50% of the chlorophyll fluorescence (F_v/F_m) has declined . *C. atrata* has the highest frost resistance and can withstand temperatures of -13.4 °C. Species that are less frost tolerant are also associated with a low osmotic concentration. The opposite can be said for the graminoids. They are located close Osm, which means they generally have a high osmotic concentration. Osm and LT$_{50}$ have a negative relationship, since the arrows are situated opposite of each other, and this means that these plants have a low LT_{50} and are more frost resistant.

F. versicolor seems to be the most different from the other species, being located on the far left of the PC1, not close to any other species. *L. glabrata, S. albicans and C. sempervirens* are located on the bottom right of the PCA, close to height, AR, and osmotic Osm. All three of these species are graminoids. All of the graminoids are located to the left and bottom (*B. alpina, C. atrata*, *L. glabrata, S. albicans, C. sempervirens* and *F. versicolor),* with *C. atrata* being located further away from the others. We can also see that they are also associated with a high osmotic concentration and LDMC.

Table 7: PCA scores *of the functional traits on the first two axes of the PCA.*

Trait	**PC1**	**PC2**
SSD	-0.013	0.012
Osm	0.520	-0.414
Chl	0.002	0.279
SLA	-0.261	-0.812
LDMC	0.807	-0.137
LT_50	-0.585	0.410
B	-0.813	0.106
Fe	0.003	0.132
K	-0.319	-0.323
Mg	-0.641	-0.347
P	-0.681	-0.475
S	-0.799	-0.269
height	0.497	-0.581
gmin	-0.417	-0.256
CWC	0.139	-0.242
tCWC	0.295	0.659
LA	0.250	-0.281
AR	0.531	-0.498
LT	-0.029	0.569
Sol	-0.530	-0.016
Suc	-0.390	0.747
Al	-0.054	0.515
Ca	-0.680	0.329
Cu	-0.088	0.274
Mn	-0.090	-0.263
Mo	-0.262	-0.150
Na	-0.548	-0.149
Zn	-0.348	-0.402
T	-0.277	-0.081
F	-0.208	-0.295
R	0.206	0.317

Nu	0.290	-0.247

3.3 Trait correlations

Pairwise correlations between the functional traits show a few different correlations (Figure 6). For the EIVs, the majority of them are only correlated with some nutrients, but F & Nu are positively correlated (r=0.59), which was also readable from the PCA (Figure 4). F is also positively correlated with LA (r=0.49). The species-wise PCA also showed a likely negative correlation between the LDMC and LT_{50}, which is also reflected in the pairwise correlations (r=-0.77).

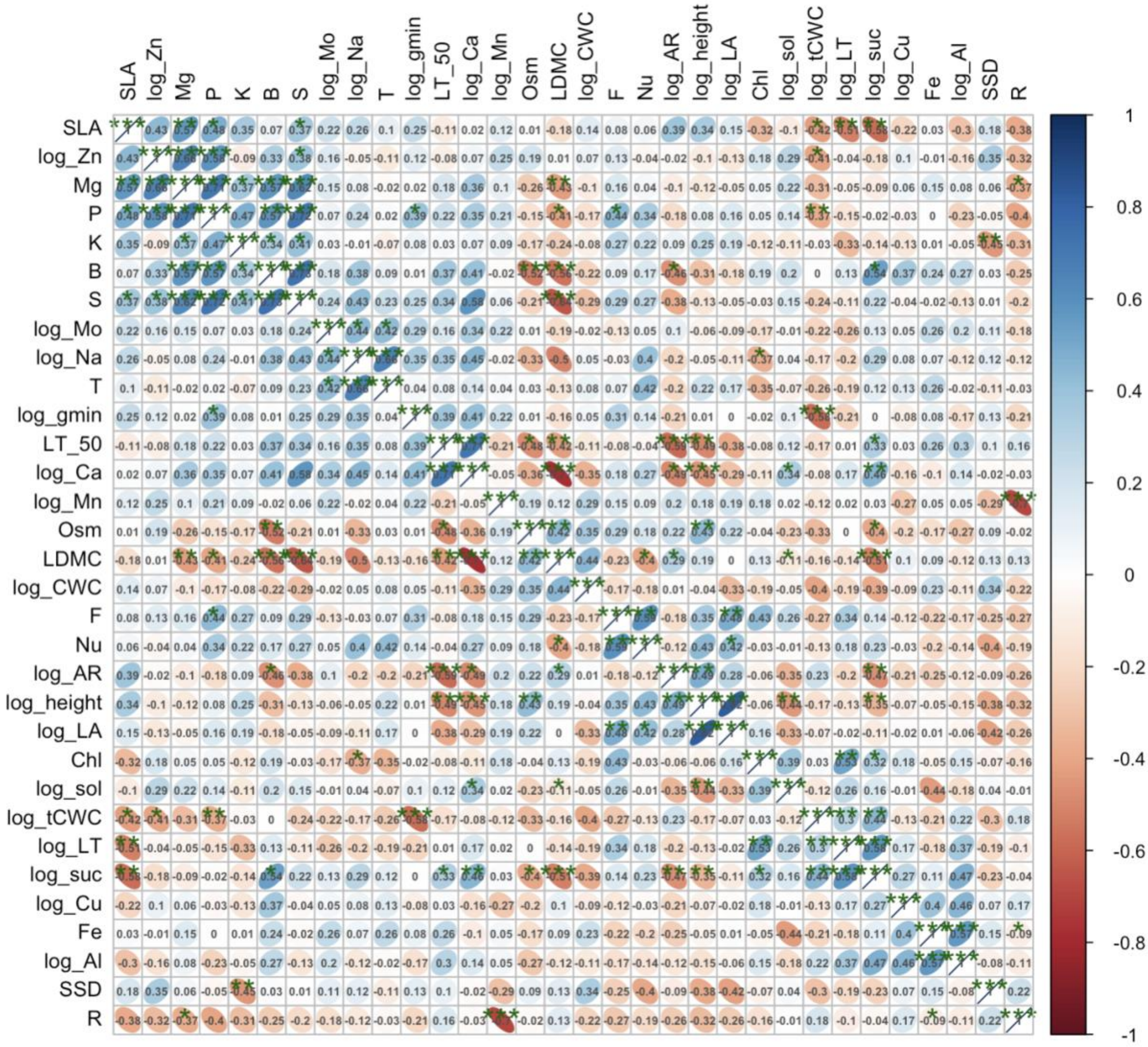

***Figure 6: Pairwise correlation plot** between the log-transformed species-wise data (n=29). Correlation matrix was made using Spearman's Rank-Order correlation, the clustering method for the corrplot was ward.D and the order of the traits uses `hclust` ordering. Plot shows all correlations, but only correlations with a star are significant. Ellipse shows the correlation: red means a negative correlation and blue means a positive correlation.*

A cluster analysis with the functional traits and the EIVs showed four different clusters of strongly correlated traits (Figure 7). Various measures of size of morphology (height, LA, LDMC, LT, succulence, SSD, AR, solidity) cluster together with CWC, g_{min}, LT_{50} ,Fe and Al. The second largest cluster contained the majority of the nutrients (Zn, Mn, Na, Mg, Mo, Ca), tCWC and all EIVs. The other two clusters are smaller, with only a few traits. SLA, K, Chl and

B are grouped together in one smaller cluster, and te S, Osm and P are grouped together and are the furthest distance away from the other traits.
In the nutrient cluster, the majority of the nutrients and the EIVs are grouped together. Both the EIV soil reaction (R) and nutrient availability (Nu) are related to the substrate and nutrient availability of the soil, which vary greatly per species (Figure 4 – Histogram of the distribution of EIVs). However, due to the fact that all of the target species were sampled in the same area, there should not be much difference in the habitat preferences of the plant species.

