

INSUBRIA UNIVERSITY

PhD. Program in Chemical and Environmental Sciences (Environmental address)

XXX cycle

MANIPULATION EXPERIMENTS FOR THE ASSESSMENT AND MONITORING OF CLIMATE CHANGE IMPACTS ON VEGETATION OF ALPINE AND POLAR ECOSYSTEMS

Ph.D. thesis by: Francesco MALFASI

Supervisor: Prof. Nicoletta CANNONE Co-supervisor: Prof. Mauro GUGLIELMIN







Cover pictures (from top to bottom): Stelvio Pass area (Central Italian Alps), looking from Stelvio Pass to north-east, with Braulio Valley on the left and Müstair valley on the right; Skua Terrace on west coast of Signy Island (northern Maritime Antarctica); Leonie Islands (Ryder Bay), about 5 km south-west from Rothera Point, and the peaks on the south edge of Adelaide Island (southern Maritime Antarctica). © PNRA

EXTENDED ABSTRACT

High altitude and latitude environments are among those areas of the Planet that are experiencing the most significant changes of the climatic conditions due to the recent global change. Terrestrial ecosystems in these regions are extremely sensitive to climate and, for this reason, well suited for the understanding, evaluation and monitoring of their responses and their modeling under different climate change scenarios. For these reasons, IPCC identifies alpine and polar regions as highest priorities and top interest for the assessment of past, present and potential future climate changes.

This thesis focused on the impacts of climate change on terrestrial ecosystems of alpine (Central Italian Alps) and Polar (Maritime Antarctica) tundra habitats. European Alps and Maritime Antarctica are indeed two of the three areas of the world where had been recorded the greatest air temperature warming since 1950.

Due to different trends of climate and anthropogenic pressures around the world, contemporary global change was characterized by a large spatial variability that makes the planning of adaptation and mitigation strategies particularly tricky. Particular habitats have been protected by international, European and national programs (the alpine study area is located within the Stelvio National Park, while the Antarctica ecosystems are protected under the Antarctic treaty and specially protected areas).

The alpine site is located at the Stelvio Pass area, a high altitude site (>2600 masl) in the central Italian Alps, where in the last decades have been reported evident impacts of climate change. Here, since the 2014 *in situ* manipulation experiments simulating potential future climate changes started, with the logistical support of the Stelvio National Park. The experiments aimed to assess the possible future responses of vegetation to: a) temperature increase (air and soil warming), b) liquid precipitation changes (water availability and soil moisture), c) solid precipitation changes (snow-depth and snow-melt time).

This PhD activity thus focused on the performance and prosecution of this monitoring, from 2015 to 2017.

The PhD project aimed to asses the effect of the manipulation experiments on: a) environmental data; b) vegetation composition and structure; c) plant phenology (as did in the frame of ITEX (International Tundra Experiment)) and, more specifically five hypothesis were formulated: (<u>*hI*</u>) a drought ability to buffer the biological answer to soil warming; (<u>*h2*</u>) a snowmelt influence on greening but not on flowering and reproductive phenological stages; (<u>*h3*</u>) a linkage between changes in species abundance and phenology; (<u>*h4*</u>) a differential responses due to functional composition, species ecology, plant communities and terrain morphology; (<u>*h5*</u>) an higher sensitivity of alpine species to extreme years rather than to gradual and constant environmental forcing.

The manipulation experiments consisted of 12 Open Top Chamber (OTC) for the air and soil warming, of 6 Precipitation Shield (PS) for the water availability and soil moisture and of 1 Snow Fence (SF) for snow-depth and snow-melt time, plus relative control plots. For what concerns the PS experiments, one plot was underneath the shield (PS; expected effect: liquid precipitation reduction and drought) and one just downslope of the PS (PSv1; expected effect: liquid precipitation increase and higher moisture) and one further downslope (PSv2; expected effect: mesic/no effect). Snow fence experiment (SF) consisted of three line of snow stakes for each side of the fence; because of the direction of the main wind, we expected an increased snow cover on the leeward side of the fence. All the experiment were replicated in two study sites (located c. 1 km from each other within the study area) and in two plant communities (alping grasslands and growtheds), expected for the growthede.

study area) and in two plant communities (alpine grasslands and snowbeds), except for the snow fence, located in one site only because of logistical constraint. Overall, 108 plots were installed and monitored.

The first step of the PhD was to assess the influence of the experiment on the environmental data. Different devices were installed in order to quantify the effect of the structures installed and their manipulation effect on soil temperature, soil moisture and snowmelt time. Ground surface temperature (GST) was recorded in correspondence of 68 plots, with particular reference to the OTC where the thermometers were installed in the middle and at the edge of the chamber, in order to detect changes in the warming effect. Soil moisture was measured in correspondence of two precipitation shieds (PS). Snow depth and snow melt in the snow fence experiment (SF) were assessed through time-lapse digital camera and validated through field surveys.

Our data confirmed that the warming effect of the OTC was comparable to that expected by the IPCC scenario RCP4.5 forecast for 2080-2100. Moreover, we identified differences in warming between edge and middle of the OTC. Precipitation shields (PS) affected the rain redistribution and decreased soil moisture underneath the cover (PS); moreover, here we found a warming effect comparable, or even more intense, than the one detected in the OTCs but still in the frame of the IPCC scenario for the 2100. Some unexpected pattern of soil moisture, due to the influence of wind during the rain events were found but, generally, in PSv1 was confirmed the increased soil moisture. The snow fence (SF) modified the pattern of snow accumulation and of snow melt. As the experiment has been set up in a typical alpine environment on a large morainic ridge, characterized by a complex topography (differently from the prevailing flat conditions occurring e.g. in the Arctic), we detected an artificially delayed snow melt (SF+) which was compared with the naturally delayed snow melt associated to the topographic relief (SFtN, SFtS).

Relating to the effect of the manipulation experiments on vegetation composition and structure, in 2013 was performed the first description of vegetation composition and structure in all the 108 plots (before the manipulation started); in 2017 we did the second survey.

Under warming condition (OTC), our data indicated a general decrease of the cryptogamic layer (mosses and lichens), especially with the co-occurrence of vascular plant species. Alpine grasslands were less sensitive to warming than snowbeds. Drought (and warm) condition (PS) decreased the coverage of the cryptogamic layer and of the forbs, while in moist conditions (PSv1) we reported an increase in graminoids. The artificially delayed snowmelt (SF+) led to a decrease in vascular coverage, mainly related to shrubs and graminoids reduction.

For what concerns the effect of the manipulation experiments on plant phenology, we monitored the onset of the phenological stages of 14 plants typical of the main communities of the study sites and representative of different growth forms. The timing of phenological events was monitored in the field on the manipulated and control plots every 3-4 days. For data analyses, we further selected a subset of 4 species, with the highest replication level in the different experiments.

Our data stressed how responses to the environmental drivers had species-specific differences, but also with plant communities and local conditions (topography) influencing most of the phenological stages. Among all, it was of relevant interest the main effect of soil warming (OTC) in determining the advance of most of the phenological stages, from flowering, to seed development and dispersal but not on leaf senescence, thus showing that the possible benefit of an extended growing season in response to a future Autumn warming will depend not only on the temperature change but also on the soil moisture.

The increase in soil moisture (PSv1) didn't influence snowmelt and greening stages, as the structures were always positioned after the snow melt due to logistical constraints. However, our results indicated species-specific differences in flowering, seeds, leaf senescence, reproductive success and plant size.

From the snow fence experiment, we confirmed that delayed snow melt (SF+) influence not only greening stages but also flowering stages, with a general decrease of reproductive success in all the species except for the most adapted. Snowmelt was less influent on seed dispersal and leaf senescence. Moreover, results indicated how the expected lengthening of the growing season as consequences of an altered snowmelt could vary even in a small area in the alpine terrain.

While in the alpine tundra vascular plants are dominant, in high latitude ecosystems, cryptogams (mosses and lichens) are the major components of terrestrial ecosystems. In particular, in Antarctica only few long-term data are available on the responses of mosses and lichens to climate change. Comparable to Alps, the Maritime Antarctica is one of the regions of the planet recording the most rapid air warming and climate change impacts on the ecosystem. For this reason at Signy Island (South Orkney Islands, northern maritime Antarctica) within an international project in collaboration with the British Antarctic Survey we installed *in-situ* manipulation experiments, comparable to those set up in the Alps, those will allow to compare in the next years the biotic and abiotic responses of different polar and alpine tundra ecosystems. The assessment of any vegetation changes was not possible, because of the logistical constrain and of the short period of manipulation (2 years); however, we presented here the experiment design and the preliminary environmental data after the first year of deployment.

The IPCC has projected a future warmer climate that has the potential to affect all the ecosystems, but still with uncertainty in the exact nature and rate, because of the possible occurrence of unexpected impacts those could exacerbate or buffer the magnitude of response. Manipulation experiments are important tools to disentangle and clarify these uncertainty and *in-situ* manipulation experiments are suggested for this purpose.

Through the identification and study of periods with analog climate to that expected in the future, also paleo-climate investigations are a valuable tool to reduce uncertainties and to assess what a changing environment could induce in the future. Paleoclimate studies provide direct evidence of how the climate system changes through time, and can be used in the validation of future impacts under different climate scenario.

Within the southern maritime Antarctica, despite the relatively well available information on recent climate change, there was a shortage and contrasting data on paleo-climate changes.

Through the analyses of organic sediment and moribund mosses collected at Rothera Point (southern maritime Antarctica), we performed paleo-climate investigation, with particular reference to Medieval Warm Period (MWA) and Little Ice Age (LIA) and we investigated moss regeneration and cryptobiosis in recently re-exposed glacier forefield. Therefore, were provided evidences consistent with MWP glacial retreat, subsequent LIA advance, and glacial retreat associated with the rapid regional warming in recent decades. Within these dynamics, we showed evidence of *in situ* moss survival for over more than 600 y in the natural habitat through cryptobiosis, and their subsequent spontaneous recovery to active metabolism after glacier retreat, persistence of active metabolism and growth in surviving exhumed mosses, regeneration with development of green stem apices from moribund stems of exhumed mosses.

The results of this study highlighted the importance of some of the environmental cues that drive climate change impacts on terrestrial ecosystems (in both alpine and Polar tundra habitats), that should be taken into account when defining models over the coming decades at the local scale, as well as at regional and global scale, and that could be integrated in the management and adaptation plans of these particularly vulnerable ecosystems.

CONTENTS

I. INTRODUCTION AND AIMS	1
II. ALPS	8
II.1. Study area	8
II.1.A. The Stelvio Pass area	8
II.1.B. Geography and climate	9
II.1.C. Geology and geomorphology	9
II.1.D. Flora and vegetation	12
III.1.D.a. Alpine landscape: general characteristics	12
III.1.D.b. Elevational belts and vegetation elements in the Stelvio Pass area	. 12
III.1.D.c. Syntaxonomic structure of the vegetation in the Stelvio Pass area	17
III.1.D.d. Climate change impact on alpine vegetation of the study area III.1.D.e. Relevance of the selected plant species in the frame of Habitat Directive	19 19
II.1.E. Pasture management and wildlife in the Stelvio Pass area	20
II.2. Materials and methods: field work and data collection	22
II.2.A. Manipulation experiments: design and aims	22
II.2.B. Environmental data collection	25
II.2.C. Species composition assessment	28
II.2.D. Phenological observations	28
II.3. Materials and methods: data elaboration and lab analyses	32
II.3.A. Climatic data	32
II.3.B. Environmental data	33
II.3.C. Effect of manipulation experiments on vegetation composition	35
II.3.D. Effect of manipulation experiments on plant phenology	35
II.4. Results	37
II.4.A. Inter-annual variability of meteorological parameters	37
II.4.B. Natural variability in micro-climate and micro-topographical conditions	40
II.4.C. Species selection and natural variability in plant phenology	45
II.4.D. Effect of manipulation deployment on environmental data	48
III.4.D.a. Open Top Chambers (OTCs)	48
III.4.D.b. Precipitation Shields (PS)	52
III.4.D.c. Snow Fence (SF)	58
II.4.E. Natural changes in plant composition and coverage	66
II.4.F. Effect of manipulation experiments on vegetation composition	69
III.4.F.a. Open Top Chambers (OTCs)	/0
III.4.F.b. Precipitation Shields (PSs)	12
III.4.F.c. Snow Fence (SF)	() 77
II.4.G. Effect of manipulation experiments on plant phenology	//
III.4.G.a. Forbs - L. helveticus	/8
III.4.G.b. Graminoids - C. curvula	10/
III.4.G.c. Forbs - A. pentaphyllea	130
III.4.G.d. Forbs - G. supinum	149
II.J. DISCUSSION	103
II.5.A. Inter-annual variability of meteorological parameters	103
11.3.D. Ivatural variability in micro-climate and micro-topographical conditions	103

II.5.C. Natural variability in plant phenology	164		
II.5.D. Effect of manipulation deployment on environmental data	165		
II.5.D.a. Open Top Chambers (OTCs)			
II.5.D.b. Precipitation Shields (PSs)			
II.5.D.c. Snow Fence (SF)			
II.5.E. Effect of manipulation experiments on vegetation composition			
II.5.E.a. Open Top Chambers (OTCs)	168		
II.5.E.b. Precipitation Shields (PSs)	168		
II.5.E.c. Snow Fence (SF)	170		
II.5.F. Effect of manipulation experiments on plant phenology	170		
II.5.F.a. Snowmelt and greening	171		
II.5.F.b. Flowering	172		
II.5.F.c. Seed development and leaf senescence	172		
II.5.F.d. Plant height	173		
II.5.F.e. Reproductive investment and success	174		
III. NORTERN MARITIME ANTARCTICA	178		
III.1. Study area	178		
III.1.A. Signy Island, South Orkney Islands	178		
III.1.B. Geography and climate	178		
III.1.C. Geology and geomorphology	178		
III.1.D. Flora and vegetation	180		
III.2. Materials and methods: field work and data collection	183		
III.2.A. Manipulation experiments	183		
III.2.A.a. Experiment design and aims	183		
III.2.A.b. Environmental data collection	186		
III.2.A.c. Species composition assessment	186		
III.3. Results	187		
III.3.A. Effect of manipulation deployment on environmental data	187		
III.3.A.a. Open Top Chambers (OTC)	187		
III.3.A.b. Precipitation Shields (PS)	188		
III.3.A.c. Snow Fence (SF)	189		
IV. SOUTHER MARITIME ANTARCTICA	191		
IV.1. Study area	191		
IV.1.A.Marguerite Bay, western Antarctic Peninsula	191		
IV.1.B. Geography and climate	191		
IV.1.C. Geology and geomorphology	192		
IV.1.D. Flora and vegetation	193		
IV.2. Materials and methods	195		
IV.2.A. Paleo-climate investigation	195		
IV.2.B. Moss regeneration and cryptobiosis	195		
IV.3. Results	197		
IV.3.A. Paleo-climate investigation	197		
IV.3.B. Moss regeneration and cryptobiosis	197		
V. CONCLUSIONS AND FINAL REMARKS			
VI. SUPPLEMENTARY MATERIALS			
VII. AKNOWLEDGMENT			
VIII. REFERENCES			

I. INTRODUCTION AND AIMS

Due to the rising atmospheric concentration of greenhouse gases, such as carbon dioxide, the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC-AR5) confirmed that global mean surface temperature has increased of 0.85°C (0.65 to 1.06°) since the late 19th century (period 1880-2012), with the decade of the 2000s as the warmest of the instrumental climatic records (**IPCC**, **2014**), despite a substantial decadal and inter annual variability and a recent decrease in the warming rate in the last 15 years (1998-2012). Moreover the late 20th century warmth detected is unprecedented at the hemispheric scales and can only be explained by anthropogenic greenhouse gas forcing (**Jones and Mann, 2004; IPCC, 2014**).

In a near term projection (2016-20135), global mean surface temperature is expected to be from $+0.3^{\circ}$ C to $+0.7^{\circ}$ C warmer then the period 1986-2005, while long term projections (2081-2100) indicates a rise between $+0.3^{\circ}$ C to $+4.8^{\circ}$ C (**IPCC**, 2014).

When averaged over land areas, between 1901 and 2008 the precipitation of the mid-latitude of the Northern Hemisphere (30°N to 60°N) has generally increased, whereas in the mid-latitude of the Southern Hemisphere (60°S to 30°S) it has limited evidence of long-term increase. Precipitation over tropical areas (30°S to 30°N) has increased over the last decade reversing the drying trend occurred from the mid-1970s to mid-1990s (**IPCC**, **2014**)

Despite the confidence in precipitation change averaged over global land areas was low prior to 1951 and medium afterwards, the time series show little change since 1901 but with a more likely increase of extreme weather events (such as drought, floods, heat waves, frosts, advanced or delayed snowmelt) especially since 1951.

In future it is expected that temperatures will continue to rise faster than average, rainfall distribution is expected to change and extreme weather events, such as torrential rain and drought, are also predicted to significantly increase in frequency (IPCC, 2014).

Hemispheric reconstructions and trends analyses, however, provide little information about regionalscale climate variations. Indeed, the patterns of climate change are not uniform over the whole planet (IPCC, 2014). High latitude and high elevation environments experienced the largest significant changes of the climatic conditions (Turner et al., 2007; Pepin and Lundquist, 2008; IPCC, 2014) and have been identified among the most sensitive and vulnerable ecosystems to climate change occurring on a global scale (IPCC, 2014). This is because climate and abiotic factors exert a greater influence than biotic factors on regulating ecosystem functions and influencing the temporal and spatial pattern of terrestrial ecosystems (e.g. Theurillat and Guisan, 2001; Diaz et al.,2003; Körner, 2003; Keller et al.,2005; Scherrer and Körner, 2011).

In the European Alps, atmospheric warming was estimated to be more than double of the planet average for the last 50 years (**Böhm et al. 2001**), reaching +1.2°C over the period 1950-2003 (**Cannone et al., 2007**), with a significant summer warming particularly severe since 1970 (**Casty et al. 2005**). The decadal rate of temperature increase was of +0.57°C during the 1975-2004 period, by far much higher than the increase of +0.135°C during the 1901-2000 period (**Rebetez and Reinard**, **2008**). There have been changes in precipitation amounts, especially in fresh snow (e.g. **Beniston et al., 2011; Beniston, 2012; Giaccone et al., 2015; Klein et al.,2016**), although they were not geographically homogeneous due to the heterogeneous morphology of the alpine region and the high complexity of alpine climate (e.g. **Serquet et al.,2011; Terzago et al.,2012; Acquaotta and Fratianni, 2013**). Earlier snowmelt dates, decreasing snow depth and an increasing proportion of winter precipitation falling as rain instead of snow have been recorded (**Marty, 2008**). Since 1975, the lowest altitude of the snowfall has increased by +200 m between December and January, +250 m in February, +450 m in March and +400m in April (**Rebetez and Reinard, 2008**).

Similarly to the Alps, Arctic experienced the greatest regional warming on Earth, with an increase in temperatures up to +3°C since 1950s (e.g. ACIA, 2005; Turner et al., 2007), affecting particularly regions such as Alaska (Hinzman et al., 2005) or Siberia (Serrezze et al., 2000). Over the 1951-2008 period, precipitation trends increased, but with a wide range of magnitude and local variability (IPCC, 2014), whereas since 1972 the annual snow cover has decreased largely due to the spring and summer deficits (Serreze et al., 2000; Liston and Hiemstra, 2011).

Climate change has been particularly evident also along the Antarctic Peninsula that, with Alaska and Siberia, is among the regions of the planet recording the most rapid atmospheric warming over the last 50 years (**Turner et al. 2007**). The largest warming has been detected on the western part of the Antarctic Peninsula (Faraday/Vernadsky Station, 65.4°S), with a statistically significant trend of +0.54°C per decade for the period 1951-2011, especially during winter season, with an increase of +1.01°C per decade (**Turner et al.,2014**). Over the longest temperature series recorded at Orcadas Station (South Orkney Islands) since 1904, the warming trend was of +0.21°C per decade. On the other side, in Continental Antarctica, trends indicated even a temperature decrease (**Doran et al., 2002; Turner et al., 2014**). As well, snowfall trends varied from region to region, with an increase along the Western Antarctic Peninsula and the coastal West Antarctica, which receive warm, moist air from lower latitudes, but with little evidence along the East Antarctic and over the continent (**Turner et al., 2014**).

Among the abiotic components of cold terrestrial ecosystems, the cryosphere, which is the dominant element of these environments, showed striking and evident responses to climate change (e.g., **Dyurgerov and Meier, 2000**; **Haeberli et al.,2007**; **IPCC, 2014**; **Chersich et al., 2015**). During the last decades, the worldwide glacier retreat and mass decrease have strongly increased above the long-term averages (**Dyurgerov and Meier, 1997, 2000**; **Haeberli et al., 1999**; **Paul et al.,2004**; **Oerlemans, 2005**; **WGMS, 2008**), with the European Alps as one of the most sensitive area (**Cannone et al.,2008**; **Diolaiuti et al., 2010, 2012**; **D'Agata et al.,2014**; **Colucci and Guglielmin, 2015**; **Giaccone et al.,2015**), coupled with a reduction of the snow cover depth (**Serrezze et al., 2000**; **Cannone et al., 2007**; **Bokhorst et al., 2016**; **Klein et al., 2016**) and its areal extension (**Thompson, 2016**), due to the increase in summer temperature (e.g. **Ohmura et al.,1992**; **Hughes and Braithwaite, 2008**) and concomitant decrease in winter precipitation (e.g. **Colucci and Guglielmin, 2015**). Similarly, Antarctica and, more in detail, the Antarctic Peninsula (AP) region showed similar glacier pattern (e.g. **Simoes et al.,2015**; **Zhao et al., 2017; Hogg et al.,2017; Petlickli et al.,2017**) with an expected future increase of the ice-free areas (**Lee et al., 2017**).

Since the 1990s, permafrost warming has been observed worldwide, both at high latitude (Osterkamp, 2003, 2007; Smith et al.,2005, 2010; Isaksen et al.,2007; Christiansen et al.,2010; Vieira et al.,2010; Guglielmin et al.,2011, 2012, 2014; Guglielmin, 2012; Bockheim et al., 2013; Farbrot et al.,2013; Guglielmin and Vieira, 2014) and at lower latitude in high mountains (Vonder Mühll, 2001; Harris et al.,2003; Guglielmin et al., 2001; Guglielmin, 2004; Gruber et al., 2004; Wu and Zhang, 2008; Gruber and Haeberli, 2009; Phillips and Mutter, 2009; Haeberli, 2013; Pogliotti et al.,2015). However warming trends have been weak in several sites, including Alaska (e.g. Osterkamp, 2008), northern Canada (e.g. Smith et al 2005) and Antarctica (McMurdo Dry Valleys) (Guglielmin et al 2011). Since the 1990s, a progressive increase of the active layer thickness (ALT) has been detected in all regions as well, with the exception of northern Alaska (Shiklomanov et al., 2010), the western Canadian Arctic (Smith et al., 2009), and western Siberia (Vasiliev et al., 2008; Christiansen et al 2012).

Permafrost warming and active layer thickening were mainly attributed to air warming, although in several cases the role of snow cover, soil properties and the overlying vegetation were emphasized (e.g. Osterkamp 2007, Romanovsky et al 2007, Fedorov and Konstantinov 2008, Haeberli et al

2010, Romanovsky et al 2010). In particular, the active layer thickness did not always follow the trend of the underlying permafrost and, in general, appeared much more closely related to the trend of summer air temperature (e.g. Osterkamp 2008, Streletskiy et al 2008) or to summer radiation (Guglielmin and Cannone, 2012).

Not only the abiotic but also the biotic components of the environment are showing ample evidence that climate change has affected a broad range of organisms with diverse geographical distributions (Walther et al., 2002; Walther, 2003; Parmesan and Yohe, 2003; Parmesan, 2006; Parmesan and Henley, 2015).

According to quantitative estimates of the biological impacts of the climate change "fingerprint" (Root et al. 2003; Walther, 2003; Walther et al., 2005), a greater amplitude is expected on ecosystems and vegetation at high latitudes (e.g. Chapin et al., 2005; Convey and Smith, 2006; Convey, 2011; Callaghan et al., 2013) and altitudes (e.g. Grabherr et al. 1994; Thuiller et al., 2005; Dirnbock et al., 2011; Engler et al., 2011; Dullinger et al., 2012), because of the greater influence of the abiotic than biotic factors on regulating the function of these ecosystems (Theurillat and Guisan, 2001; Körner, 2003; Keller et al., 2005; Scherrer and Körner, 2011, Le Roux et al., 2013), strongly constrained by low temperature, short growing seasons and frequent freezing events during the snow-free period (Chapin et al., 2000).

In the European Alps, climate change impacts affected different levels of ecological organization (plant species, community and landscape) with consequences on physiology (**Brancaleoni et al., 2007; Carbognani et al., 2012; Mondoni et al., 2011, 2012**), phenology (**Menzel et al., 2006; Cleland et al., 2007; Shevtsova et al., 2009; Hülber et al., 2010; Cornelius et al., 2012; Wolkovich et al., 2012; Frei et al., 2014; Gallinat et al., 2015; Schuster et al., 2014; Wheeler et al., 2016**), spatial distribution (**Grabherr et al., 1994; Cannone et al., 2007; Parolo e Rossi, 2008; Frei et al., 2010; Erschbamer et al., 2009, 2011; Stöckli et al., 2011; Wipf et al., 2013**) colonization rates and vegetation dynamics (**Cannone et al., 2008; Cannone and Pignatti, 2014**), ecosystems processes including the biogeochemical cycles (**Koch et al., 2008; Gerdol et al., 2008; Zeeman et al., 2010; Zweifel et al., 2010**).

A growing body of literature documented similar vegetation changes both in Arctic (e.g. Molau et al.,2005; Hoye et al., 2007; Bokhorst et al., 2011; Daniels et al., 2011; Myers-Smith et al.,2011; Elmendorf et al.,2012; Callaghan et al.,2013; Ernakovich et al., 2013; Semenchuk et al., 2013; Barret and Hollister, 2016; Moffat et al.,2016; Prévey et al.,2017) and Antarctic ecosystems (e.g. Fowbert and Smith 1994; le Roux and McGeoch, 2008; Convey, 2011; Favero-Longo et al.,2011, 2012; Park et al., 2013; Guglielmin et al., 2014; Cannone et al., 2016).

Along the ground observations within the long term monitoring, more recently and in the frame of the International Tundra Experiment (Molau and Mølgaard, 1996), plot-scale *in situ* manipulation experiments of one or more environmental drivers have started in order to predict future impacts of climate change on vegetation, from the alpine regions (e.g. Erschbamer, 2007; Carbognani et al., 2016; Wipf and Rixen, 2010), to the Arctic (e.g. Danby and Hick, 2007; Wipf, 2010; Wipf and Rixen, 2010; Bokhorst et al., 2013; Elmendrof et al., 2015) and the Antarctica (e.g. Bokhorst et al., 2007, 2011, 2013).

Air and soil temperature are manipulated mainly through different types of passive warming green house, the most used are the ITEX designed Open Top Chambers (e.g. Molau and Mølgaard, 1996; Erschbamer, 2007; Hudson et al., 2011; Elmendrof et al., 2012; Bokhorst et al., 2011, 2013, 2016; Cornelius et al., 2014; Carbognani et al., 2016), through active heating (e.g. Dunne et al., 2003; Cleland et al., 2006; Sherry et al., 2007; Anadon-Rosell et al., 2014; Rosa et al., 2015; Xu et al., 2015) or through transplantation (e.g. Meng et al., 2017).

In the Northern Hemisphere, warming induced an increase in shrubs and graminoids abundance, whereas non-vascular plants (mosses and lichens) decreased their coverage (Walker et al., 2005; Elmendorf et al., 2012), combined with larger leaf size and increased plant height (Hudson et al., 2011), and an advance of reproductive phenology (Cornelius et al., 2014) especially for late-flowering species (Carbognani et al., 2016). In the Southern Hemisphere, even if the number of manipulation experiments is low (Bokhorst et al., 2011), grasses and lichens appeared more negatively affected by temperature increase than dwarf shrubs and mosses (Bokhorst et al., 2007), especially if associated with increases in winter snow depth or duration (e.g. Bokhorst et al., 2016).

Plant responses to water availability and soil moisture are analyzed through water table manipulations (e.g. Weltzin et al., 2000; Strack et al., 2006; Cornelius et al., 2014), precipitation shields or "drought experiments" (e.g. Wu et al., 2011; Beier et al., 2012; Cherwin and Knapp, 2012; Cornelius et al., 2013; Swidrak et al., 2013; Gugger et al., 2015; De Boeck et al., 2016) or through water supply (e.g. Wasley et al., 2006; Sherry et al., 2008; Darby et al., 2011; Han et al., 2015; Li et al., 2015; Kong et al., 2013).

Despite only few studies are located in tundra ecosystems (Beier et al.,2012), here water manipulation experiments had lower influence than warming on the onset and the duration of reproductive stages of plants (Cornelius et al., 2013, 2014), especially at the higher elevation (Gugger et al.,2015). However in alpine grassland communities, when heat waves coincided with drought, the plants showed clear signs of stress, resulting in vegetation browning and reduced phytomass production (De Boeck et al., 2016). Drawdown in water table or lowered water availability induced changes in many ecosystems, such a modification of carbon fluxes in water related peatlands (Strack et al., 2006), a reduction in aboveground growth in forests (Swidrak et al., 2013) and a restriction of the Antarctic terrestrial bryophyte communities. Similarly, water supply increased the aboveground biomass (Kong et al., 2013), especially when combined with nitrogen deposition (Li et al., 2015), and delayed the browning timing leading to an expanded growing season (Han et al., 2015) in the semiarid grasslands, where the lowest mean annual precipitation occurs (e.g. Cherwin and Knapp, 2012).

Due to the occurrence of many uncertainties and the uneven distribution of study sites (**Beier et al.**, **2012**), there is a need for new experiments addressing water availability and soil moisture.

Because its influence on plant activity in habitats with a persistent snow cover such as polar and alpine regions, another topic largely studied by experiments is the snow cover depth and the snowmelt time (Wipf and Rixen, 2010), both manipulated through fences (i.e. Cooper et al., 2011; Johansson et al., 2013; Rumpf et al., 2014; Legault and Cusa, 2015; Semenchuk et al., 2016), OTCs (i.e. Bokhorst et al., 2013; Suzuki, 2014), reflective or absorbing cloth covers (e.g. Livensperger et al., 2017) or manual removal or addition (shoveling) (e.g. Bombonato and Gerdol, 2012; Gerdol et al., 2013; Petraglia et al., 2014; Sherwood et al., 2017).