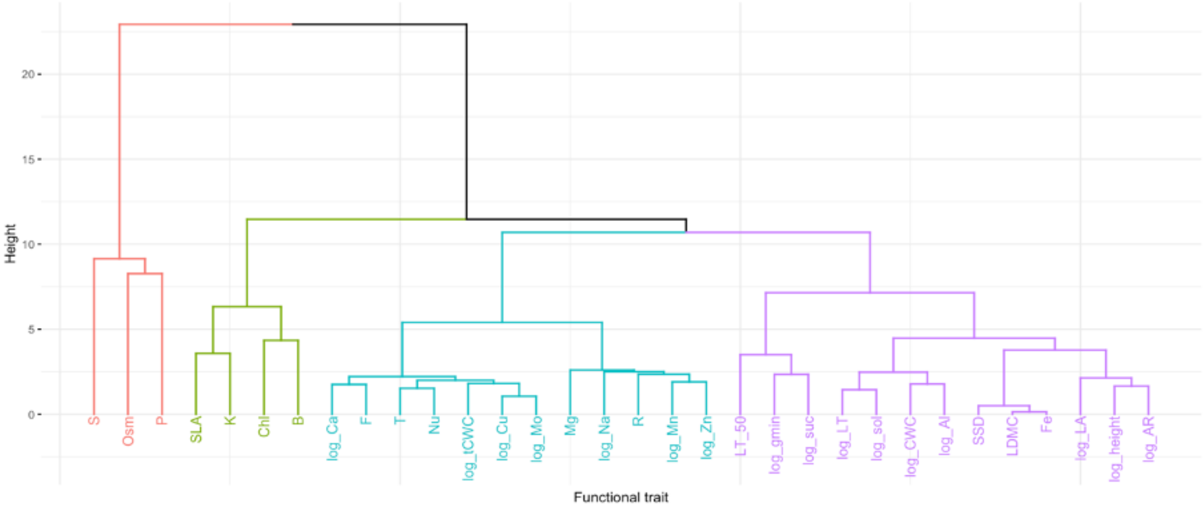

***Figure 7: Cluster dendrogram** showing hieratical relationships of the log-transformed functional traits of the species wise data (n=29). The method used for the clustering was 'ward.D. '*

Pairwise correlations between the functional traits and the EIVs confirm the findings of the PCA and the cluster dendrogram (Figure 7). While the majority of the nutrients were grouped together in the same cluster, they are not all correlated to each other. While T and Nu are clustered closely together, they are not significantly correlated.

3.4 Changes in species abundance

The results of the GLORIA ecological survey show that the majority of the plant species at Hochschwab changed in species abundance between 2001 and 2015, with 14 species increasing in abundance by more than 50%, and 19 species decreasing in abundance by more than 50% between the years 2001 and 2015 (Figure 8). Among the target species *S. albicans* and *L. hispidus* have increased by >50% in cover while *H. discolor* has decreased by45%. *C. firma*, one of species with the highest average cover, has not changed much between 2001 and 2015. The only species with a relatively high average cover that has changed >25% is *F. versicolor*, which has decreased in abundance. The species with the highest average cover in all three years were *C. firma* (cov_{avg} of 2019 %), *D. octopetala* (cov_{avg} of 457%) and *P. mugo* (cov_{avg} of 333%), while Campanula cochleariifolia was the least commonly found species (cov_{avg} of 0.00050%). The average cover of *P. mugo* decreased between 2001 and 2015 (66% to 56%), while the sum of the cover increased (331% to 335%). The average cover of C. firma increased (34% to 36%), and the sum of the cover also (1983% to 2055%). This indicates that *C. firma* was present at many plots but had a lower coverage in all of them (in 2001, it appeared in 54 out of 64 plots) , while *P. mugo* was more dominant in the plots that it appeared but appeared in less plots overall. *P. mugo* was third most common species with average cover, but it was interesting to see that this species was only found at one summit: Zinken, which is the lowest summit at Hochschwab (1910 m). It stayed very constant and only increased by 0.6% between 2001 and 2015.

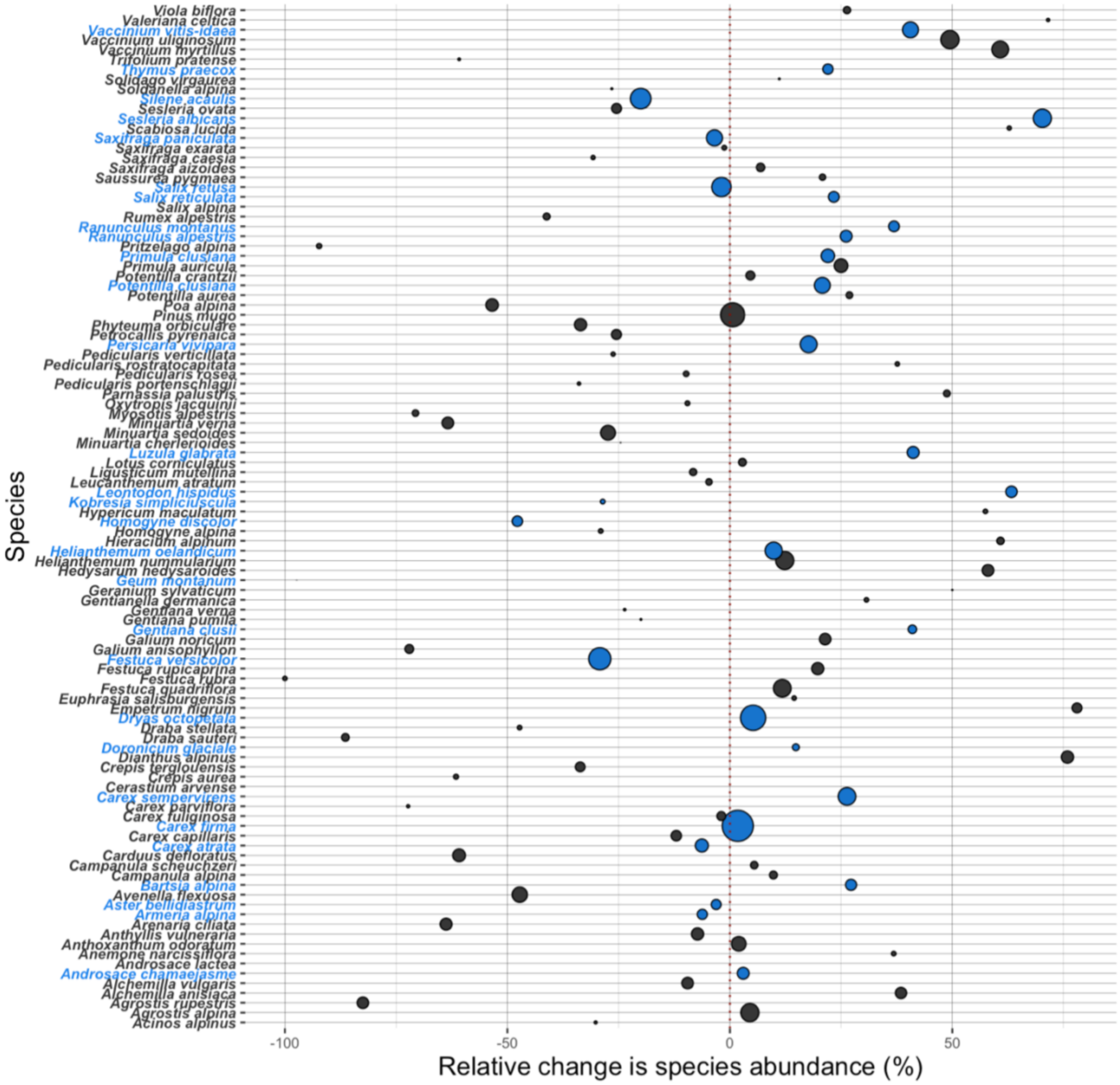

Figure 8: Relative change in species abundance (%) *for all the species (only species with cov*$_{avg}$ *> 0.5 are shown) between the years 2001 and 2015. Species colored blue are the target species for this study and bubble size represents the average cover of the species.*

3.5 Changes in species attributes

Changes in species community are visualized by NMDS that shows patterns in the community composition for 2001, 2008 and 2015 (Figure 9). The NMDS shows that some plots have a directional shift in the same direction, with the community data in the year 2015 having shifted to the top or the right. However, this shift is not significant; an ANOSIM test showed no significant difference between the three years (R = -0.009, p-value = 0.9).