Changes in snow cover characteristics can considerably influence plant growth and performance. The response to delayed snowmelt time appeared to be species specific, with lichens and graminoid species generally decreasing in abundance in response to added snow and later snowmelt, whereas forbs and dwarf shrubs remained neutral or increased in abundance (**Wipf and Rixen, 2010; Gerdol et al., 2013**). The temporal niche of a species' phenological development could be a predictor of its response to snowmelt time, with earlier flowering species resulted more affected by an advanced snowmelt date than later flowering species, despite flowering duration was not influenced by snowmelt timing (**Wipf, 2010; Petraglia et al., 2014**). However an advanced snowmelt alone led to early leaf emergence, but often had limited effects on above ground production (**Livensperger et al., 2016**), whereas the combination of advanced snowmelt and warming largely enhanced the development of vegetation (**Huelber et al., 2006; Petraglia et al., 2014**; **Rumpf et al., 2014**; **Suzuki, 2014**). Other changes could be induced by the indirect effect of an increased snow accumulation, such an increase in the active layer thickness (**Johnasson et al., 2013**). Experimental studies of snow cover

changes can improve our understanding of ecosystem changes those have been observed in recent years, such as the increase in shrub cover (i.e. **Sturm et al., 2001, 2005; Tape et al., 2006; Cannone et al., 2007**).

The main feedbacks of terrestrial ecosystems to global change regard biogeochemical cycles (gas fluxes and net carbon storage), that can be altered following a modification of plant communities composition and thus of dominant plant growth forms (Wookey et al.,2009; Cannone et al.,2012; 2015; 2016; García-Palacios et al., 2015). However, the response of the terrestrial carbon cycle to global change remains one of the main uncertainties in current climate change predictions (IPCC, 2014). Plant traits drive carbon inputs and outputs, firstly trough modifications in leaf litter decomposition rates (Cornelissen et al., 2007; Cornwell et al., 2008), or soil temperatures (e.g. Klene et al., 2001; Cannone and Guglielmin, 2009) and thus on soil carbon sequestration under global changes (de Deyn et al., 2008; Schmidt et al., 2011). Concerns are thus for the future projections, that show evidences that global changes will continue to threat terrestrial ecosystems, exacerbating such impacts on biodiversity (IPCC, 2014).

Aims of the study

The analysis of the dynamics of terrestrial ecosystems in regions that are facing different climatic, biological and land-use changes, may improve the understanding and evaluation of the impacts of climate change, emphasizing the different sensitivities and peculiarities for such threatened terrestrial ecosystems. This can contribute to better quantify the real vulnerability of ecosystems to climate change and the construction of more realistic and predictive models, that could be used for adaptation and mitigation policies, which have been recognized as main elements of the climate policy at the international level (e.g., UNFCC – United Nations Framework Convention of Climate Change), as well as at the EU level (e.g., EU adaptation strategy). The identification of conservation actions and monitoring plans is thus the priority for such threatened environments, to ensure a correct management of the biodiversity and of the ecosystem services that they can provide.

In this study, we focused on the impacts of climate change on the terrestrial ecosystems of alpine (European Alps) and polar (Maritime Antarctica) tundra habitats, by analysing two regions of the world that are now facing the greatest rise in air temperature at least in the last 50 years but those experienced also past climatic changes.

In cold-environments is necessary to disentangle the impacts and interactions of multiple drivers (climate, permafrost, biotic interactions) and to identify the complex processes (competition, facilitation) those can lead to high spatial heterogeneity of species responses (**Callaghan et al., 2013**).

The European Alps are one of the three areas of the planet experiencing the most intensive warming on the period 1950-2000 (**Böhm et al., 2001**) and predicted to be among the most vulnerable and sensitive to the potential impacts of actual and future climate change (**IPCC, 2014**). Indeed, during the last 60 years, the vegetation that surrounds the Stelvio pass (central Italian Alps) experienced evident impacts of recent climate, both at species as well as at community level (**Cannone et al., 2007; Cannone and Pignatti, 2014**). This area falls within the alpine and nival belts and is representative of the main vegetation occurring at this elevation along the entire alpine range. Moreover, this area is included in the Stelvio National Park and hosts several habitats of community interest. Therefore, the improvement of the knowledge about the ecological dynamics can help to enhance the proper management and to identify the best climate change adaptation plans. In this frame, since summer 2014 we started *in-situ* manipulation experiments which involved two of the most representative and sensitive plant communities (and at higher regression risk due to climate change impacts): alpine grasslands and snowbeds. The experiments aimed to assess the possible future responses of vegetation to: a) air and soil warming, b) water availability and soil moisture, c) snow-depth and snow-melt time.

This PhD activity thus focused on the performance and prosecution of this monitoring, from 2015 to 2017

In the Alps, the main topic of the PhD project can be divided in subgroups that will be described in details in the furthers chapters: a) effect of the manipulation deployment on environmental data; b) effect of the manipulation experiments on vegetation composition and structure; c) effect of the manipulation experiments on plant phenology, in the frame of ITEX (International Tundra Experiment) (see Chapter III).

More specifically, after four years of experiment manipulations, has been hypothesized that: (<u>*h1*</u>) drought is able to buffer the biological answer to soil warming of alpine species; (<u>*h2*</u>) delayed snowmelt affects greening but not flowering and reproductive phenological stages; (<u>*h3*</u>) changes in species abundance cope with the phenological answers; (<u>*h4*</u>) functional composition, species ecology, plant communities and terrain morphology modify the effects of the experiment manipulations.

Moreover, during the years of monitoring we experienced two contrasting extreme years (2014 and 2015), which exceeded the normal summer air temperature and rain ranges. Thus we also hypothesis that (<u>h5</u>) extreme years affect differently plant phenology than a gradual and constant environmental forcing.

IPCC identified as main priorities both alpine and polar regions being at highest vulnerability to climate change. Antarctica is the largest protected area of the world; it is under the Antarctic treaty, which has among the objectives the preservation of the flora, fauna and the ecosystems. In Antarctica, the research activities allowed us to analyse two geographically remote areas belonging to the northern and southern maritime Antarctica, one of the three regions of the planet recording the most rapid atmospheric warming over the last 50 years according to Turner et al. (2009).

As previously reported for the alpine part, also in maritime Antarctica have been recently recorded several evidences of climate change impacts on many components of the terrestrial ecosystems (e.g. Waluda et al., 2010; Guglielmin et al., 2012; Cannone et al., 2016). However, unexpected responses to climate change were found as well because of the possible occurrence of disturbance drivers (Cannone et al., 2016).

In this frame, during two different Antarctic campaigns of the Italian Antarctic Research Program (PNRA) and in collaboration with the British Antarctic Survey (BAS) we installed at Signy Island (southern maritime Antarctica) *in-situ* manipulation experiments, comparable to those set up in the Alps. This will allow to compare in the next years the biotic and abiotic responses of different polar and alpine tundra ecosystems. More in details, the experiments aimed to assess the possible future responses of mosses and lichens dominated vegetation to: a) air and soil warming, b) water availability and soil moisture, c) snow depth and snowmelt time (see Chapter IV).

Moreover, within the southern maritime Antarctica, despite the relatively well available information on recent climate change, there is a shortage and contrasting data on paleo-climate changes.

In the frame of the PhD activity we analyzed samples of organic sediment and moribund mosses rexexposed after a glacier retreat at Rothera Point (southern maritime Antarctica, Eestern Antarctic Peninsula). With these analyses we were able to: a) perform paleo-climate investigation, with particular reference to Medieval Warm Period (MWA) and Little Ice Age (LIA); b) investigate moss regeneration and cryptobiosis (see Chapter V). In these remote areas (Antarctica as well as Stelvio Pass area), we could observe the natural dynamics and evolution of ecosystems. For this reason, the evaluation and quantification of climate change impacts on these ecosystems based on ground observation, could contribute to create more realistic models for the adaptation policies, also in other areas of the world with human settlements and where vegetation is no longer left at its natural evolution.

II.1. STUDY AREA

II.1.A. The Stelvio Pass area

The study area is located around Stelvio Pass (46°31′ N, 10°25′ E; elevation 2230–3095 m asl), a high alpine site in Stelvio National Park in the Italian Central Alps. Along the Braulio Valley runs the upper part of the Stelvio National road (SS38 "dello Stelvio") built at the begin of the XIX century to connect Lombardy and Tyrol territories though the Stelvio Pass (46°31'N, 10°25'E).

The study area is a typical alpine environment characterized by the presence of different habitats of high quality: from fens vegetation, pastures, grasslands and shrublands up to the characteristic habitats of the snowbeds and pioneer communities of the high alpine and nival belts. Indeed, it has been recognized as a site of community importance (SIC "Valle del Braulio-Cresta di Reit"; IT2040010) by the European Union, in the frame of the ecological network "Nature 2000", established under the Habitats Directive (92/42/EEC). These habitats constitute a source of livelihood for the alpine fauna that colonize the area. It is easy to observe protected species, like eagle (*Aquila chrysaetos*), bearded vulture (*Gypaetos barbatus*) and many other species typical of alpine environments, like chamois, bighorns and marmots.

The study area is also characterized by a great morphological diversity with several glacial and periglacial features, relict, inactive or still active. Permafrost has a patchy and discontinuous distribution (**Guglielmin and Siletto, 2000; Guglielmin et al.,2001; Cannone et al.,2003**). Nevertheless, 1 km East from the study area at 3000 m the permafrost thickness exceeds 200 m (**Guglielmin et al.,2001**).



Fig. II.1.A.1 - Location of the study area (black dotted line) and of the two study sites (red stars). Grey line = contour 100 m asl. Orthophoto: Regione Lombardia, 2012; WGS84/UTM32N)

II.1.B. Geography and climate

The Stelvio Pass area is located in the north-eastern sector of Italian Central Alps (Alps Retiche), in Upper Valtellina, north from Bormio. It lies in the province of Sondrio (SO), in the country of Bormio and is included in the table D1d5 of regional technical maps (CTR) of Lombardia region. The study area is situated on the hydrological left side of upper part of Braulio valley, in the western and northern glacial cirques and slopes of Mt. Scorluzzo (3095 m asl). At the norh-east edge of the study area, the Stelvio pass links the Valtellina and Braulio valley with the Venosta valley and the province of Bolzano, whereas at the north-west edge the Umbrail pass (46°31'N, 10°25'E; 2503 m asl) connects with the Müstair valley and the Swiss Confederation. The whole study area is about 5.8 km², with an elevation range of about 900 metres, and spreads from the main road SS38 up to the summit of Mt. Scorluzzo. The hydrological catchment is defined by the Braulio river basin, which feeds the river Adda.

The climate is characterized by a continental regime (**Ceriani and Carelli, 2000**), with a highly variable values of precipitation because of the complex orography. Climate data for the 1978-2017 period from the nearest available meteorological station at Cancano (10.32076 E, 46.51729 N, 1948 m asl, 9 km to E-SE) indicate a mean annual air temperature (MAAT) of $+3.3 \pm 0.75$ °C; January is the coldest and driest month (-5.2 ± 1.8 °C; 38.9 mm) and July is the warmest and wettest (12.2 ± 1.6 °C; 94.7 mm). Mean annual precipitation sum is 810 mm, 56 % of which falls between May and September. Snow can fall at any time, but lay continuously for 6 months, from mid November to May, reaching a mean maximum depth of 133 cm.

II.1.C. Geology and Geomorphology

The geology and the lithology of the Stelvio pass area is complex, because several units of the Upper Austroalpine domain overlay and interact in this part of the Central Alps (**Montrasio et al., 2010**). The study area is part of the Umbrail-Chavalatsch fault that belongs to the Upper Austroalpine domain. The bedrock is mainly acidic consisting of granitic and granodioritic ortogneiss and of biotic or two micas paragneiss, with some outcrops of dolomite in the south-west corner of the study area, from Bocca del Braulio (2280 m asl) upward to the south slope of Filone dei Mot (**Giacomini and Pignatti, 1955; Guglielmin and Tellini, 1992**) and a localised outcrop of andesite-basalts about 700 m west from Mt. Scorluzzo (**Montrasio et al., 2010**).

Glacial deposits, mainly composed of ablation till, are common and widespread up to 2850 m asl, whereas inactive eluvial-colluvial and fluvial-stream deposits are mainly located below 2500 m asl. Because the edges of the cirque scarps of Mt. Scorluzzo have been remodelled by degradational landslide processes, active scree slopes and talus cones are common within the study area at the base of the main rocky crest-line, especially in the western glacial cirque (**Pozzi et al., 1990; Montrasio et al.,2010**).

The soils are mainly Regosols, with only localized areas with Leptosols and mixed soils underneath the dolomite deposits and along the ridges that bring to M. Scorluzzo, respectively (**Carta Pedologica Lombardia 1:25000; FAO, 2006**).

The Brauio valley is characterized by several geomorphological features (Pozzi et al., 1990; Fig. II.1.C.1), as evidenced by the presence of transversal U-shape section (upward than Bocca del Braulio), glacial circle edges and moraines in the valley and in their lateral tributaries. A large number of glacial, periglacial and gravitational features are reported by several authors (Giorcelli and Pietracaprina, 1954; Nangeroni, 1954; Albertini, 1955; Pietracaprina, 1963; Guglielmin and Tellini, 1992; Guglielmin et al.,2001, 2003, 2004; Cannone et al.,2003; Pozzi et al.,1990; Montrasio et al.,2010).



Fig. II.1.C.1– Sketch of geomorphological map of the study area (from Pozzi et al., 1990). Red star = study sites. Legend: 1) limestones; 2) gneisses; 3) micashists; 4) surface affected by rill wash; 5) gully; 6) low water channel; 7) lake; 8) fluvial and/or stream deposits; 9) alluvional fan; 10) edge od degradational and/or landslide scarp; 11) edge of cirque scarp remodelled by degradational or landslide processes; 12) edge of glacially eroded scarp remodelled scarp remodelled by degradational or landslide processes; 13) gap and/or avalanche track; 14) small landslide; 15) scree slope and/or talus cone; 16) eluvial-colluvial deposit; 17) solifluction; 18) protalus rampart; 19) rock glacier; 20) special landforms due to discontinuous frost action and/or to snow; 21) rocky crest-line; 22)edge of cirque; 23) edge of erosional scarp; 24) Holocene moraine ridge; 25) Pleistocene moraine ridge; 26) Holocene glacial deposit; 27) Pleistocene glacial deposit; 28) karst form; 29) glacier and/or semipermanent snow field boundary.

The study area is composed of two glacial cirques, on the north and west side of Mt. Scorluzzo (**Fig. II.1.C.2**), those are characterized by the presence of several Holocene and Pleistocene moraine rigdes and of glacial erosional landforms (rocky crest-line, edge of cirques, roches moutonnées). Notwithstanding the abundance of historical documents, the Holocene glacial evolution of the Stelvio pass area is not well known. Historical maps show that Platigliole and Vedretta Piana glaciers joined until 1872 (Hoffmann and Haushofer, 1872), with a maximum documented glacier extent in 1866 (**Payer, 1868**). The minimum elevation of the Little Ice Age (LIA) ice margins was 2780 m asl in Platigliole Valley and 2680 m asl in Trafoi Valley. It was not possible to date the glacier advance by dendrochronological, lichenometric or ¹⁴C methods, but the ¹⁴C age of AD 1580 obtained for the contiguous glacier of Trafoi Valley (**Cardassi, 1995**) provides an approximate date for the Vedretta Piana glacier maximum during the Little Ice Age. From geological and geomorphological surveys, the maximum extension of the glaciers during the Holocene reached 2610 m asl (**Cannone et al., 2003**). During this phase, the Vedretta Piana glacier crossed the Stelvio pass and joined with Scorluzzo glacier. A frontal morainic ridge at 2690 m asl represents the maximum advance of



Fig. II.1.C.2 – Overview from West to East of the western glacial cirque of Mt. Scorluzzo (3095 m asl) and of the Braulio valley, with Stelvio National road (SS38 "dello Stelvio") at the bottom. In the background, on the top right, the Cristallo glacier.