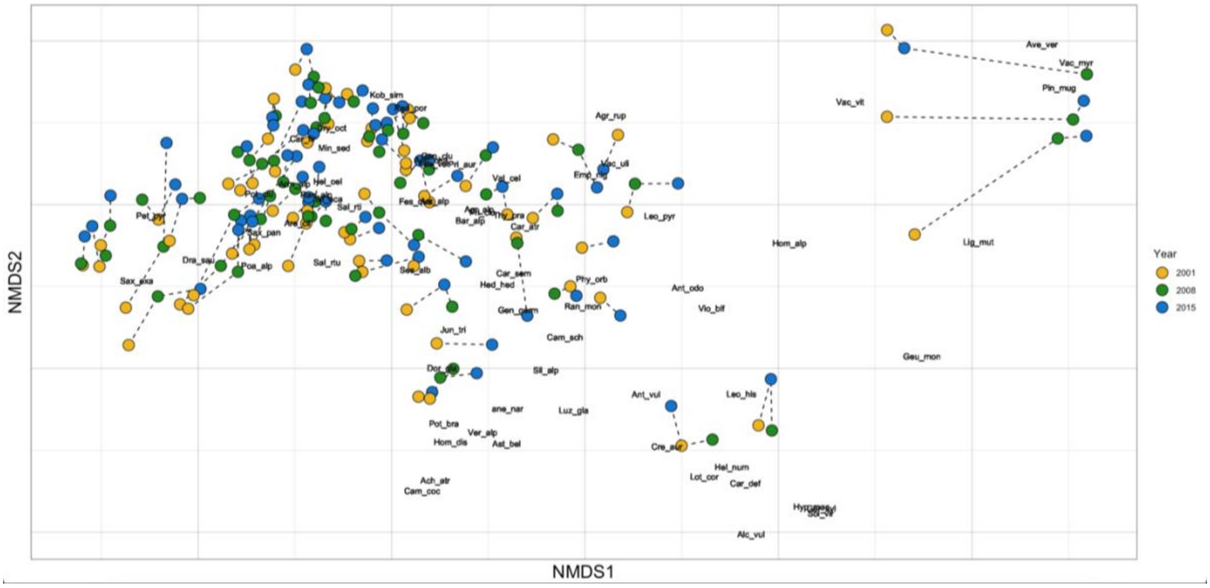

*Figure 9: **NMDS of the community data** of all GLORIA plots with the changes between 2001, 2008 and 2015 for all species (n=126). Circles represent individual plots and circles connected broken lines are show the same plot in the different years.*

The PC1 is explained by LDMC, SSD, CWC, and the nutrients Mg, Zn, Mn, S, Na and B, and explains 44.9% of the total variation in the data. The PC2 is explained by height, Cu, Fe, AR, Al, LT, and tCWC and explains 20.6% of the total variation. The functional traits that explain the PC1 and PC2 of the community-weighted means (Figure 10) are different then the traits that explain the PC1 and PC2 of the species-wise data (Figure 4). The variation on the first axis is also higher in the CWM PCA than the species-means PCA: 44.9% against 19.1% respectively. We checked for significant differences between the years in the PCA data, but a MANOVA showed there were none (p-value =0.92).

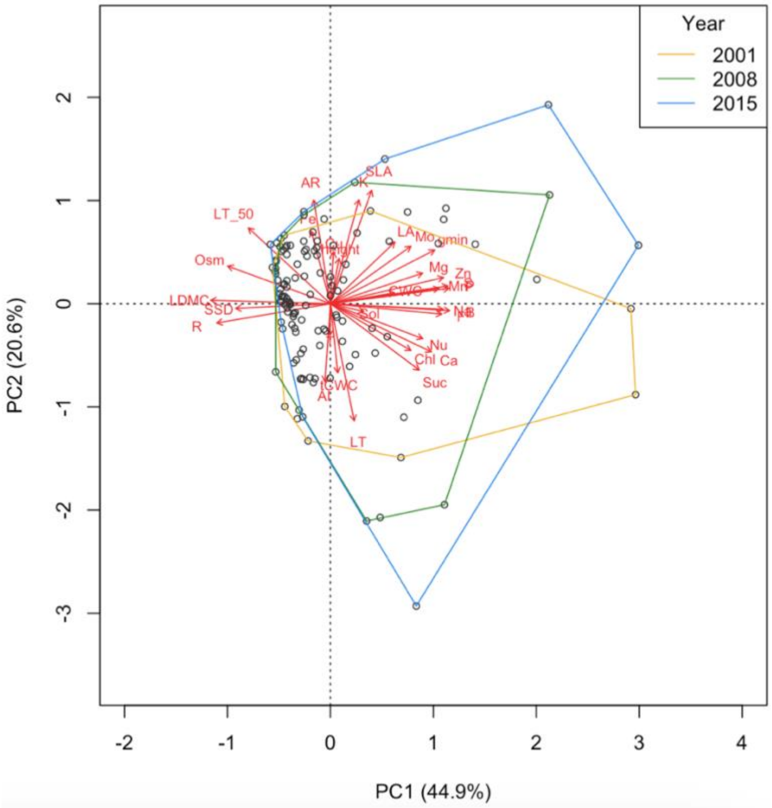

Figure 10: PCA of CWM

We then checked if the CWM of the functional traits had changed. The CWM between the years shows if there has been a change in species with a certain trait, if the CWM of a trait increased, then it means that there was an increase or decrease in species with those traits. However, the trait composition of the communities stayed very similar: an ANOVA test showed that none of the CWM traits differed significantly between 2001, 2008 and 2015 (Figure 11).

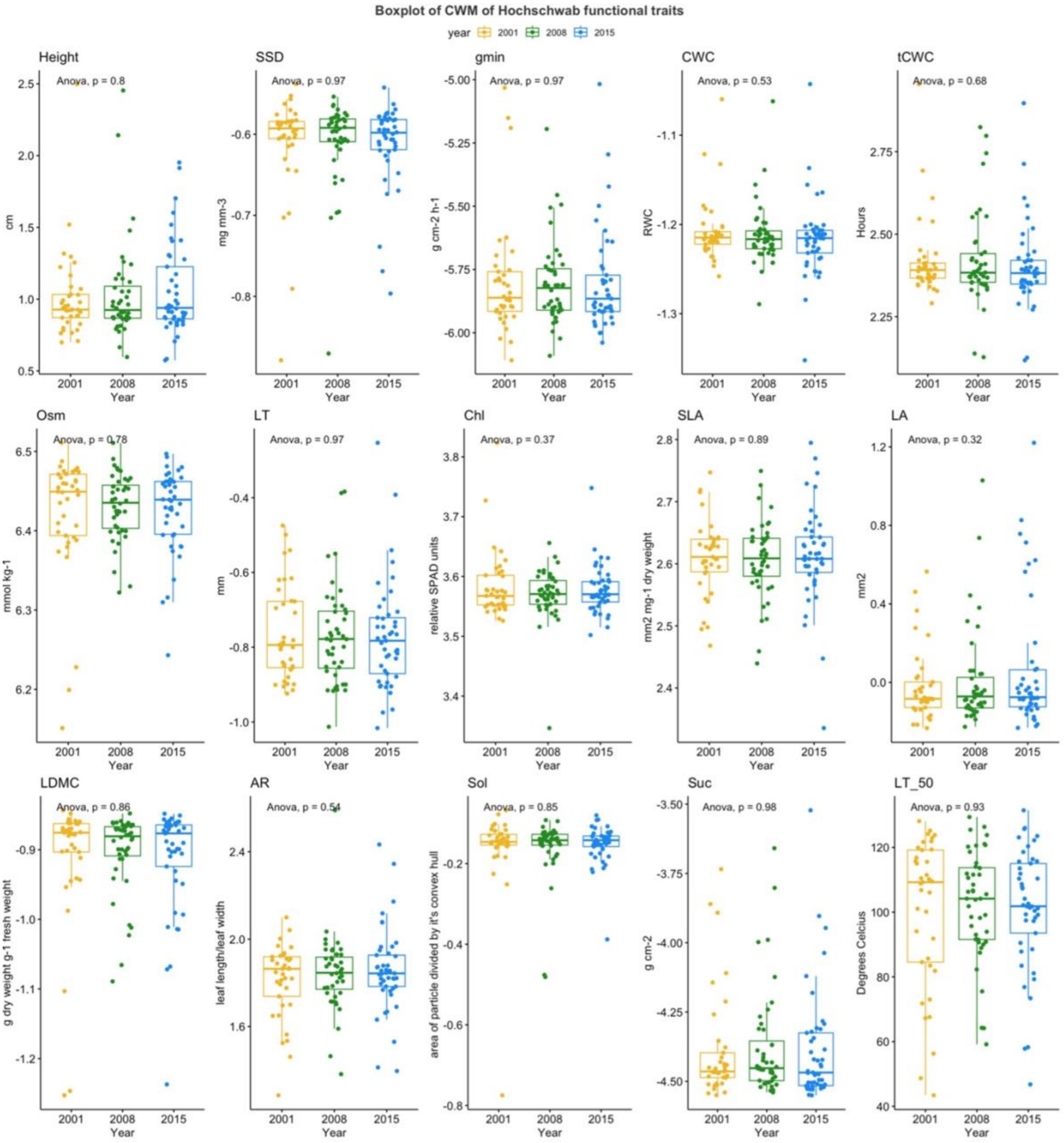

Figure 11: Boxplots of the transformed CWM *(n=192) with an ANOVA testing for difference between the 3 years for the functional traits without nutrients.*

3.6 Modelling predictors for change in species abundance

While there have been changes in species abundance, we found no evidence of changes in the overall communities or in CWM trait values. These results, however, do not address the question if traits or other information can inform on which species increased or decreased in abundance. In order to predict which functional traits and EIVs can explain those changes, we explored different linear models to see which model best explains the change.

First, we checked if any of the EIVs can explain the changes in species abundance. There appears to be a weak trend when comparing the changes in species abundance with the species-wise EIVs (Figures 12 & 13). When looking at trends for only the target species, there is a weak trend, but it is significant ($R^2 = 0.19$, p-value = 0.019) with species with a higher Landolt T increasing in abundance. However, this is only true for our target species. When looking at the overall change in species abundance (Figure 13), the trend disappears ($R^2 < 0.01$, p-value = 0.47). Something interesting can be seen for the Landolt R values. Our target species show in increase in species with a higher R value, while all the species show a decrease in species with a higher R value.

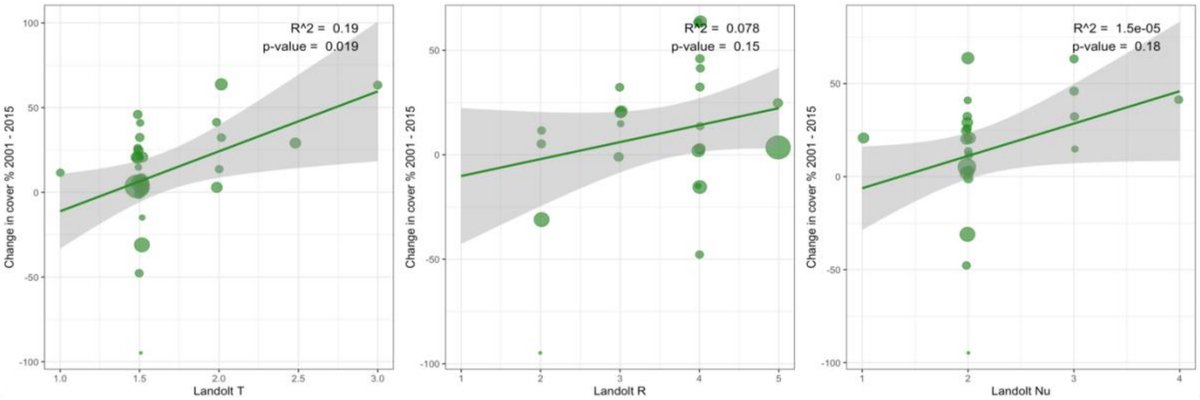

Figure 12: Relative change in species abundance (%) *plotted against the Landolt values T, F and Nu for the target species (n=29).*

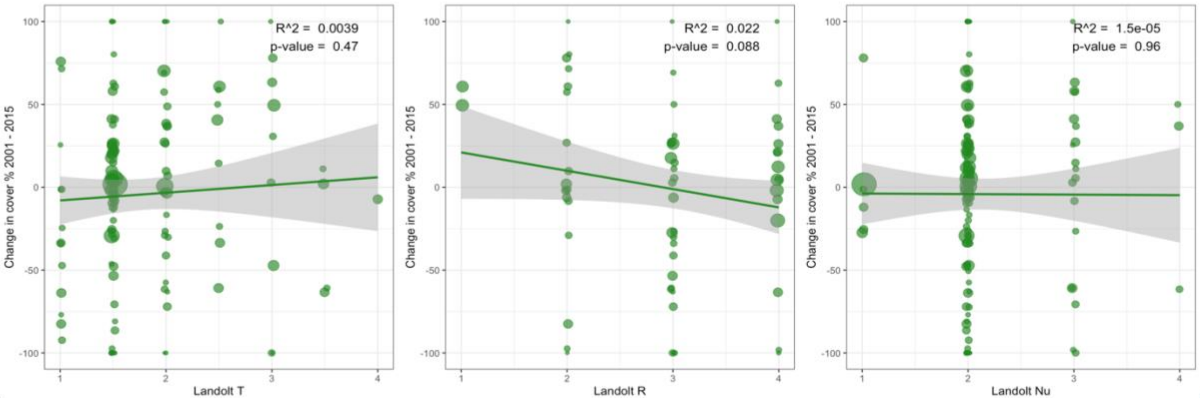

Figure 13: Relative change in species abundance (%) *plotted against the Landolt values T, F and Nu for all species (n=135)*

Alternatively, we tested if a combination of functional traits might explain change in (Table 9). In total the model accounted for 44.6% of the total variation. The variation inflation factor (VIF) of the three variables is < 3, thus multicollinearity is not a problem for the model.

***Table 8: Linear model** explaining the change in species abundance from a reduced model containing the LT_{50}, solidity and height. The adjusted R^2 is 0.446 and the p-value for the model is 4.536E-04.*

Coefficients:	**Std. Error**	**t-value**	**Pr(>|t|)**	**VIF**
LT_{50}	1.393	1.492	0.148	1.68
Height	5.874	3.851	7.26E-04***	2.73
Solidity	6.618	4.863	5.32E-05***	2.07

We then tested the PC1 and PC2 axis of the species-wise data to test . While PC1 was not related to cover change (p = 0.9), PC2 was significantly (p = 0.045, $R^2 = 0.10$) related to cover change of the target species (Table 10). The traits that best explained the PC2 were SLA, Suc, tCWC, height & LT, and explained 10.4% of the total variation.

***Table 9: Linear model** explaining the PC1 and PC2 with the changes in species abundance.*

Coefficients:	**Std. Error**	**t-value**	**Pr(>|t|)**	**Adj. R^2**
PC1	0.021	0.162	0.873	-0.03475
PC2	0.013	2.098	0.045*	0.1049

4. Discussion

Functional traits are important for determining species fitness and vital rates and can exhibit different attributes under contrasting environmental conditions. But even in the same environment, those attributes can vary greatly. We first look at variation of the functional traits and ecological indicator values (EIVs) of the plant species that were measured at Hochschwab, Austria. Afterwards, we investigate patterns and correlations between the functional traits and the changes in species abundance, and finally, we explore the relation between changes in alpine vegetation and functional traits.