Scorluzzo glacier during the LIA. From the historical documents it is possible to say that this position is lower than that reached in 1866 and documented by Payer (1868), which seems to correspond to the frontal morainic ridge at 2705 m asl. The presence of Scorluzzo glacier in the north cirque was documented until 1937 when the front reached a minimum altitude of 2820 m a.s.l. (**Pelfini, 1992**), in agreement with iconographic document reported by **Garancini (1930)** (**Fig. II.1.C.3**); around 1910 there were still two small tongues, reaching 2720 and 2730 m asl. In the west glacial cirque of Mt. Scorluzzo the iconography documented the presence of a glacier until 1872, further substituted by the actual active rock glacier (**Guglielmin et al., 2007**). Eventually, the reconstruction of the glacial history, especially the more recent, was investigated through vegetation survey (**Cannone et al., 2003**).

Permafrost distribution in the Stelvio pass area was little know before the PACE European Permafrost Monitoring Network project (Harris et al., 2001) commenced at Stelvio-Livrio area in 1998 (Guglielmin et al., 2001). Based on the distribution of active and inactive rock glaciers, the areas with actual potential permafrost occurrence are located above 2500-2600 m asl, whereas those between 2300-2500 m asl represent the potentially permafrost distribution in the Holocene (Guglielmin and Siletto, 2000). In the upper part of Trafoi Valley, around Stelvio pass and in Platigliole valley, BTS measurements showed a patchy permafrost distribution above 2850 m asl. From the first year of data (1998-1999) of the 100.3 m deep PACE borehole located at 3000 m asl (46°30'N 10°28'E), permafrost shows a mean annual ground surface temperature (MAGST) of -1.9°C and an active layer thickness (ALT) of about 2.5 m (Guglielmin et al., 2001). Mean annual permafrost temperature (MAPST) was -1.7°C with a total thickness of about 200 m (Guglielmin, 2004). Two DC resistivity tomographies were carried out between 2680 and 2750 m asl on an active rock glacier and along the slope of the old glacier, where the ground was partly reworked for the construction of a ski run, in the north cirque of Mt. Scorluzzo. Results confirmed a patchy permafrost distribution, with an active layer ranging from 3 to 5 m and a permafrost thickness between 10 and 20 m (Cannone et al., 2003). Moreover, in the same area, a permafrost map was obtained by combining the ecological indications of vegetation. The areas with "permafrost absent" are mainly located in the lower part of the area, while areas with "possible permafrost" occurrences are distributed all over the area, without any particular link to altitude, even though the highest percentage of this category occurs between 2720 and 2750 m asl. "Permafrost present" has a patchy distribution. In particular, in the ski area, permafrost occurs both in the upper and in the lower part, the middle area being "possible permafrost". On the rock glacier, permafrost is mainly in an area between 2700 and 2750 m asl and on a little patch on the western side of the very upper part (close to 2800 m asl).

Despite its limited extent, the study area is rich in periglacial features. Seven rock glaciers (4 active and 3 of uncertain activity; **Guglielmin and Smiraglia**, 1997; **Guglielmin and Siletto**, 2000), protalus ramparts, patterned grounds, turf hummocks (mainly localised on the northern slope of Le



Fig. II.1.C.3- Picture of the small glacier still present in 1930 on the northern side of Mt. Scorluzzo (Garancini, 1930). Source: http://www2.sgl.cluster.it:81/archivio_storico/ortles/ortles_ghiacciaio.asp?nome=Scorluzzo

Rese ridge, at 2500-2600 m asl) and several solifluction lobes (**Pozzi et al.,1990**). Small block streams are also present in the western glacial cirque (**Albertini, 1955**).

Characteristics are also landforms and deposits due to gravity: edge of cirque scarps and of glacially eroded scarps remodelled by degradational and landslide processes, active scree slopes and talus cones, active debris flows (**Pozzi et al.,1990**). Lot of gullies due to running water also characterize the area.

II.1.D. Flora and vegetation

II.1.D.a. Alpine landscapes: general characteristics

The study area is located in the alpine and in the nival belts (**Ellemberg, 1988**), the latter colonised by discontinuous pioneer communities, especially by *Androsacetum alpinae, Oxyrietum digynae*, and by the more evolved *Luzuletum spadiceae*. In the alpine belt, the climatic condition is reflected by the continuous alpine meadow *Caricetum curvulae* and in chionophilous conditions by *Salicetum herbaceae*.

II.1.D.b. Elevation belts and vegetation elements in the study area

The Stelvio pass area is a suitable example of the characteristic vegetation of the central Alps and of all the vegetation belts typical of high elevation landscapes: subalpine (2200-2400 m a.s.l.), alpine (2400-2800 m a.s.l.) and snow belts (>2800 m a.s.l.). Moreover the study area it is a unique case study where it has been possible to detect and assess the recent climate change impacts on the alpine flora and vegetation (**Cannone et al., 2007; Cannone and Pignatti, 2014**). The broad lineaments of the vegetation were drawn for the first time by **Giacomini and Pignatti (1955)** and, more recently, by **Cannone et al. (2007)**.

In 1953 a phytosociological map of the Stelvio pasture area was performed at scale 1:12500 (Giacomini and Pignatti, 1955), without the support of aerial photographs, and completed by phytosociological relevés describing all the communities observed. In 1953 no precedent examples of similar investigations on the alpine vegetation in the mountain regions of Italy existed, so that the aim of the investigation was to give a pioneering classification of the vegetation. In 2003 (Cannone et al.,2007) and 2014 (unpublished data) the vegetation of the Stelvio pass area was re-sampled to build new vegetation maps of the study area and new phytosociological relevés of the vegetation were performed.

Because of the differences in elevation, morphological features and edaphic conditions (e.g. long permanence of the snow cover, or limited drainage of water with consequent stagnation, or the presence of instability of the surface), the vegetation is composed of a mosaic of different plant communities, mainly belonging to the high elevation alpine geosigmetum on acidophilous soils (*Caricion curvulae, Festucion variae, Androsacion alpinae, Caricion fuscae, Salicion herbaceae, Loiseleurio-Vaccinion, Nardion strictae*) and on calcareous soils (*Seslerio variae-Caricetum sempervirentis, Caricetum firmae, Potentillion caulescentis, Thlaspion rotundifolii*) (Blasi et al.,2010; Biondi and Blasi, 2013; Biondi et al.,2014).

Hereafter the main plant communities of the study area will be briefly described, on the base of past and recent vegetation and floristic assessments (Giacomini and Pignatti, 1955; Pirola, 1959; Pedrtotti et al.,1974; Cannone et al.,2007; Cannone and Pignatti, 2014). For a detailed syntaxonomic frame and nomenclature of the vegetation assemblages, please refer also to the next chapter.

Elevation (m asl)	Belt	Vegetation communities
>3000	Snow belt	pioneers Androsacion alpinae and Androsacion vandelli
		Cryptogameae communities (algae, fungi, mosses and lichens)
2800-3000	Sub-Snow belt	Discontinuous grasslands Caricion curvulae
2600-2800	High-Alpine	grasslands Caricion curvulae
		snowbeds Salicion herbaceae
		pioneers Androsacion alpinae
		pioneers/rocky substrate Androsacion vandellii
2400-2600	mid-Alpine	grasslands Nardion strictae
		dwarf shrublands Loiseleurio precumbentis-Vaccinion microphylli
2200-2400	low-Alpine	shrublands Rhododendro ferruginei-Vaccinion myrtilli
		grasslands Aveno-Nardetum
2000-2200	Subalpine	Coniferous forests Piceion excelsea

Tab. II.1.D.b.1 - Typical elevation belts on high mountains siliceous substrate.

Concerning the subalpine belt, the vegetation dynamics would lead, in the absence of environmental disturbance, to the formation of acidophilous shrub communities with the *Rhododendro ferruginei-Vaccinion myrtilli* alliance. Dominant species are *Rhododendron ferrugineum*, *Vaccinium myrtillus*, *Vaccinium uliginosum*. In the study area this shrublands have a patchy distribution, mainly located in gully and in area with enhanced snowdepth in order to protect leaves from winter frost damages, and forms mosaic with alpine grassland communities where *R. ferrugineum* has been encroaching since 1953 in response to recent climate change.

In areas with little snow cover accumulation especially in winter time (such as on wind ridges), the exposure to low temperatures and cold winds enhance the risk of frost damage. Here silicicolous, alpine heaths composed of nano-phanerophytes, dwarf shrubs and lichens of the *Loiseleurio procumbentis-Vaccinion microphyilli* alliance dominate. *Kalmia procumbens* (syn. *Loiseleuria procumbens*) is the distinctive and dominant species, with *Luzula lutea, Hieracium alpinum* aggr., *Juncus trifidus* and *Vaccinium vitis-idaea* as other diagnostic species. Moreover, high hedges of macrolichens of *Cetraria* spp. and *Cladonia* spp. that characterized the dwarf alpine shrubland, especially in the most xeric and wind exposed sites where the lichen coverage and thickness increase considerably. Depending on the local topographic and edaphic conditions between the dwarf alpine shrubland and other alpine communities: in areas where the snow cover tends to increase, there are transition mosaics between *Loiseleurio procumbentis-Vaccinion microphyilli* and *Rhododendro ferruginei-Vaccinion myrtilli* as well as with alpine grassland at the higher elevation where the dwarf shrubs have recently expanded in the study area. Here it cover a wide area between 2300and 2600 m asl, where *Oreochloa disticha, Primula daonensis, Phyteuma hemisphaericum, Polygonum viviaprum, Leucanthemopsis alpina* and *Avenula versicolor* also occur frequently.

The tree-species line (Korner & Paulsen, 2004) in this area is formed mainly by sapling of *Larix decidua* Mill. and *Pinus mugo* Turra, with few individuals of *Picea excelsa* Link.

Concerning the alpine belt, the vegetation dynamics would lead, in the absence of environmental disturbance and with constant climatic conditions, to the formation of high-alpine grasslands, which constitute the natural potential vegetation. However due to local topographical and edaphic features, the most common condition is the presence of a mosaic of different plant communities.

In fact, where the snow cover persists for more than eight months a year (for example in the hollows or on slopes facing north) snowbeds community develops; but where the water drainage is reduced and the degradation of organic matter is prevented, marsh areas form with typical fens vegetation.

At sites with bare soil or surface instability, such on scree slopes and talus cones, the vegetation presents pioneering traits, which enable it to colonize new substrates and/or to tolerate the disturbance of surface movements.

The alpine meadow occupies, with continuous coverage, most of the glacial circues and the gentle slopes of the study area, and is mainly constituted by the alliance of Caricion curvulae, which dominant and representative species is *Carex curvula*. Among the characteristic species, there are several graminoids (Graminaceae, Cyperaceae and to a less extent Juncaceae) including Agrostis rupestris, Festuca halleri, Juncus trifidus, Oreochloa disticha and many other vascular plants including Hieracium glaunduliferum, Minuartia recurva, Pedicularis kerneri, Phyteuma hemisphaericum, Primula daonensis, Senecio incanus, Veronica bellidioides. Other frequent abundant species are Leontodon helveticus and Trifolium alpinum. The typical alpine grassland is the association Caricetum curvulae, which develops mainly at 2600 m asl in the study area, despite its altitudinal range spans from 2400 to 2800 m asl. Several variants of the association have been proposed by different authors (Braun-Bluanquet, 1949-50; Giacomini and Pignatti, 1955). Where the snow remains longer or the soil moisture is higher, it is observed the hygrophilic variant Caricetum hygrocurvuletosum, which represents a transition between the Caricetum curvulae and Salicetum herbaceae and is characterized by the presence of Salix herbacea, Alchemilla penthaphyllea, Ligusticum mutellina, Luzula spicata and Bistorta vivipara. On the more exposed sites, the macrolichens and Kalmia procumbens coverage can increase and the variant cetrarietosum and loislerietosum appears. The variant elynetosum can occurs on calcareous soils under acidification, and is characterized by the presence of Kobresia myosuroides (syn. Elyna myousuroides).

Between 2650 and 2750 m asl on the south exposed scree slope of Le Rese ridge, Giacomini and Pignatti (1955) identified the alliance of *Festucion variae*, characterized by the occurrence of *Festuca varia* aggr. and *Poa variegata*. This plant community has been reported in the 1953 phytosociological map only.

In areas subject to grazing in the past, it is observed the *Nardion strictae*, typical alliance of subalpine and alpine pastures dominated by *Nardus stricta*, and its transition to the *Caricion curvulae*, the *Curvulo-Nardetum*. In the study area, it is mainly located between 2350 and 2550 m asl, further upward than its typical distribution, and it is particularly rich in *Trifolium alpinum*.

Snowbed communities of the alliance *Salicion herbaceae* are observed where the snow cover remains on the ground for at least 8 months. Several are the diagnostic species and, depending on the duration of snow cover, snowbeds have different plant composition. The main association in the study area is the *Salicetum herbaceae*. Characteristic species are *Salix herbacea*, *Alchemilla pentaphyllea* and *Soldanella pusilla* and, on the base of their relative abundance, *Salicetum salicetosum* and *alchemilletosum* variants can be distinguished. This association is present mainly between 2550 and 2750 m asl, both with continuous and discontinuous coverage, in the glacial cirques of Mt. Scorluzzo. Where the snow remains for longer periods (up to about 10 months a year), mosses dominate the plant communities, with the selection of *Polytrichetum sexangularis* association. It was reported in a very localized area next to the Stelvio pass at about 2700 m asl only in the 1953 phytosociological map.

The vegetation of rocks and instable siliceous screes, loose debris substrata, slope deposits, moraines and stream terrace includes three main plant associations of the *Androsacion alpinae* alliance, (characterized by a different degree of evolution in the succession dynamic series and different characteristics (function of elevation and snow): *Androsacetum alpinae*, *Oxyrietum digynae* and *Luzuletum spadiceae*.

The *Androsacetum alpinae* represents the initial stage of vegetation development and the typical variation of the highest elevations. Its existence in the study area is reported only in some areas of the scree slopes and talus cones, above 2700 m of elevation. The species characteristic of this association are *Androsace alpina* and *Saxifraga seguieri*.



Fig. II.1.D.1.– Examples of plant species from Snow (>3000 m asl) to low-Alpine (2200-2400 masl) belts in the study area. *Androsace alpina* (L.) Lam., *Ranunculus glacialis* L., *Phyteuma globulariifolium* Sternb. & Hoppe s.l., *Sempervivum montanum* L., *Pulsatilla vernalis* (L.) Mill., *Eriophorum scheuchzeri* Hoppe, *Kalmia procumbens* (L.) Gift, Kron & Stevens ex Galasso, Banfi & F. Conti, *Rhododendron ferrugineum* L..

The Oxyrietum digynae characterizes stages of greater maturity and evolution in the succession and is present with different variants, mainly on moist, low nutrient and north exposed sites, with a snow coverage lasting more than 8-9 month. The species characteristics of this association are Oxyria digyna, Geum reptans, Cerastium uniflorum and Cerastium pedunculatum accompanied by species of superior orders such as Ranunculus glacialis, Poa laxa, Saxifraga bryoides and Cardamine resedifolia and from companion species Leucanthemopsis alpina, Sedum alpestre, Veronica alpina and Senecio incanus ssp carniolicus. In the study site, this association cover most of the scree and deposits the upper part of glacial cirques of Mt. Scorluzzo, up to 2800-2850 m asl.