Functional traits

Our results show that the functional traits of the plant species that were measured differed strongly among plant species. This is expressed as the coefficient of variation (CV), for which the highest CV are for AR (117%), LA (98%) and height (90%). This indicates that there is strong variation between the plant species concerning these traits, which are all physical traits on the global spectrum of plant form and function (Díaz, et al. 2016). This is because certain environments have certain leaf shapes and sizes and in mountainous areas, plants have generally small leaves, as the conditions are very harsh. We expected less variation in the LA, but alpine plant leaves are still highly diverse and adapted to many environmental challenges (Körner 2021). The variation in our data most likely comes from the different growth forms that we sampled: mean species height varied from 0.58 cm (*S. acaulis,* cushion plant*)* to 22.20 cm (*F. versicolor,* grass*).* This is similar to another study on Schrankogel (Austria) where plant height varied between 1.00 cm and 23.40 cm (n=59) along a much larger elevational gradient (2140 – 3150 m) (Paetzolt 2022). Plant height depends on the growth form of the species: dwarf shrubs are close to the ground, with heights recorded between 10-30 cm just above the tree line, and 1-5 cm at higher alpine elevations. Graminoids are taller, even at higher elevation, and can range from >0.5m to a few cm (Körner 2021).

Due to climate change, plants are expected to experience more frequent drought in the future. Therefore, we looked at traits related to drought tolerance: g_{min}, succulence, tCWC, CWC and the osmotic potential. From these traits, g_{min} shows a high variation among species (77%), which shows that there are big differences in the drought resistance of plant species in the same environment. It is influenced by succulence (61%), which reflects the amount of water that can be stored per leaf area. Duursma, et al. (2018) reviewed literature to try and explain variation in g_{min} between different plant species. The data they compiled (n=221) for g_{min} measurements had a mean value of 4.5 mmol m^{-2} s^{-1}, which is 0.029 g cm^{-2} h^{-1}; the unit used for our data. This is much higher than for our data: our maximum value is 0.0157 g cm^{-2} h^{-1}. This is likely due to the environment that our species live in: alpine plants are considered to live in extreme conditions. A low g_{min} is an adaptation many plants in arid environments have, with plants having xerophytic adaptations like small leaves, thick cuticles, and a high succulence, which are all traits that are geared towards reducing water loss (Smith, Monson and Anderson 2012). They also found that besides grasses having a higher g_{min}, it is very difficult to explain variation. The species with the highest g_{min} in our data were *R. montanus* (0.015 g cm^{-2} h^{-1}), a deciduous perennial and *K. simpliciuscula* (0.012 g cm^{-2} h^{-1}), a grass. (Landolt 2010). Effects of the environment on g_{min} have also been researched, and studies have found that water limitation and a lower relative humidity decrease g_{min}, while a higher temperature increases g_{min} (Duursma, et al. 2018, Fanourakis, Heuvelink and SM. 2013, Drake, et al. 2018).

Another trait that may influence how well plants will be able to cope with climate change is tolerance to frost. During the winter, plants are hardened and can survive extreme low temperatures and in Austria, the temperature can drop to extreme values (-37.4 °C, measured at Mt. Sonnblick, 3105m) in alpine areas (Neuner 2014). And even with global temperatures increasing, in the spring and summer, the temperature can still drop below zero. In the nival zone of the Tyrolian Alps, the air temperature can drop to -6°C at the treeline, and -15.6 °C in the nival zone (Neuner 2014). In the summer, the frost resistance of leaves is the lowest. Therefore, LT_{50} and osmotic potential are crucial for determining if plants can survive summer frost. The lowest frost resistance was measured in *S. acaulis* (-4.8 °C) and the highest in *C. atrata* (-13.4 °C). The PCA showed that graminoids, like *C. atrata,* are likely to be more frost resistant then other species, which is a pattern shown more often (Taschler and Neuner 2004, Pescador, et al. 2016) Resistance to summer frost in alpine plants has been studied, and ranges from -4.5 (*Vaccinium myrtillus*) to -14.6 °C (*Poa alpina*) on Mt. Patscherkofel (1950m), near the University of Innsbruck, and in the foreland of the Hintertuxer Glacier (2660m) (Taschler and Neuner 2004). These values for LT_{50} are very similar, as well as the elevation at which the plants were sampled. An interesting difference between our studies is that for *Vaccinium vitis-idea*, LT_{50} that we measured was -11.3 °C. We sampled this species at the Zinken summit, which has an elevation of 1950m. Taschler and Neuner (2004) sampled this species at an elevation of 2700 and measured an LT_{50} of only -5.5 °C. Resistance to summer frost is related to elevation, with frost resistance increasing with elevation, but that was not the case for this specific species. Intraspecific variation of species is therefore also something interesting to focus on in further research.

Ecological indicator values

Besides functional traits, we also looked at the ecological indicator values (EIVs), which are ordinal values that are derived from previous observations and expert judgment that reflect species habitat preferences (Diekmann 2003). Community-weighted mean EIVs of the target species (n=29) indicate a fresh soil moisture (F = 2.26), with a neutral to alkaline (R = 4.41) and nutrient infertile soil (Nu = 1.48), with an air temperature characteristic to lower alpine and subalpine zones (T = 1.64). Overall, the set of target species represent the community well when it comes to EIVs. The high Landolt R value is likely linked to the calcareous parent's rock and the Wetterstein limestone soils which predominate in the study area (Hochschwab). It contains lime materials like calcium carbonate ($CaCO_3$) and causes the soil to have a more alkaline pH, which represents a stressful habitat for plants and has an effect on the nutrient availability (Romeo, et al. 2015). This is also reflected in the Landolt Nu value, which is very low and is thus nutrient infertile. The pH of the soil and the nutrient availability have a strong effect on the species community composition and distribution (Romeo, et al. 2015).

4.2 Coordination and correlation of traits

The pairwise correlations and the cluster dendrogram show correlations between the functional traits and EIVs: the cluster dendrogram and pairwise correlation showed which traits were most correlated to each other. Our results showed that, due to the amount of nutrients in our dataset, there were many correlation between functional traits and nutrients. The dendrogram showed that the traits are grouped together in four different clusters. The majority of the functional traits and some of the nutrients are together in one large cluster, the rest of the nutrients and the Landolt values in another, and two small clusters containing only a few traits.

The AR, height, and LA are in one sub-cluster. These are all traits that are on the global spectrum of plant form and function and explain the plants size and shape (Díaz, et al. 2016). The aspect ratio reflects leaf shape and is related to leaf area, which is important for light capture, height is important for competition for light. Height is also correlated to LT_{50} (r=-0.49) and osmotic potential (r=0.43). The negative correlation between height and LT_{50} indicates that taller plants are more frost resistant, which is also what a study in Venezuela found (Squeo, et al. 1991). In winter, small alpine plants are protected by snow, while taller plants may not be fully covered and thus have to be more tolerant to lower temperatures (Körner 2021). The positive correlation with osmotic concentration could mean the same, as taller plants have lower osmotic potential. As more negative osmotic potential reduces the freezing point and thus delays ice inside the cells, this also means they are more frost tolerant (Snyder and Melo-Abreu 2005).

It is therefore rather surprising that the osmotic potential clusters in the dendrogram with the nutrients S and P only and this cluster has the greatest distance to the other clusters. However, in pairwise correlations, Osm is among other things negatively correlated to LT_{50} (r=-0.48) and succulence (r=-0.4). The correlation between the osmotic concentration and LT_{50} indicates that plants with a high osmotic concentration have a low LT_{50} and can thus recover from lower temperatures. This is also explained by Snyder & Melo-Abreu (2005), who found that an increased concentration of solutes in the protoplasm can help reduce the formation of ice crystals.

Another sub-cluster included LT_{50}, g_{min} and succulence. LT_{50} and Suc are positively correlated in both the species-wise (r=0.38) and CWM (r=0.60) correlations. Since the values for LT_{50} are negative, this indicates that species with a higher succulence a have a higher LT_{50} and are less frost tolerant. This is confirmed by Griffiths & Males (2017), who explain that, while succulence increases tolerance against drought, most succulents are susceptible to frost damage at lower temperatures. That succulence is g_{min} are clustered together is likely because the succulence is used to calculate g_{min}. There was little variation between the plant species when it came to g_{min}, which is also confirmed by literature (Duursma, et al. 2018). tCWC is positively correlated to the LT (r=0.37), g_{min} (r=-0.62), and Suc (r=0.49). The tCWC is calculated from the g_{min} and the succulence and are likely correlated to each other due to this. However, plants with a higher tCWC, will have more water available since succulent species can store water in their leaves. This allows them to can survive longer without water (Farooq, Wahid, et al. 2009).

The second largest cluster contains the majority of the nutrients and all EIVs, as well as tCWC. The correlation between nutrients and Nu and R was expected, since the Landolt Nu value explains the nutrient availability (mainly N, P and K) and the R is the soil reaction, which is strongly related to the availability of nutrients. However, the soil moisture (F) and temperature (T) are also grouped in this cluster, but there seems to be no previous research into this relationship. Landolt F and Nu are correlated with each other (r=0.59), which means that when the nutrient availability increases, the soil humidity also increases. As these are the correlations between the species-means, this indicates that species with a high humidity requirement are likely to prefer nutrient rich soils.

The two final small clusters are quite interesting. One contains SLA, Chl, B and K. SLA is a ratio between the leaf area and the dry mass, which reflects the relative growth rate of the plant (Mengzhou Liu, et al. 2017). It is a morphological trait, that can influence the resistance to drought. A previous study has found a negative correlation between SLA and CWC, in 14 plant species that were sampled from different habitats (Reich, Walters and Ellsworth 1992). In our data, the SLA was negatively correlated with the tCWC (r=-0.42). This indicates that species with a high SLA then to have a lower CWC. Since a high SLA reflects a high relative growth rate, this suggests that plants with a high SLA invest more resources into growth, and thus require more water (Reich, Walters and Ellsworth 1992). Alpine plant species are generally characterized as having a low SLA and are therefore likely to be more drought resistant than species at lower elevations (Körner 2021).

4.3 Changes in species attributes

Changes in species abundance

Our results showed that there were changes in species abundance between 2001 and 2015. We assumed that with the change in species abundance, there would also be a change in the trait composition of alpine communities. We tested if the composition of the species community changed using an anosim test, and it showed that the composition of the species community did not change between 2001, 2008 and 2015 (p-value = 0.9).

We also looked at if the functional trait community changed. We performed a MANOVA on all of the CWM trait data between the three years, which found no significant difference (p-value=0.92), and an ANOVA per functional trait, which also did not show significant difference. This is likely due to the fact that the most dominant species*, C. firma*, has not changed much in abundance between 2001 and 2015 (increased by 1.8%). Because *C. firma* was the species with the highest average cover, it is likely to have biased the CWM output since it is present on almost all of the plots and changed very little between 2001 and 2015. A study by Zelený (2017) explains that, in certain cases, the use of CWM results in highly biased results in terms of the significance of the relationship between the CWM and the sample attributes, in our case the EIVs and functional traits, and the effect size.

Modelling of species attributes

Because there was no change in the community-weighted means of the functional traits, we also wanted to look at if the traits could explain the changes in individual species' abundance. Therefore, in addition to the visualization of the trait clusters and correlations, we also checked if any functional traits or EIVs were significant in explaining the change in species abundance in a regression model.

For the EIVs, we wanted to check if any of the Landolt values were significant in explaining changes in species abundance. Out of all the Landolt values, only T was significantly related to change in species abundance. The linear model showed that, for the target species, the abundance of species with a higher temperature preference were increasing (R^2=0.19, p-value = 0.019). This has also been determined by previous studies (Lamprecht, et al., 2018; Steinbauer, et al., 2018).

As an alternative to looking at EIVs, we also looked if functional traits could explain changes in species abundance and composition. An ANOVA that tested for difference between the CWM of the three years showed that the functional trait composition did not change. As explained, this is likely due to the fact that the most dominant species, *C. firma*, stayed very constant over the years. We therefore also looked at if the functional traits of the species means could explain the change in species abundance. We started with a reduced model, containing SLA, LT_{50}, height, LA, LT, and Sol, to avoid over-parameterization, and found some traits that are significant. A stepwise reduction by `AIC` resulted in the best model that includes LT_{50}, height and Sol leaf solidity and height having a highly significant effect. This model was only significant when weighing by average cover ($cov^{0.5}$), but not in an unweighted model. By weighing our model, species with low occurrences do not count equally to species with a high occurrence. Therefore, the change in species abundance can be explained by the increase in both plant height and solidity. This could mean that leaves with more leaflets or deep lobes (low solidity) become less common. Plant height is important for species growing in harsh conditions, as increased snow cover, low temperatures, and hard winds. Typical growth forms of Alpine plants include rosette, creeping and cushion plants (Körner 2021). But, with increasing temperatures, previous research has found that there will be a pronounced shift to taller plant communities with more resource investment (high SLA) as a result of long-term warming (Bjorkman, et al. 2018). Bjorkman, et al. (2018) found that over 27 years of monitoring, the plant height was the only CWM trait that changed significantly at nearly every site they sampled as a result of temperature increase. This is likely due to the migration of taller species from lower, warmer microclimates that move upwards, rather than the loss of short species (H. Pauli, et al. 2012, Bjorkman, et al. 2018, Parolo and Rossi 2008).

Soudzilovskaia, et al., (2013) also did research into explaining changes in species abundance using functional traits and found that alpine plant species with high resource input traits, like a low SLA and thick leaves, increase in abundance with an increase in temperature. The functional traits SLA and LT were thus significant in explaining the change in species abundance. Their study took place in the Caucasus mountains in Russia, where they measured the functional traits of 50 different plant species. The study differs from ours in the way that they measured the species abundance: GLORIA measures species by cover (%), while Soudzilovskaia, et al., (2013) measured by the annual abundance (number of shoots per m^2). This could also have an influence in calculation of which, if any, functional traits can explain changes in abundance.

As an alternative to a model with individual functional traits, we also regressed the change in species abundance with the two main components of a PCA and found that the PC2 reflects the change in abundance the best. Our data-set contains many functional traits, with almost half of them being nutrients. This affects the outcome, with many nutrients scaling along the PC1. Therefore, the more important functional traits generally scale on the PC2. The traits with the highest & lowest scores on the PC2 are SLA, Suc, tCWC, height & LT.

4.4 Reflections and outlook

We set out to explain if changes in alpine vegetation are reflected in their functional traits. Our results showed that, while there were changes in abundance for the individual species, the overall community did not change significantly. This likely affected the community-weighted means, which were dominated by the species *C. firma*. Due to that bias, the CWM of the functional traits did not show changes. We were however able to show that the changes in abundance for our target species are reflected by the trait's height and solidity. The EIV Landolt T also reflected the change in abundance. It was surprising that none of the traits related to drought resistance (succulence, g_{min}, tCWC, CWC) were among the predictors of changes in species abundance in the regression model. Since the Landolt values are species related, there could be a difference in habitat preference between the same species growing at lower vs. higher altitudes. The Landolt T value as a predictor for the change in species abundance may therefore be weak since the values are ordinal and subjective and may not reflect the actual preferences of the sampled species. The Landolt values are also not likely to change, as they are static, and using them to explain dynamic changes (change in species abundance) is also something that must be taken into consideration.

Another important part of this study were the data transformations and working with the linear models. By changing the transformations of data (log vs. power transformation), the results of the linear models changed. By adding the average cover of the species as weight to the model, some predictors turned out to be significant, when without the weight they were not. This makes sense, since the model is weighted by the species that were more abundant and had more influence in the community. The same happened for the transformations. The model with the Landolt T value was significant with a power$^{0.5}$ transformation, but not with a log+1 transformation. We chose standard transformations for the present analysis but given that these details of the analyses are to some extent subject to individual choices, obtaining the same outcome with alternative tests would provide more confidence.

A limitation in this study was the small dataset. Many traits were measured for a few species, which increases the chances of false positive effects (type I errors). Therefore, we started with a reduced trait set in the linear models to reduce the over-parameterization of the model. With a larger list of species, the chances for these effects decreases, and true underlying patterns may be observed more easily.