The *Luzuletum spadiceae* grows in areas where the slope increases. This association has a more advanced dynamic term than *Oxyrietum digynae*; moreover, being characterized by a greater permanence of snow cover is considered a transitional phase between the pioneers and the snowbeds, of which could share some species such as *Salix herbacea* and *Soldanella pusilla* (**Pedrotti et al.,1974**). The characteristic species is *Luzula alpinopilosa*. It extends from 2300 up to 2800 m asl in many part of the study area.

Moreover, in some location, there are patches with series of transition between the main pioneers associations and variants with typical species of alpine meadows such as *Agrostis schraderiana*, *Anthoxantum alpinum*, *Homogyne alpine*, *Deschampsia caespitosa and Potentilla aurea* (Credaro and Pirola, 1975).

In conditions of water stagnation, it occurs a shortage of oxygen in the soils, which makes difficult the processes of degradation of the organic substance, causing the accumulation of peat in the surface layers of the soil. It is in this environmental context that are formed bog environments, characterized by the presence of species whose ecological characteristics are high hygrophilous (total and continuous flooding), very acidic pH (both that of the water and of the soil) and the partial anoxia of the terrain.

The main plant alliance that grows in this context is the *Caricion nigrae*, which represent communities composed of oligotrophic to mesotrophic small sedges and bryophytes that grow in acid fens. The most abundant species are *Carex nigra*, *Eriophorum scheuzeri*, *Juncus triglumis*, *Juncus filiformis* with the *Sphagnum warnstorfii*. Two are the associations in the study area, that differ for species composition and abundance: *Caricetum nigrae* and *Eriophoretum scheuchzeri*. The *Caricetum nigrae* can be generated as regression of pastures vegetation respect an increase in the amount of water stagnation or flow or as a decrease of water depth in an *Eriophoretum scheuchzeri* association.

Eventually, in the phytosociological map of 1953, Giacomini and Pignatti reported the alliance *Cardamino amarae-Montion fontanae* in two sites in the glacial plan on the west side of Mt. Scorluzzo, at about 2600 m asl. This is a community that occurs along streams close to cold springs with acidic water, dominated by bryophytes but with many vascular diagnostic species like *Carex frigida, Epilobium nutans, Saxifraga aizoides, Cardamine amara* and *Saxifraga stellaris*. It has not been recorded in the further vegetation maps in 2003 and 2014, substituted by *Caricion nigrae* and *Salicion herbaceae* communities.

Because the occurrence of calcareous outcrops and deposits, basophilous vegetation is also present in the study area, despite localized between 2230 and 2500 m asl at the south-west corner of the study area, next to the Bocca del Braulio and the Stelvio national road.

The alliance *Caricion firmae* is the primary, pioneer, discontinuous, from subalpine to alpine swards that grows on calcareous or dolomite slopes, on cool ravines and valleys. Diagnostic and frequent species are *Carex firma* and *Dryas octopetala*, that with their roots system are the first able to stabilize the surface and to start the soil development.

More evolved stages are those of the alliance *Seslerion caeruleae*, a meso-xerophilous or xerophilous communities of the stabilized basophilous south exposed slopes, with *Sesleria caerulea, Carex*

sempervirens and *Hormium pyrenaicum* as most abundant species. Nowadays the areal extent of this community in the study area is occupied by a high coverage stage of *Dryas octopetala*.

Windy ridges on neutro-basic substrata with acidic leached soils, dominated by perennial small graminoids and pulvinate chamaephytes, are characteristic of the alliance *Oxytropido-Kobresion myosuroidis*, a transitional stage between basophilous and acidophilous vegetation. Diagnostic and frequent species are *Kobresia myosuroides (syn Elyna myousuroides)* and *Festuca quadrifolia*, with many other characteristic species. The association *Elynetum myosuroidis* was recorded in 1953 close to Bocca del Braulio at 2450 m of elevation, but it has further evolved within other plant communities that belong to the *Seslerion caeruleae* and *Loiseleurio procumbentis-Vaccinion microphyilli* alliances.

II.1.D.c. Syntaxonomic structure of the vegetation in the study area

In this section is reported the syntaxonomic frame of the main elements of the vegetation in the study area, according to the Italian vegetation prodrome (**Biondi and Blasi, 2013; Biondi et al.,2014**): <u>http://www.prodromo-vegetazioneitalia.org/introduzione</u>.

• *Alpine shrublands and dwarf shrublands*

Cl: LOISELEURIO PROCUMBENTIS-VACCINIETEA MICROPHYLLI Eggler ex Schubert 1960 Ord: RHODODENDRO FERRUGINEI-VACCINIETALIA MICROPHYLLI Br.-Bl. in Br.-Bl. & Jenny 1926

All: Loiseleurio procumbentis-Vaccinion microphylli Br.-Bl. in Br.-Bl. & Jenny 1926 Ass: Loiseleurieto-Cetrarietum Br-Bl 1926
All: Rhododendro ferruginei-Vaccinion myrtilli A. Schnyd. 1930 Ass.: Rhododendretum ferruginei Rübel 1911

• Alpine meadow acidophilic vegetation

Cl: *CARICETEA CURVULAE* Br.-Bl. 1948 nom. cons. propos. Rivas-Martínez, Diaz, Fernández-González, Izco, Loidi, Lousa & Penas 2002 Ord: *CARICETALIA CURVULAE* Br.-Bl. in Br.-Bl. & Jenny 1926

All: Caricion curvulae Br.-Bl. in Br.-Bl. & Jenny 1926 Ass. Festucetum halleri Br-Bl 1926 Ass. Festucetum variae Rübel 1911 Ass: Caricetum curvulae (Kerner,1863) Borckm.-Jer.,1907 Ass: Caricetum hygrocurvuletosum Br.-Bl.,1948-50 All: Nardion strictae Br.-Bl. in Br.-Bl. & Jenny 1926 Ass: Nardetum alpigenum Br.-Bl.;1949 All: Agrostion schraderianae Grabherr 1993 Ass: Pediculari recutitae-Agrostitetum schraderianae Grabherr 1993

Alpine meadow basophilous vegetation

CI: CARICI RUPESTRIS-KOBRESIETEA BELLARDII Ohba 1974
 Ord: OXYTROPIDO-KOBRESIETALIA MYOSUROIDIS Oberdorfer 1957 nom. mut. propos.
 Rivas-Martínez, Diaz, Fernández-González, Izco, Loidi, Lousa & Penas 2002
 All: Oxytropido-Kobresion myosuroidis Br.-Bl. (1948) 1949 nom. mut. propos. Rivas-Martínez, Diaz, Fernández-González, Izco, Loidi, Lousa & Penas 2002
 Ass: Elynetum myosuroidis Br.-Bl. 1913

Cl: *FESTUCO-SESLERIETEA* Barbéro-Bonin 1969 Ord: *SESLERIETALIA CAERULEAE* Br.-Bl. in Br.-Bl. & Jenny 1926 All: *Seslerion caeruleae* Br.-Bl. in Br.-Bl. & Jenny 1926 Ass: *Seslerio-Semperviretum* Br.-Bl. in Br.-Bl. et Jenny 1926 All: *Caricion firmae* Gams 1936 Ass: *Caricetum firmae* Rübel 1911

Snowbeds vegetation

Cl: SALICETEA HERBACEAE Br.-Bl. 1948 Ord: SALICETALIA HERBACEAE Br.-Bl. in Br.-Bl. & Jenny 1926 All: Salicion herbaceae Br.-Bl. in Br.-Bl. & Jenny 1926 Ass: Polytrichetum sexangularis (Rübel, 1912) Br.-Bl.,1926 Ass: Salicetum herbaceae Rübel 1911

• *Alpine vegetation on scree and debris*

Cl.: THLASPIETEA ROTUNDIFOLII BR.-BL. 1948
Ord.: ANDROSACETALIA ALPINAE Br.-Bl. in Br.-Bl. & Jenny 1926
All.: Androsacion alpinae Br.-Bl. in Br.-Bl. & Jenny 1926
Ass.: Androsacetum alpinae Br.-Bl.;1918
Ass.: Oxyrietum digynae (Lüdi,1921) Br.-Bl.;1926
Ass.: Luzuletum spadiceae (Brockm.-Jer.,1907) Br.-Bl.;1926

• Alpine vegetation on rocks

Cl: ASPLENIETEA TRICHOMANIS (Br.-Bl. in Meier & Br.-Bl. 1934) Oberdorfer 1977
Ord: ANDROSACETALIA VANDELLII Br.-Bl. in Meier & Br.-Bl. 1934 corr. Rivas-Martínez, T.E. Díaz, Fernández-González, Izco, Loidi, Lousã & Penas 2002
Sub-Ord.: ANDROSACENALIA VANDELLII Loisel 1970
All: Androsacion vandellii Br.-Bl. in Br.-Bl. & Jenny 1926 corr. Rivas-Martínez, T.E. Díaz, Fernández-González, Izco, Loidi, Lousã & Penas 2002
All: Androsacion vandellii Br.-Bl. in Br.-Bl. & Jenny 1926 corr. Rivas-Martínez, T.E. Díaz, Fernández-González, Izco, Loidi, Lousã & Penas 2002
Ass: Androsacetum vandelii Br.-Bl.;1918

Marsh and spring vegetation

Cl: SCHEUCHZERIO PALUSTRIS-CARICETEA NIGRAE nom. mut. propos. ex Steiner 1992 Ord: CARICETALIA NIGRAE Koch 1926 nom. mut. propos. All: Caricion nigrae Koch 1926 em. Klika 1934 nom. mut. propos. Ass: Caricetum nigrae Br.-Bl. 1915 nom. mut. propos. Ass. Eriophoretum scheuchzeri (Brockm.-Jer. 1907) Rubel 1912

Cl MONTIO FONTANAE-CARDAMINETEA AMARAE Br.-Bl. & Tüxen ex Klika & Hadac 1944 Ord. MONTIO FONTANAE-CARDAMINETALIA AMARAE Pawlowski in Pawlowski, Sokolowski & Wallisch 1928

All. Cardamino amarae-Montion fontanae Br.-Bl. 1926 Ass. Montio-Bryetum schleicheri Br.-Bl. 1926

II.1.D.d. Climate change impact on alpine vegetation of the study area

The Stelvio pass represents an unique area where to study the recent environmental change and their impacts on the alpine vegetation on the Alps. This is because of the historical data on plant community distribution and composition available since 1953 (Giacomini and Pignatti, 1955) that were compared with more recent information (Cannone et al., 2007; Cannone and Pignatti, 2014), allowing to detect the vegetation changes in the last few decades.

Therefore, Cannone et al. (2007) underlined how the alpine vegetation above the tree line experienced changes in area between 1953 and 2003, with rapid shrubs expansion (+5.6% per decade at altitudes between 2400 and 2500 m asl) and upward displacement of many communities, but also with unexpected patterns of regression of vegetation coverage and dynamics mostly associated with increased precipitation and permafrost degradation, as the increase of bare ground between 2800 and 2900 m asl. Among all communities, alpine grasslands are those most subjected to a distributional reduction and to the shrubs invasion, despite the ability of late successional species (like C. curvula) to migrate, as well as to an upward shift, replacing the snowbeds communities. The contraction of the snowbeds and their shift to higher altitudes is consistent with the shortening of the snow cover season. At the species level, Cannone and Pignatti (2014) confirmed that since 1953 all plant communities experienced a high species turnover, leading to an overall increase in species richness (+32.4 %) as new species (n = 34) exceeded species losses (n = 10). The main recruitment sources are neighbor communities within the same elevation belt, performing biotic exchanges with other plant communities in the same altitudinal belts, confirming that in the last decades range filling and downward shifts exceeds the upward shifts in the species composition dynamics. However, all communities exhibited a coverage decline of the most dominant species, allowing the potential expansion of competitors and/or of new species. The low number of extinct species was related to the extinction debt (Jackson and Sax, 2009), defined as the number of persistent species undergoing to population decline (about the half of the 60 persisting species in the study area). Alpine grasslands and snowbeds species were those more subjected to the extinction debt process.

These results indicate that both the increase in mean annual air temperature of 1.0-1.2°C between 1950 and 2003, the summer warming of 0.4-1°C per decade after 1980, and the shortening of the snow season in the study area may activate feedback mechanisms (**Chapin et al. 2005**), able to cause vegetation changes both at community and species levels. Moreover, in addition to expected effects (e.g. species and community displacement), warming of air temperature could lead unexpected effects, with unpredictable consequences for biodiversity.

The authors highlighted how the research priorities should aim to understand the response of the most sensible community to recent and future climate change. This is because one of this PhD activities deals with *in situ* manipulation experiments, to assess the response of alpine grasslands and snowbeds phenology and biodiversity to different environmental drivers.

II.1.D.e. Relevance of the selected plant species in the frame of Habitat Directive

In the supplementary materials (**Tab.S2**) is given a list of the phenology monitored species at the in situ manipulation experiments.

The selected species are representative of the most widespread vegetation communities of the study area, as well as of the most sensible communities to climate change: alpine grasslands and snowbeds. Moreover, these species are characteristic of habitat of community importance, as identified by the Habitats Directive (92/43/EEC), as indicated by the Italian Vegetation prodrome (**Biondi & Blasi, 2013; Biondi et al., 2014**) and by the Italian manual of interpretation of the Habitats Directive (**Biondi et al., 2010**).

Siliceous alpine and boreal grasslands (Habitat 6150)

Microtherm, siliceous alpine and boreal grasslands that grow in high elevation and/or stations with prolonged snow cover, from the Alps (and rarely in the northern Apennines) to the Carpathians and Scandinavia ranges, developed on soils derived from silicate or decalcified substrates. They include plant communities of the *Caricion curvulae* (mainly represented by *Carex curvula*) as well as of the alpine snowbeds of the *Salicion herbaceae* (mostly represented by *Salix herbacea*).

The physiognomic combination comprises other important species, such as: *Agrostis rupestris* All., *Alchemilla pentaphyllea* L., *Avenella flexuosa* (L.) Parl, *Avenula versicolor* (Vill.) Laínz, *Euphrasia minima* Jacq. ex DC., *Festuca halleri* All. s.l., *Geum montanum* L., *Gnaphalium supinum* L., *Juncus trifidus* L. s.l., *Leontodon helveticus* Mérat emend. Widder, *Ligusticum mutellina* (L.) Crantz, *Luzula alpinopilosa* (Chaix) Breistr., *Nardus stricta* L., *Oreochloa disticha* (Wulfen) Link), *Phyteuma hemisphaericum* L., *Potentilla aurea* L., *Primula daonensis* (Leyb.) Leyb.), *Senecio incanus* L. (incluso subsp. *carniolicus* (Willd.) Braun-Blanq.), *Sibbaldia procumbens* L., *Soldanella pusilla* Baumg, *Trifolium alpinum* L., *Veronica alpina* L., *Veronica bellidioides* L.. Other companion species of this habitat are: *Bistorta vivipara* (L.) Delarbre, *Leucanthemopsis alpina* (L.) Heywood, *Poa alpina* L..

II.1.E. Pasture management and wildlife in the Stelvio Pass area

At the elevation of our study area, the only anthropogenic land use is extensive summer pasturing. Transhumance is still practice and during summer (from mid-June), cattle herds are taken to the highland, while at the begin of autumn (late-September – early-October) they are brought back to the lowland.

The study area lays within the territory of Alpe Stelvio pasture. Here the livestock population size was reconstructed from official historical archives of the Mountain Community of Alta Valtellina, from literature (**Giacomini and Pignatti, 1955**) for the period before the 1993 and from personal communication with pasture holder and local shepherd since 1994 to 2013.

On average the livestock population size is of 374 individuals, with the highest value occurred in 1842 with 1937 individuals (448 bovine, 864 ovine, 625 equine) and the smallest value occurred in 1994 with 117 individuals (all bovine). Despite the scattered information available (years: 1842, 1901, 1903, 1950), since the mid of the 19th century up to the 1950s livestock population has decreased of 55% (from 1937 to 868 individuals) and more than 60% of the cattle herd was composed of sheep (**Fig. II.1.E.1**). After a further decrease of 29% and the complete disappearance of sheep, from 1978 to nowadays the grazing pressure has remained constant and the livestock population has composed exclusively by cows, with an average population size of 196 individuals (min: 117; max:



Fig. II.1.E.1– Grazing disturbance in the study area. Cows (from the local pasture) on the more gentle slope; chamoises and ibexes on the more steep slopes and along the ridges

305). In correspondence of our study sites, the herds used to graze for about 15-20 days, between mid-August and September.

However, the cattle herd size brought to graze in the Alpe Stelvio pasture each year since the last four decades was almost constant. For this reason, we can exclude any impact of sheep grazing/nutrient input on the study period concerning the vegetation communities' dynamics.

One more possible grazing effect is that from local wildlife (**Fig. II.1.E.1**). Since 1999 population of large herbivores, ibex (*Capra ibex* L.) and chamois (*Rupicapra rupicapra rupicapra* L.), didn't show any significant trend in the population size (data not shown, Stelvio National Park personal communication). In mid-summer during the whole study period, ibexes were frequently observed separately, in small groups composed on average of 3-4 heads (maximum 10 heads), mainly on the slopes and the higher ridges of the glacial circues and less frequently down on the grasslands of glacial plains and moraines. Chamois are less frequently observed, always as single individual or small groups of 2-3 heads. Information on small herbivores, such as rodents or mountain hares, are not available; however, marmots (*Marmota marmota* L.) are frequent visible and abundant in the whole study area. Tracks of brown bear (*Ursus arctos* L.) were personally recorded and further confirmed by Stelvio National Park expertize in the west circue of Mt. Scorluzzo before the snowmelt in May 2015.

Due to this, wildlife has a small impact (compared to climate forcing) on the vegetation dynamics in the study area and sites.

II.2. MATERIALS AND METHODS: FIELD WORK AND DATA COLLECTION

II.2.A. Manipulation experiments: design and aims

In 2013 and 2014 in the Stelvio pass study area two sites were selected for the set-up of *in situ* manipulation experiments that focus on different drivers of the climate change and their impacts on terrestrial ecosystems and on plants phenology and biodiversity.

We replicated these experiments in 2 sites located at the same elevation but with differences in their general aspect (**Fig. II.1.A.1; Fig. II.2.A.1**):

- Site 1 Tornante: 46.53123 N, 10.44109 W (WGS84; dd.ddd^o) 2603-2635 m asl, next to the SS38 road, it is located on an Holocene moraine (**Pozzi et al.,1990**) with a N-NW exposition.
- Site 2 Scorluzzo: 46.52656 N, 10.42757 W, 2626-2630 m asl, on the western glacial cirque of Mt. Scorluzzo, at the foot of the debris scree slopes of Le Rese ridge and next to few small glacial lakes, with a general S-SW exposition.

The manipulations were performed through different structures that influenced the local characteristics (plot scale) in different ways, with a mono-factorial design: air and soil warming; water availability and soil moisture; snow-depth and snowmelt time.

Except for the latter, the experiments were replicated in each site and among two of the most widespread vegetation communities and those at the highest vulnerability to recent climate change and showing the highest regression risk (**Cannone et al., 2007; Cannone & Pignatti, 2014**): snowbeds (*Salicetum herbaceae* – SH) and grasslands (*Caricetum curvulae* – CC). Within the same site, the sub replicates among each communities were almost at the same elevation and on the flattest areas possible, in order to get comparable data, unbiased from topographically effects.

1) Open Top Chamber (OTC) - air and soil warming.

To passively warm small vegetation plots, the International Tundra Experiment (ITEX) designed hexagonal green house was used, that typically increases the mean daily summer air temperature by 1-3°C (**Molau and Mølgaard, 1996; Marion et al., 1997).** The chamber was constructed of 3-mm thick polycarbonate panels with an inclination of 60°. It is 50 cm tall and the distance between parallel sides is 208 cm at the base and 105 cm at the top. The open-top design allows free air exchange and is said to minimize undesirable chamber effects as changes in precipitation, gas and humidity concentrations, and exclusion of pollinators (**Marion et al. 1997**), despite a need remains to quantify OTC effects on water availability and wind speed (affecting, for example, drying rates and water stress) (**Bokhorst et al., 2013**). OTC was kept in their position year round. Within the OTC a 2x1 m



Fig. II.2.A.1 – Overview of the of the two study sites: Site 1 (left) and Site 2 (right)



Fig. II.2.A.2 – Picture and plot scheme of Open Top Chambers (OTC) soil warming experiment. OTC = warming; OTCc = control plot.

plot was delimited. In the near vicinity of each OTC, a 1x1 m control plot was established. Hence, two treatments were applied: Open Top Chambers (OTC-CC*n*; OTC-SH*n*) and control plots (OTC-CC*n*c; OTC-SH*n*c) (**Fig. II.2.A.2**).

OTCs were placed in July 2014 (Site 1: 15 July; Site 2: 28 July) and left in the field year round.

2) Precipitation Shield (PS): water availability and soil moisture (e.g. Wu et al.,2011; Beier et al.,2012; Cherwin and Knapp, 2012; Cornelius et al.,2013; Swidrak et al.,2013; Gugger et al.,2015; De Boeck et al., 2016; Kreyling et al.,2017).

A 2x2 m polycarbonate sheet, 3-mm thick, installed at 150 cm above the ground, subparallel to the soil surface and open on the borders was used to recreate drought and moist scenarios.

As the PS collects the precipitation and moves it on the down slope side of the structure, for each precipitation shield, four treatments were expected to be applied: drought (under the precipitation shield; PS-CC*n*; PS-SH*n*); great water increase and erosion (next to the PS on the down slope side, it gets the precipitation that are intercepted by the shield; PS-CC*n*v1; PS-SH*n*v1); moderate water increase (about 2 m down slope from PS; PS-CC*n*v2; PS-SH*n*v2) and control plots (located up slope than the shield; PS-CC*n*m; PS-SH*n*m) (**Fig. II.2.A.3**). The size was 2x1 m for PS plot, whereas for PSv1, PSv2 and PSm were 1x1 m. The PSs were placed in July 2014 (Site 1: 15 July; Site 2: 28 July) and to avoid the influence on the winter snow falls and to prevent damages duo to excessive snow weight, the polycarbonate sheet of the PSs was keep on place during the summer season only, approximately from mid June to early October.

3) Snow Fence (SF): snow depth and snowmelt time (e.g. Wahren et al.,2005; Wipf and Rixen, 2010; Cooper et al.,2011; Johansson et al., 2013; Rumpf et al., 2014; Legault and Cusa, 2015; Semenchuk et al.,2016).

A woody barrier, 2.5 m tall and 12 m long, aligned perpendicular to the main north-eastern winds, was used to manipulate the snow depth and the snowmelt time due to the wind effects (snow erosion, transport, accumulation). The expected effect of the snow fence is associated to the action of wind which redistributes the snow cover, eroding the snow cover (and hence reducing its thickness) on the windward side (where the wind speed increases) and redistributing it on the leeward side (where the



Fig. II.2.A.3 - Picture and plot scheme of Precipitation Shield (PS) water availability and soil moisture experiment. In the picture, is well visible the effect of the PS on a late summer snow precipitation (mid-September 2015). PS = drought (under precipitation shield); PSv1 = great water increase and erosion; PSv2 = moderate water increase; PSc = control plot.

wind speed decreases). On each side of the SF, were located three 20 m-long lines, each composed of 10 plot of 1x1 m (n. 60 plots total, size 1x1 m: 30 on the leeward and 30 on the windward side of SF). In correspondence of each plot, a graduated 205 cm tall snow stake has been located, in order to assess the snow-depth both in the field and thought images collected by digital cameras (see next paragraph). Reference heights were located at 10, 20, 30, 40, 50, 80, 100, 150, 200 cm. Distance between one plot and the next one (as well as between one line and the next) was 2 m. The plots experience different snow accumulation and snowmelt time conditions depending on the distance from the SF and on the combined redistribution effort of snowfalls (in terms of temporal and spatial distribution) and wind speed and direction. Hence, at the begin of this experiment and without any previous information on the effect of SF in the study site, we hypothesized two main treatment: plots on the leeward side (SFnS) that undergone to snowdrifts events and delayed snowmelt and plots on the windward side (SFnN) that undergone to snow erosion and advanced snowmelt (Fig. II.2.A.4). The SF was placed in October 2014 for the first time and therefore it started to influence the snow pack formation and melting since winter 2014/2015. The monitoring of pattern of snow accumulation and depth (see next paragraph for see devices and methods employed) began in November 2013, one year before the SF placement, in order to characterize the natural winter snow accumulation and summer snowmelt gradient without disturbance of the fence. In order to avoid affecting summer wind regimes and shading the vegetation, the SF was retrieved in summer and replaced in early-October every year.

All the delimited plots received a code, result of the combination between the treatment type, the plant community and the replicate number to which the plot belongs to (i.e.: OTC-CC1 = Open Top Chamber, *Caricetum curvulae* community plot, replicate number 1).

The configuration of the structure for each site was as follow. Site 1: 6 OTCs (CC: n = 3; SH: n = 3), 4 PS (CC: n = 2; SH: n = 2), 1 SF (CC: n = 1); Site 2: 6 OTCs (CC: n = 3; SH: n = 3), 2 PS (CC: n = 1; SH: n = 1).

Overall, the investigated plots were 108 (Site 1: 88; Site 2: 20), of which 84 were on *Caricetum curvulae* community (Site 1: 74; Site 2: 10) and 24 were on *Salicetum herbaceae* community (Site 1:



Fig. II.2.A.4 - Picture and plot scheme of Snowfence (SF) snow-depth and snowmelt time experiment. Top panel: overview of the SF experiment after an early-summer snowfall (30th June 2017); bottom left: scheme of manipulated plots; bottom right: snow fence structure.

Down-slope

14; Site2: 10). The difference in plots number between Site 1 and Site 2 as well as between CC and SH communities stay in the 60 plots of the SF treatment, located on the CC community of Site 1 only. A more detailed list of the investigated plots, with relative dimensions, topographic characterization, treatment and community type within each study site, is reported in **Tab. S1** in Supplementary materials.

II.2.B. Environmental data collection

Plot limit - sector aimed by snowcameras

Plot limit Data logger Air Temperature

In order to know the effect of the structures installed and their manipulation effect on soil temperature, soil moisture and snowmelt time different devices were installed.

The ground surface temperature (GST; °C) is a good proxy for the evaluation of the soil thermal regime (e.g. **Cannone & Guglielmin, 2009; Gubler et al., 2011; Guglielmin et al., 2012**). Since the summer 2014, in both the study sites, soil temperature was derived from Hobo Pro V2 U23-003 thermistor by Onset and from Tinytag Plus 2 TGP-4520 and TGP-4020 thermistors by Gemini (accuracy \pm 0.2°C, resolution \pm 0.02°C). Temperature loggers were installed at 2 cm depth (according to **Guglielmin, 2006**) in correspondence of 68 vegetation plots and were programmed to record and store data every hour. Dataloggers sensor were distributed in the middle of each treatment plots (e.g. **Cornelius et al., 2013**), whereas in each OTCs, soil temperature was recorded both in the middle and at the edge (10 cm far from the polycarbonate panels) of the chamber, in order to evaluate the

occurrence of any "edge effect". In the SF manipulation, dataloggers were located in all the plots of the first line only.

The thermistors were placed parallel to the surface, avoiding as much as possible the noise due to the roughness of the ground, according to the standard procedure developed for polar regions, from Arctic (**Osterkamp, 2003**) to Antarctica (**Guglielmin, 2006**) and also applied in the mid latitude mountain regions (**Hoelzle et al, 2003; Pogliotti et al, 2015**).

It must be noted that during the study period, for maintenance reasons, at some plots the data loggers have been replaced also changing the model and the type of thermistor. Moreover, in some years there were missing data, due to the malfunctioning of data loggers, mainly during the winter periods.

The soil volumetric water content (VWC; m^3/m^3) and total precipitation (TP; mm) monitoring were the tools used to evaluate the effect of the PS treatment on the amount of rain excluded trough the precipitation shields and on the increase of soil moisture in the downslope plots. During the summer period only of 2015 (from 22 June to 29 September) and continuously year round since summer 2016 (28 June), the VWC was measured with a five channel EM50 datalogger by Decagon Devices. Four channels were equipped with 5TM soil moisture sensors (accuracy: \pm 0.03 m³/m³ or \pm 3% VWC; resolution: 0.0008 m³/m³ or 0.08% VWC) at 2 cm-depth and one was equipped with an ECRN100 high resolution rain gauge sensor at 160 cm above the ground (resolution 0.2 mm; funnel size 17 x 14.2 cm). Data were recorded every 10 mins. Following the removal procedure of the polycarbonate sheet from the PS, the rain gauge was in place from mid-June to early-October, in order to detect and assess the summer precipitation only.

Soil VWC sensors and rain gauge were installed in one Precipitation Shield (PS) treatment for each site (Site 1: PS-CC2; Site 2: PSs-CC1) and relative control plots, both on the *Caricetum curvulae* community.

Following the procedure mentioned above of the thermistors, sensors were placed parallel to the surface, avoiding as much as possible the noise due to the roughness of the ground.

Since the year 2014, the thickness of the snow cover from mid May to the snowmelt has been manually monitored with a snow pole (5 cm resolution) every week, in order to assess the snow depth at the end of winter and the melt out date on each manipulated and control plots of both the study sites.

In correspondence of each plot a woody pole (60 cm height) was installed to indicate with high accuracy at the end of the snowmelt period the position of the plot under the snow pack, especially the OTCs (50 cm height) and control plots, that were the last emerging from the snow pack. Snowfence (250 m height) and relative graduated snow stakes (205 cm height) were usually well visible since the first monitoring date in May, whereas precipitation shields (150 cm height) and OTCs came out about 10 days later. When the snow was higher than 2 meter or when the manipulation structure were not visible, the GPS (\pm 3m) was used to get to the plot position. In that case, at least three different measures were made randomly within a circle 3 meters radius.

In total 17 days of measurements were carried out before the first plot melted out during year 2014, 2015, 2016 and 2017, which corresponded to 1836 data.

In order to know the snowpack dynamic in terms of thickness and melt-out date due to the occurrence of the snowfence manipulation, a system for automatic time-lapse photography of snow was set-up at the Site 1 (e.g. Floyd and Weiler, 2008; Parajka et al.,2012; Ide and Oguma, 2013) (Fig. II.2.A.4; Fig. II.2.B.1). This system required two components: digital cameras and reference point (gradated snow stakes) to be used for snow thickness evaluation.