This study was an initial attempt for the location Hochschwab, but the approach taken in study can also be used more widely. Other studying are trying to explain if changes vegetation can be related to functional traits. Another ecological survey was performed on Hochschwab in 2022, which will provide more robust data on directional changes in abundance. The past 8 years were the warmest years on record, with 2016, 2019 and 2020 being in the top three (World Meteorological Organization 2023). It is therefore important to include the latest changes in alpine vegetation and relate these changes to functional traits. In the Italian Alps, ecological surveys found that the species composition has changed over the past 20 years, with the proportion of thermophilic species increasing (Porro, et al. 2022). In Sweden however, surveys done in 2001, 2008 and 2019 found only minor changes in the community composition, with vascular plants having a remarkable steady assemblage

(Hagenberg, et al. 2022). This shows that plant species and communities react differently to climate induced changes everywhere, which is why it is important to keep monitoring in the future as well.

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Appendix

A. Table with target species

S1: List with target species. *The species name, the summits that the species can be found at, and the summit that the species were collected.*

Species	GHK	WEK	ZAK	ZIK	number of summits Hochschwab	Collected at:
Androsace chamaejasme Wulfen	1	1	1	1	4	ZAK
Armeria maritima (Mill.) Willd. subsp. alpina (Willd.) P.Silva	1	1	1	1	4	GHK
Aster bellidiastrum (L.) Scop.	1	1	1	1	4	GHK
Bartsia alpina L.	1	1	1	1	4	GHK
Carex atrata L. subsp. atrata	1	1	1	1	4	GHK
Carex firma Host	1	1	1	1	4	WEK
Carex sempervirens Vill.	1	1	1	1	4	WEK
Doronicum glaciale (Wulfen) Nyman	1		1		2	GHK
Dryas octopetala L.	1	1	1	1	4	WEK
Festuca versicolor Tausch subsp. brachystachys (Hack.) Markgr.-Dann. (zu F. varia agg)	1	1	1	1	4	WEK
Gentiana clusii E.P.Perrier & Songeon	1	1	1	1	4	WEK
Geum montanum L.	1		1	1	3	GHK
Helianthemum oelandicum (L.) DC. subsp. alpestre (Jacq.) Breistr. syn H.alpestre	1	1	1	1	4	WEK
Homogyne discolor (Jacq.) Cass.	1	1	1	1	4	GHK
Kobresia simpliciuscula (Wahlenb.) Mack.		1			1	WEK
Leontodon hispidus L.	1			1	2	WEK
Luzula glabrata (Hoppe) Desv.		1	1	1	3	GHK
Polygonum viviparum L.	1	1	1	1	4	GHK
Potentilla clusiana Jacq.			1	1	2	ZAK
Ranunculus alpestris L. subsp. alpestris	1	1	1	1	4	GHK

Ranunculus montanus Willd.	1	1	1	1	4	GHK
Salix reticulata L.	1	1	1	1	4	GHK
Salix retusa L.	1	1	1	1	4	GHK
Saxifraga paniculata Mill.		1	1	1	3	WEK
Sesleria albicans Kit. ex Schult.	1	1	1	1	4	WEK
Silene acaulis (L.) Jacq. subsp. acaulis	1	1	1	1	4	GHK
Thymus praecox Opiz subsp. polytrichus (A.Kern. ex Borb s) Jalas	1	1	1	1	4	GHK
Vaccinium vitis-idaea L. subsp. vitis-idaea	1	1		1	3	WEK

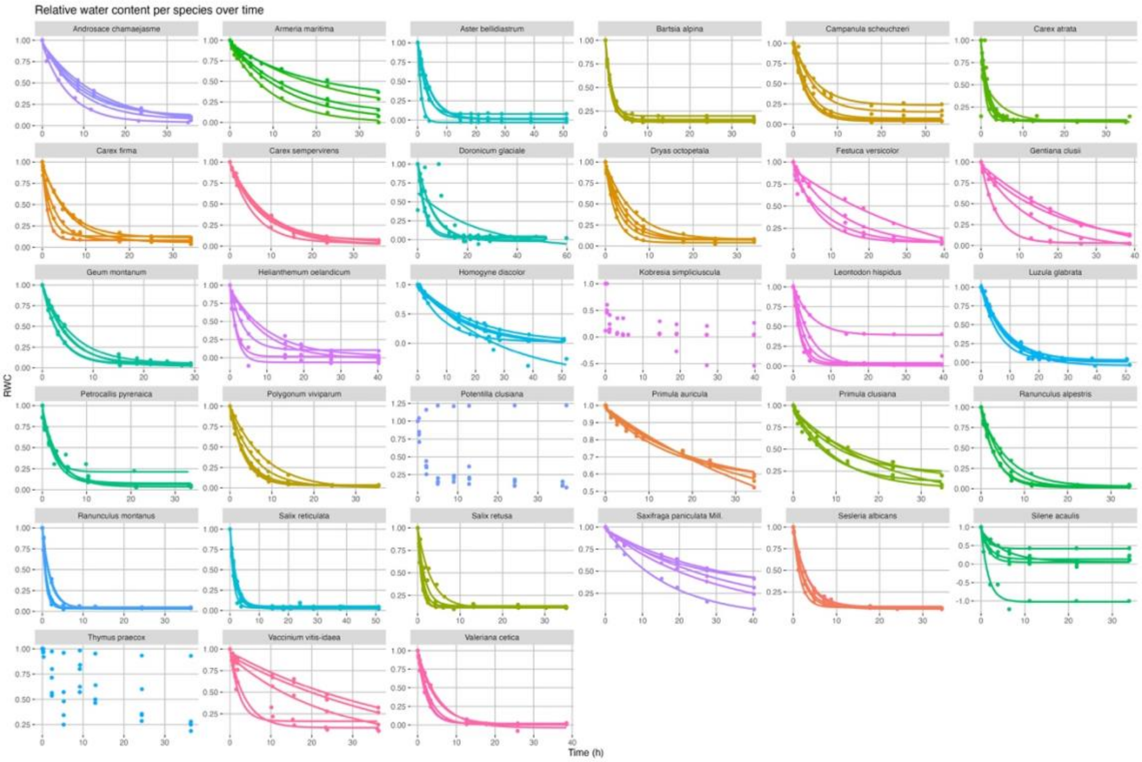
B. Table with additional information on target species

S2: Ecological indicator values (Landolt 2010) and abbreviations used in Fig. 8 for the target species.

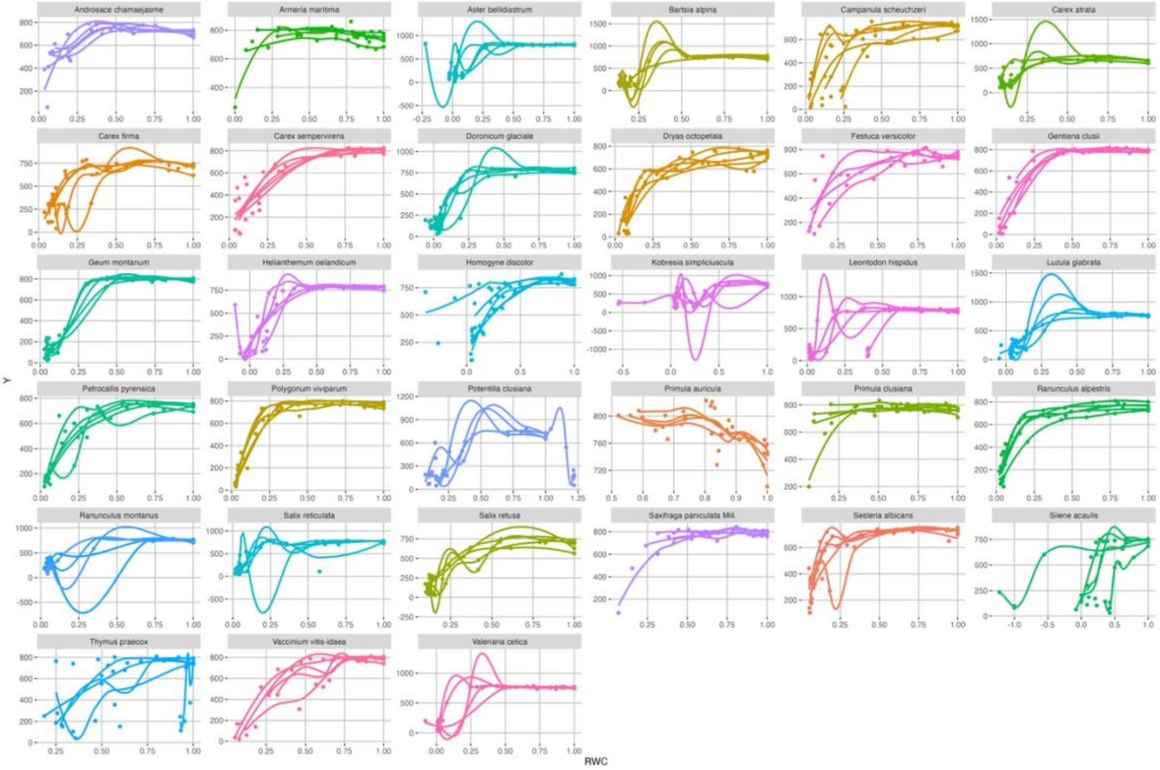
Species name	**sp_code**	**Family**	**T**	**F**	**R**	**Nu**
Androsace chamaejasme	andr_cham	Primulaceae	1.5	2	2	5
Armeria maritima	arme_mari	Plumbaginaceae	1	2	2	2
Aster bellidiastrum	aste_bell	Asteraceae	2	3.5	4	2
Bartsia alpina	bart_alpi	Orobanchaceae	2	4	3	3
Campanula scheuchzeri	camp_sche	Campanulaceae	1.5	2.5	3	3
Carduus defloratus	card_defl	Asteraceae	2.5	2	4	3
Carex atrata	care_atra	Cyperaceae	1.5	3.5	3	2
Carex firma	care_firm	Cyperaceae	1.5	2	5	1
Carex sempervirens	care_semp	Cyperaceae	1.5	2	3	2
Crepis aurea	crep_aure	Asteraceae	2	3	3	4
Doronicum glaciale	doro_glac	Asteraceae	1.5	3.5	3	3
Dryas octopetala	drya_octo	Rosaceae	1.5	2.5	5	2
Empetrum nigrum	empe_nigr	Ericaceae	3	3.5	2	1
Festuca versicolor	fest_vers	Poaceae	1.5	2	2	2
Gentiana clusii	gent_clus	Gentianaceae	1.5	2.5	5	2
Geum montanum	geum_mont	Rosaceae	1.5	3	2	2
Hedysarum hedysaroides	hedy_hedy	Fabaceae	1.5	3	4	3
Helianthemum oelandicum	heli_oela	Cistaceae	1.5	2	5	1
Homogyne discolor	homo_disc	Asteraceae	1.5	3.5	4	2
Kobresia simpliciuscula	kobr_simp	Cyperaceae	1.5	4	4	1
Leontodon hispidus	leon_hisp	Asteraceae	3	2.5	4	3
Ligusticum mutellina	ligu_mute	Apiaceae	1.5	3.5	2	3
Luzula glabrata	luzu_glab	Juncaceae	1.5	3.5	4	3
Petrocallis pyrenaica	petr_pyre	Brassicaceae	1.5	2	5	1

Phyteuma orbiculare	phyt_orbi	Campanulaceae	2.5	3	4	2
Polygonum viviparum	ploy_vivi	Polygonaceae	1.5	3	3	2
Potentilla clusiana	pote_clus	Rosaceae	1.5	1.5	5	1
Primula auricula	prim_auri	Primulaceae	1.5	3.5	5	2
Primula clusiana	prim_clus	Primulaceae	1.5	2.5	5	2
Ranunculus alpestris	ranu_alpe	Ranunculaceae	1.5	3.5	4	2
Ranunculus montanus	ranu_mont	Ranunculaceae	2	3.5	4	4
Salix reticulata	sali_reti	Salicaceae	1.5	3	5	2
Salix retusa	sali_retu	Salicaceae	1.5	3	4	2
Saxifraga paniculata Mill.	saxi_pani	Saxifragaceae	2	2	4	2
Sesleria albicans	sesl_albi	Poaceae	2	2	4	2
Silene acaulis	sile_acau	Charyophyllaceae	1	3	4	1
Thymus praecox	thym_prae	Lamiaceaae	4.5	1	4	2
Vaccinium myrtillus	vacc_myrt	Ericaceae	2.5	3	1	2
Vaccinium uliginosum	vacc_ulig	Ericaceae	3	4	1	2
Vaccinium vitis-idaea	vacc_viti	Ericaceae	2.5	2.5	1	2
Valeriana celtica	vale_celt	Valerianaceae	1	2	2	2
Viola biflora	viol_bifl	Violaceae	2	4	3	4

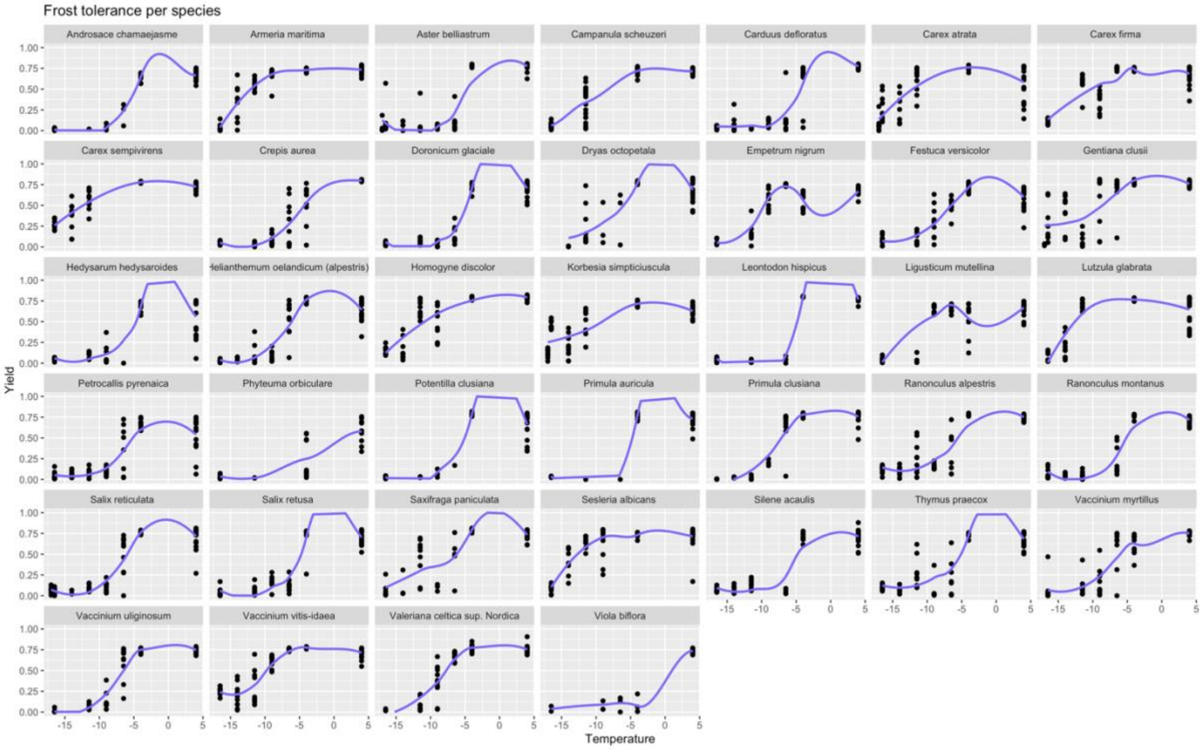
C. Leaf drying curves



S3: *Relative water content (RWC) over time (in hrs) from the weight-loss curves.*



S4: *Shows the chlorophyll fluorescence (F_v/F_m) against the relative water content (RWC) from the weight-loss curves.*



S5: Effect of temperature on chlorophyll fluorescence *F_v/F_m (Yield) used to assess frost tolerance.*