Since October 2013, two X80 series cameras by BuckEye Cam were installed on a woody pole at 280 cm of height, about 10 m upslope than the upper edge of the snowfence. Since 12 November 2015 they were connected to an 18Ah 12V battery and a solar panel (12V 50W 3.15Ah; 750 x 524 x 34



Fig. II.2.B.1 - Images collected by the snowcameras along the 2015/2016 winter season. Left panel: windward side of the snowfence; right panel: lee-ward side of the snowfence. Date of pictures: 01/11/2015, 01/01/2016, 21/03/2016, 27/06/2016.

mm; 4.6 kg), that ensures them to work year round. The cameras operated in Standalone mode and images were taken on an 8 hours basis (three images per day at 8:00, 16:00; 24:00). One of the camera aimed to the windward side of the snowfence (snowcam_wind), the other to the leeward side (snowcam_lee). The images were saved in JPEG format with a resolution of 1.2 M pixels (1280 x 960 pixels). The pixel size on the ground was about 0.5-1 cm, at the snow stakes level. It must be noted that before the installation of the solar panel on 12 November 2015, the images of the entire winter period are not available because of the run out of the battery life and the impossibility to reach the site for substitute it until early May.

As a reference for snow depth assessment, six line of 10 graduated blue and red snow stakes (205 cm height) were installed from early October to the complete snowmelt at the SF manipulation experiments. Tree lines were on the wind ward side and three lines were on the leeward side of the snowfence, in correspondence of the manipulated plots of 1x1 m. Reference height of each snow stake are at 10, 20, 30, 40, 50, 80, 100, 150 and 200 cm, as a black belt of 2-cm thickness. Narrower or more dense markers wouldn't be detected by the images due to the resolution limits of the images. Moreover because of the compromise between wideness of lens and resolution of the cameras, not all the manipulated plots were reported on the images. The snow stakes aimed by each cameras are shown in **Fig. II.2.B.1**.

II.2.C. Species composition assessment

In order to detect the future reactions of alpine vegetation to climate change in a mid- to long-term scenario, a base-line description of the vegetation in all manipulated and control plots (n=108) was performed in summer 2013 (Site 1) and 2014 (Site 2), before the manipulation has started (year 2014). The vegetation was described analyzing a total of 108 plots, of which 90 of 1x1 m (Site 1: 78; Site 2: 12) and of 18 of 2x1 m (Site 1: 10; Site 2: 8). Within every plot, total vegetation cover and the cover of each vascular species were estimated visually, with the support of a 5x5 cm or 10x10 cm cell-size grid. Cover estimates were also given for cryptogams, which were not identified as species but grouped into three broad categories: mosses, epilithic lichens, and ground lichens. Vascular plant species have been identified in accordance with **Pignatti (1982**); the nomenclature of species is according to **Conti et al. (2005)**.

GPS coordinates [dd.ddddd°N; dd.dddd°E], elevation [m asl], slope [degree], aspect [°N] and surface texture [% of each grain size] were recorded for each plots as well.

II.2.D. Phenological observations

The phenological data collected during this PhD thesis field activity focused on the most representative vegetative and reproductive stages of 14 plant species of the alpine-tundra environment, following the ITEX protocol (Molau and Mølgaard, 1996) and its further implementation by different authors (Tab. II.2.B.1).

The species were selected at the beginning of the manipulation experiments but after the species composition assessment, according to the following criteria: a) they were the most representative of the selected vegetation communities (grasslands and snowbeds), b) they were representative of different growth forms and c) they were present at least in three replicates of each treatment per community type per year, in order to gain a more robust analysis.

Among the plant species identified in the experimental and control plots (n=47), phenological observations were conducted on 14 species namely: *Agrostis rupestris* All., *Alchemilla pentaphyllea* L., *Carex curvula* All. s.l., *Euphrasia minima* Jacq. ex DC., *Gnaphalium supinum* L., *Homogyne alpina* (L.) Cass., *Leontodon helveticus* Mérat emend. Widder, *Leucanthemopsis alpina* (L.) Heywood, *Ligusticum mutellina* (L.) Crantz, *Luzula alpinopilosa* (Chaix) Breistr. subsp. *alpinopilosa*, *Phyteuma hemisphaericum* L., *Poa alpina* L. subsp. *alpina*, *Salix herbacea* L., *Soldanella pusilla* Baumg. subsp. *alpicola* (F.K. Mey.) Chrtek.

The selected species belong to the main community types of the study sites (snowbeds and grasslands) and to different growth forms, well widespread over the study area and typical of alpine environment as well (a list of the selected species and a brief ecological contextualization is given in supplementary material, **Tab. S2**). Moreover, they belong to **5** main growth forms, according to **Elmendorf et al**.
(2012): grasses (2 ssp; *A. rupestris, P. alpina*), rushes (1 spp: *L. alpinopilosa*), sedges (1 ssp: *C. curvula*), forbs (9 ssp: *A.pentaphyllea, E. minima, G. supinum, H. alpine, L. helveticus, L. alpine, L. mutellina, P. hemisphaericum, S. pusilla*), deciduous dwarf shrubs (1 ssp: *S. herbacea*). As "dwarf shrubs", we intend here all those shrubs with vegetation height less than about 20 cm (Myers-Smith et al., 2015).

Of the 108 permanent plots of the two study sites, a sub-set of 64 (38 in year 2014, when monitoring was performed in Site 1 only) was used for the phenological observation (24, 24 and 16 related to OTCs, PS and SF treatments, respectively), performed in an sub-plot of 50x50 cm size for a total of 2498 replicates (species x plots x years) among the study period (2014-2017).

The timing of phenological events was monitored in the field on the manipulated and control plots every 3-4 days, according to the ITEX protocol (Molau & Mølgaard, 1996), starting from the snow

Phenophase	Brief description	Main reference
SM - snow melt	Complete absence of snow on the Complete absence of snow on the ground	Webber & Walker, 1991
FS -first shoot	First vegetative shoot (herbs) or start of buds elongation (shrubs)	Anadon-Rossel et al., 2014; Cooper et al., 2011 Cornelius et al., 2012;
NL - new leaves	First leaf fully expanded	Anadon-Rossel et al., 2014; Cooper et al., 2011; Cornelius et al., 2012; Haggerty & Mazer, 2008; Molau & Mølgaard, 1996
MS - main shoot	Start of shoot elongation (only for shrubs)	Anadon-Rossel et al., 2014; Molau & Mølgaard, 1996
FB - flower bud	First flower budding: inflorescence or flower buds visible, but not in anthesis	Anadon-Rossel et al., 2014; Cooper et al., 2011; Denny et al., 2014; Dorji et al., 2013; Hulber et al., 2006; 2010; Ladining et al., 2013; Molau & Mølgaard, 1996
FF - first flower FSt - First stigma FA - First anthers	First flower open: stage which flowers begin anthesis or is receptive to pollen. Stigma and/or anthers are visible	Abu-Asab et al., 2001; Anadon-Rossel et al., 2014; Anderson et al., 2012; Caradonna et al., 2014; Cook et al., 2012; Cooper et al., 2011; Cornelius et al., 2012; 2013; Craine et al., 2011; 2012; Crimmins et al., 2009; Denny et al., 2014; Dorji et al., 2013; Haggerty & Mazer, 2008; Hulber et al., 2006; 2010; Iler et al., 2013; IPG guide; Ladining et al., 2013; Molau et al., 2005; Molau and Molgaard, 1996; Schwartz et al. 2003
MF - main flowering	Maximum number of flowers, obtained by counting the number of flower at each plot for each census	Anderson et al., 2012; Caradonna et al., 2014; Haggerty & Mazer, 2008; Iler et al., 2013; Schwartz et al., 2003
WF – wilting flowers	Senescence of the stigma or anthers	Cooper et al., 2011; Molau and Mølgaard, 1996
SR - seed ripening	One or more ripe fruits/seeds are visible, ripe color and/or beginning of fruit dropping	Hulber et al., 2006; 2010; Ladining et al., 2013; Schwartz et al., 2003
Sdisp – seed dispersal	Dispersal of ripe seeds/dropping of ripe fruits	Cooper et al., 2011; Denny et al., 2014; Molau & Mølgaard, 1996; Schwartz et al., 2003
LS - leaf senescence	Date on which the leaves on an individual plant start to change color	Kolarova et al., 2014; Schuster et al., 2014; Panchen et al., 2015
LF - leaf fallen	Beginning of leaf fall	Denny et al 2014; Haggerty & Mazer, 2008; Schwartz et al., 2003

Tab. II.2.B.1 - Schematic description of the main phenological events monitored at the study sites.

melt (SM) until the beginning of the permanent snow cover in Fall, from summer 2014 in Site 1 and Summer 2015 in Site 2.

The phenological observations were recorded in 50x50 cm sub-plot in each manipulated and control plots and it included both qualitative and quantitative data, with the implementation of the original ITEX protocol, in order to improve some key information on the selected phenophases.

The qualitative data were the timing (DOY, Day Of the Year) of appearance of the following phenological stages: First Shoot (FS), New Leaves (NL), Main Shoot (MS), Flower buds (FB), First Flowering (FF), First Anthers (FA), First Stigma (FSt), Main Flowering (MF), Seed Development (SD), Seed Ripening (SR), Leaf Senescence (LS). The qualitative data implementation concerned the recording of seed dispersal (SDisp), flower senescence (WF, wilting flower) and leaf falling (LF) timing (**Tab. II.2.B.1**).

The quantitative data relied on the observations of a) the duration and b) the quantitative features of each phenophase.

The duration of each phenological stage is the number of days between the first and the last day of appearance of the phenophase (e.g. **CaraDonna et al., 2014; CaraDonna and Inouye, 2015**). Over the season, we obtained the start, maximum and the end DOY for each phenological events. The entire distribution over the time was recorded for each phenophase, allowing us to study the behavior of the phenology over the course of the season.

The quantitative features were represented by the size of the object of each phenophase (e.g. shoot length, leaves number, flowers number, seeds number, etc...), the plant height (**Cornelissen et al.**,



Fig. II.2.D.1 - Example of phenological stages of *Leontodon helveticus* (Asteraceae), from first shoot (FS) to seed dispersal (Sdisp). a) FS; b) NL, FB; c) FF; d) MF; e) WF; f) SD; g) SR; h) Sdisp.

2003), a parameter found to be a significant indicator of global warming (**Walker et al., 2006; Elmendorf et al., 2012**), and the intensification of the senescence stages and autumn processes, identified as the most neglected in climate change literature (**Gallinat et al., 2015**). For the latter, we reported the percentage of leaves of the current year in senescence as well as the decolouring percentage of the leaves lamina: 0-10%, 25-50%, 50-75%, 90%, and 100% (similarly to: e.g. **Cooper et al., 2011; Rosa et al., 2015**) and for deciduous species, we observed also the timing of leaves abscission.

In total, during the course of this PhD (2014-2017) were executed 72 days of phenological observation (2015: 29 days; 2016: 21 days; 2017: 22 days + 4 not reported in this thesis), that, considering the replicates of each year corresponded to 49717 observations.

II.3. MATERIALS AND METHODS: DATA ELABORATION

II.3.A. Climatic data

Since summer 2014, in our study sites we are recording meteorological data, such as air temperature (AirT), summer precipitation (pp) and snow fall (snowH; measured as snow thickness at the ground).

In order to validate the data and to assess if they were representative of the local/regional scale, we performed a linear regression between daily data recorded in our sites and those of others two automatic weather stations (Fig. II.3.A.1):

- Valdisotto-Oga S.Colombano AWS: 2295 m asl, 10 km SW from our study area (ARPA Lombardia; station ID 848);
- Zaufenkofel-Madriccio AWS: 2825m asl, 15 km E-SE from our study area (Bolzano Province; station ID 0609).

Despite the occurrence of many other AWS, we chose these two because they were the only in a 15 km radius from our study area those have been recording without gaps all the following parameter: air temperature (°C), precipitation (mm), snowfall (cm), wind speed (m/s), wind direction (degree North). Data were available with hourly frequency, or less.

Wind speed and wind direction are the only two parameters that we are not recording in the study sites. Wind data collected in correspondence of these two AWSs did not deserve exact information of speed and direction in our study sites, but provide information on their changes in frequency and amplitude

These data were used in the analysis of the snow fence experiment and on the evaluation of its influence on snow redistribution.



Fig. II.3.A.1 –Automatic weather stations (AWS) around our study area. Legend: 1 = ARPA Lombardia AWS; 2 = Bolzano Province AWS; 3 = Meteo Swiss AWS. a = Valdisotto-Oga S.Colombano AWS; b = Zaufenkofel-Madriccio AWS. Dashed black line represent our study area. Bing Aerial photography, 2017.

More in details, we computed frequency statistics for each wind directions and mean wind speeds for winter (DJF) and spring (MAM) seasons, the periods when most of the snowfalls occurred.

II.3.B. Environmental data

Ground surface temperature (GST), air temperature (AirT), summer precipitation (pp) and soil volumetric water content (VWC) were recorded at 1 hour interval or less, averaged at daily means (for temperature and soil moisture) and sums (for precipitation) and then used for the further analyses. Snow cover thickness in the sector aimed by the two snowcameras of the SF experiment was reported through images collected three times a day, on an 8 hours basis (three images per day at 8:00, 16:00; 24:00). From these images, we first excluded dark or covered pictures. Then with the remaining, a daily mean snow thickness (snowH) was assessed for each snow stakes.

Growing season (GS) is here defined as the period between the snowmelt in late Spring/early Summer and the begin of the permanent snow cover in early-Autumn.

Analyses of GST, AirT, pp and VWC were computed for the growing season (GS) of the four years of study (2014, 2015, 2016, 2017), three or which (from 2015 to 2017) within the period of this PhD thesis. Analyses of snowH were computed using season and hydrological year (1st October-30th September) (e.g. **Cannone & Guglielmin, 2009; Gruber et al., 2011; Pogliotti et al., 2015**).

Manually measures of snow cover were not available for each day and, therefore, we were not able to assess visually the exact snowmelt date for each of the monitored plots.

For this reason, the presence or absence of snow cover have been assessed using the daily GST time series obtained by temperature loggers, as already showed by several authors (Schmidt et al., 2009; 2012; Danby and Hick, 2007; Gadek and Leszkiewicz, 2010; Mutter and Phillips, 2012; Jean and Payette, 2014).

Moreover similarly to other authors (e.g. Floyd and Weiler, 2008; Ide and Oguma, 2010, 2013) in Site 1 we could reconstruct the temporal pattern of snow cover using the two snowcameras installed in October 2013 at the top of a 280 cm height wooden poles.

Through the combination of field measurements, GST time series and digital images we were able to identify the period of permanent snow cover and the snow free period, hereafter called growing season (GS).

For what concerns OTC, PS and SF experiments, the soil temperature data recorded at 2 cm of depth (e.g. **Pogliotti et al.,2015**) were elaborated to estimate the mean ground surface temperature for the growing season (hereafter defined as GST_GS) for each of the equipped plot in each of the years of study.

From these data we were able to: a) assess the natural variability of GST_GS among years, sites and plant communities and b) quantify the difference in GST_GS between manipulated and control plots.

For what concerns the PS experiments, because the polycarbonate cover had been removed in winter and replaced every next summer in the two precipitation shields equipped with soil moisture sensors, we analyzed the volumetric water content (VWC; m^3/m^3) for the whole growing season (VWC_GS) and for the period with polycarbonate cover deployment (VWC_PS).

Moreover to evaluate the occurrence or not of a warming effect in soil temperature, analyses on GST were carried out for the days with the polycarbonate cover deployment. The ground surface temperature during this period will be hereafter called GST_PS.

To test if the environmental parameter (SM, GST_GS, GST_PS, VWC_GS; VWC_PS) had changed as a consequence of manipulation experiments, we run the following analysis:

- Descriptive statistics including: minimum, maximum, median, 25% percentile, 75% percentile, mean (±SE and ±1.96 SE) (e.g. Gubler et al., 2011; Gill et al.,2015; Rosa et al., 2015);
- One-way analyses of variance (ANOVA; p<0.05), with environmental parameter as dependent variables and treatments (OTC, OTCc, PS, PSv1, PSv2, PSc, SFN*n*, SFS*n*, SFc) as factors; for OTC, the difference between the edge and middle of the chamber was assessed as well;
- Tukey HSD post-hoc test, were used for multiple comparison between treatment, where possible.

The analyses of the effect of each treatment (predictive factor) on environmental data (dependent variable) were carried out identical for each of the groups defined by the following variables: 1) Site; 2) Plant community; 3) Year; 4) Year*Plant community; 5) Site*Plant community; 6) Year*Site*Plant community; 7) Year*Site.

For what concerns the OTC experiment, the aim was to verify the occurrence and to quantify the warming effect in the OTC compared to OTCc and, more in detail, between edge (OTC edge; expected: warm+) and middle (OTC middle; expected: warm++) of the chamber and control.

For what concerns the PS experiment, the aim was to verify the occurrence and to quantify the moisture gradient between PS (expected: dry), PSv1 (expected: wet), PSv2 (expected: mesic), PSc (control) (e.g. Wu et al.,2011; Beier et al.,2012; Cherwin and Knapp, 2012; Cornelius et al.,2013; Swidrak et al.,2013; Gugger et al.,2015; De Boeck et al., 2016).

For what concerns the SF experiment, in order to verify the influence of the fence on snowH and SM and to identify the plots along the snow stake lines (SFNn, SFSn) most influenced, the difference between each plot and the controls was performed for the two variables in 2014 (before the SF deployment) and in 2015-2017 period (with the SF deployment).

Changes in the difference reported before and after 2014 indicated the extent and the direction of the SF deployment on snowH and SM allowing to identify the occurrence or not of delayed and/or advanced snowmelt (e.g. Wahren et al.,2005; Cooper et al.,2011; Johansson et al., 2013; Rumpf et al., 2014; Legault and Cusa, 2015; Semenchuk et al.,2016).

The verification of the presence/absence of the abiotic effects of such expected treatments was the baseline for the assessment and the explanation of any of the further biological responses in terms of species composition and plant phenology.

II.3.C. Effect of manipulation experiments on vegetation composition

Changes between the two years of survey (2013, 2017) under the three manipulation experiments were analysed for different vegetation features: percentage cover of the main vegetation layers (total vegetation cover, cov_total_%; vascular plant cover; cov_vascular_%; cryptogamic cover, cov_cryptogamic_%); percentage cover of the main growth forms (graminoids: cov_graminoids_%; forbs: cov_forbs_%; shrubs: cov_shrubs_%), total number of species and number of species of the main growth forms (sp_n_total; sp_n_graminoids; sp_n_forbs; sp_n_shrubs) (e.g. Elmendorf et al., 2012; 2015; Dorji et al., 2013; Brancaleoni and Gerdol, 2014). Moreover, percentage cover changes were evaluated at the species level (e.g. Cornelius et al., 2014; Baruah et al., 2017; Alatalo et al., 2017).

To test if vegetation parameters had changed as consequence of treatments, for each of the manipulation experiments we run the following analysis:

- Descriptive statistics for each year of survey (2013 and 2017) including: minimum, maximum, median, 25% percentile, 75% percentile, mean (±SE and ±1.96 SE) (Maggini et al.,2011);
- Three-way analyses of variance (ANOVA; p<0.05), with year, site and treatment as factorial variables and all the above mentioned parameters as dependent variables (except for those related to shrubs, because of the low frequency of occurrence and the uneven distribution among the treatments) (e.g. **Wang et al.,2012; Brancaleoni and Gerdol, 2015**);
- Tukey HSD post-hoc test, were used for multiple comparison between treatment, where possible (e.g. **Bragazza et al., 2012; Smith et al.,2012; Cornelius et al.,2014**).

II.3.D. Effect of manipulation experiments on plant phenology

For the assessment concerning the effect of manipulation experiments on plant phenology, 4 of the 14 monitored species were selected for statistical analysis: *Alchemilla pentaphyllea* L., *Carex curvula* All. s.l., *Gnaphalium supinum* L., *Leontodon helveticus* Mérat emend. Widder.

These species were chosen because are representative of the selected vegetation communities (grasslands and snowbeds) and occurred in more than 80% of the monitored plots for each year of study, in order to have the maximum level of replication.

We focused on phenological events that were recorded since the first survey in 2014.

For the qualitative data, we selected: snowmelt (SM), first shoot (FS), new leaves (NL); flower bud (FB), peak of main flowering (MF), seed development (SD), seed dispersal (Sdisp), leaf senescence (LS).

For these parameters, observed dates were converted in Julian date or Day of Year (days after 31 December) before data elaboration.

Along qualitative data, we selected also some quantitative data that are good indicator of plant growth and fitness and can track environmental changes (**Cornelissen et al.,2003**): mean height (Hmean; mm, DoY), maximum height (Hmax; mm, DoY), number of reproductive individuals (flowers_n), and reproductive success (rep_succ; %).

For the further analyses, these key stages were clustered in five groups:

- a. snowmelt and greening: SM, FS, NL;
- b. flowering: FB, MF;
- c. seed development and leaf senescence: SD, Sdisp, LS;
- d. plant growth: Hmean, Hmax;
- e. reproductive investment and success: flowers_n rep_succ.

To test if phenology had changed as consequence of treatments, for each of the manipulation experiments we run the following analyses for the selected phenological stages:

- Descriptive statistics including: minimum, maximum, median, 25% percentile, 75% percentile, mean (±SE and ±1.96 SE) (Maggini et al.,2011);
- One-way analyses of variance (ANOVA; p<0.05), with key phenological stages as dependent variables and treatments (OTC, OTCc, PS, PSv1, PSv2, PSc, SFNn, SFSn, SFc) as factors (e.g. Bragazza et al.,2012; Abeli et al.,2017).
- Tukey HSD post-hoc test, were used for multiple comparison between treatment, where possible (e.g. **Bragazza et al., 2012; Smith et al.,2012; Cornelius et al.,2014**).

The analysis of the effect of each treatment (predictive factor) on phenology (dependent variable) were carried out identical at inter- and intra-specific levels for each of the groups defined by the following variables: 1) Site; 2) Plant community; 3) Year; 4) Year*Plant community; 5) Site*Plant community; 6) Year*Site*Plant community; 7)Year*Site.

All statistical analyses were performed using the package STATISTICA (release 10; StatSoft Inc., Tulsa, OK, USA).

II.4. RESULTS

II.4.A. Inter-annual variability of meteorological parameters

Within the study period, in the two study sites the growing season (GS) length, referred as the period between the snowmelt and the begin of the permanent snow cover, lasted on average 114 days, with 101 (2016) and 138 (2015) as the longest and shortest ones. The begin of SM was recorded between early June (4 June; DoY 155, in 2015) and late-June (1 July; DoY 183, in 2016).

Air temperature (measured at 160 cm above the ground) has been recorded in both study sites every hour, since 03/11/2013 in Site 1 and 29/07/2014 in Site 2 and it is still ongoing. Only Site 1 has a complete data series, whereas Site 2 shows several data gaps due to damages of the dataloggers before the snowmelt or because of bad functioning during the summer season. For the available periods of data overlap, Site 1 and Site 2 didn't show any significant difference in air temperature, as tested by linear regression (R² = 0.91; p = 0.01) (**Fig.II.4.A.1**). Therefore, data from Site 1 could be considered as representative of both the study sites and were used for all the further analyses.

Considering all the four complete hydrologic years (Oct 2013-Sep 2017), the mean air temperature was 0°C, ranging between -0.3°C (2013-2014) and 0.3°C (2014-2015), but without any statistical difference among years, as tested by one-way ANOVA (F=0.59; p=0.62) (**Fig.II.4.A.2**). Minimum and maximum daily air temperature ranged between -17.3°C and -20.4°C and between 11.5°C and 15.3°C, respectively.

Considering the four growing season periods, the mean air temperature was of 6.8° C, ranging between 5.4°C (2014) and 7.3°C (2017) and showing statistical differences among year, as tested by one-way ANOVA (F=6.57; p<0.01). Minimum and maximum daily temperature ranged between -2.3°C and



Fig. II.4.A.1- Relation of mean daily air temperature [AirT; °C] and total daily precipitation [pp; mm] between Site 1 (S1) and Site 2 (S2), as tested by linear regression.



Fig. II.4.A.2 - Patterns of daily air temperature (top panel) and descriptive statistics of air temperature from 01/10/2013 to 27/09/2017, for full hydrologic year (bottom left panel) and growing season only (bottom right panel). Thick horizontal lines = means, boxes = SE, whiskers = 1.96*SE.

_	Ai	r Tempe	rature - Hy	ydrologi	c year [°	C]	_	Ai	r Tempe	rature - Gi	rowing s	eason [°	C]
Year	Ν	Mean	Median	Min	Max	SD	_	Ν	Mean	Median	Min	Max	SD
all	1454	0.0	0.0	-20.4	15.3	6.4	-	456	6.8	6.7	-2.3	15.3	3.5
2013_2014	365	-0.3	0.2	-17.5	11.5	5.6		103	5.4	5.6	0.1	11.5	2.5
2014_2015	365	0.3	0.0	-17.3	15.3	6.8		138	7.1	6.4	-0.5	15.3	3.9
2015_2016	366	0.0	0.4	-18.9	12.1	6.2		101	7.1	7.6	0.1	12.1	2.5
2016 2017	358	-0.1	-0.7	-20.4	15.3	7.1		114	7.3	8.2	-2.3	15.3	4.3

Tab. II.4.A.1- Non-parametric statistics of air temperature from 01/10/2013 to 27/09/2017, for full hydrologic year and growing season only at Site 1.

0.1°C and between 11.5°C and 15.3°C, respectively. The patterns of daily air temperature and their descriptive statistics are reported in **Fig.II.4.A.2** and **Tab.II.4.A.1**.

Concerning the summer precipitation, rain gauges were in place from mid-June to late-September/early-October of year 2015 (22 Jun-29 Sep), 2016 (28 Jun-6 Oct) and 2017 (27 Jun-27 Sep), in order to assess the summer precipitation and to evaluate the effect of the PS treatment on the amount of rain excluded through the precipitation shields. At Site 2, data gap occurred for part of the summer season because cable damages by local marmots.

Overall the study period, the mean total summer precipitation ranged between 316 mm (2016: Site 1: 316 mm; Site 2: 54 mm, incomplete data) and 467 mm (2017: Site 1: 434 mm; Site 2: 498 mm). Maximum total daily precipitation ranged between 43.2 mm (2015) and 65.6 mm (2017) (**Fig.II.4.A.3**).



Fig. II.4.A.3- Patterns of daily liquid precipitation at Site 1 (top panel) and Site 2 (bottom panel) from 01/10/2013 to 27/09/2017. Red lines = precipitation recorded at the study sites; grey lines = precipitation at Valdisotto Oga-S.Colombano AWS (2295 m asl; ARPA Lombardia, ID 835); R2 Site 1 = 0.82, R2 Site = 0.75, as tested by linear regression.

Site 1 and in Site 2 didn't show any significant difference, as tested by linear regression ($R^2=0.87$; p=0.00) (**Fig.II.4.A.1**).

From the above-mentioned data, the two study sites resulted completely comparable in terms of meteorological parameters, such as air temperature and summer precipitation. Moreover, they are comparable in other environmental variables, such as mean altitude (2612 vs 2638 m asl), mean slope (10 vs 11°), lithology (acidic glacial deposits) and plant community types (*Caricetum curvulae* and *Salicetum herbaceae alchemilletosum*).

Significant differences were detected in ground surface temperature (GST; °C; 2 cm depth), both on yearly and on growing season levels, as tested by full factorial ANOVA and Tukey post hoc test (p<0.05). However these differences are related to micro-topographical features at the local scale and reflect the natural environmental variability of the study sites those, therefore, were considered as true replicates within the experiment design.

GST of the growing season (GST_GS) of the four years of study (2014-2017) showed significant differences at year (F=239.0; p=0.00), site (F=69.9; p=0.00) and plant community levels (F=59.3; p=0.00), as tested by Factorial ANOVA.

Among the study sites, Site 1 (GST_GS=9.1°C \pm 3.5) is significantly cooler than Site 2 (9.9°C \pm 4.0), in both alpine grasslands (CC: Site 1: 9.5°C \pm 3.9; Site 2: 10.2°C \pm 3.6) and snowbeds (SH: Site 1: 8.6°C \pm 4.0; Site 2: 9.5°C \pm 3.4°C) (**Fig.II.4.A.4**).

			М	ean (GST -	growing	seaso	n [°C]				
			CC						SI	Н		
Year	Site	Ν	Mean	SD	Diff	р	Site	N	Mean	SD	Diff	р
2014	1	498	7.3	2.7	0.6	0.71	1	408	6.4	2.8	1 1	<0.01
2014	2	255	7.9	2.2	0.0	0.71	2	255	5 7.5	2.1	1.1	<0.01
2015	1	538	10.6	4.4	0.5	0.77	1	432	2 10.0	4.3	0.5	0.02
2015	2	317	11.2	3.8	0.5	0.77	2	292	2 10.5	3.9	0.5	0.95
2016	1	527	9.6	3.6	0.0	<0.05	1	420	8.6	3.7	1.0	<0.05
2016	2	319	10.5	3.4	0.9	<0.03	2	308	9.5	3.1	1.0	<0.03
2017	1	497	10.3	3.8	0.6	0.64	1	316	5 9.4	3.9	1.2	<0.05
2017	2	306	10.9	3.6	0.0	0.04	2	254	10.5	3.2	1.2	<0.03
all years	1	2060	9.5	3.9	07	<0.01	1	1576	6 8.6	4.0	1.0	<0.01
all years	2	1197	10.2	3.6	0.7	<0.01	2	1109	9.5	3.4	1.0	<0.01



Fig. II.4.A.4 - Intra-community variability of the natural ground surface temperature of the growing season (GST_GS; °C; 2 cm depth) in the control plots between Site 1 and Site 2. Dots = means, whiskers = ± 0.95 Conf. Interval. Diff = difference in GST between Site 1 and Site 2. p = significant differences (p < 0.05, Tukey post hoc test).

Among plant communities, grasslands showed higher GST_GS (+0.7°C) compared to snowbeds (CC: GST_GS=9.7°C \pm 3.8; SH: 9.0°C \pm 3.8), difference that was maintained in both study sites (Site 1: +0.9; Site 2: +0.7°C) and for all the years of study.

Within the same plant community, the maximum difference between study sites was reported in 2016 for grasslands (+0.9°C; Site 1: 9.6°C ± 3.6; Site 2: 10.5°C ± 3.4) and in 2017 for snowbeds (+1.2°C; Site 1: 9.4°C ± 3.9; Site 2: 10.5°C ± 3.2) (p < 0.05, Tukey post hoc test).

Among the study period, 2015 was the warmest year (GST_GS=10.5°C \pm 4.2) both at Site 1 (10.3°C \pm 4.4) and Site 2 (10.8°C \pm 3.9), whereas 2014 was the coolest year (GST_GS= 7.2°C \pm 2.6), with lower temperature at Site 1 (6.9°C \pm 2.8) than at Site 2 (7.7°C \pm 2.1).

II.4.B. Natural variability in micro-climate and micro-topographical conditions

Differences in GST between sites could be related to local features like a) vegetation, b) snow depth and/or snowmelt, c) soil water content or d) microtopography, as reported by several authors from polar to alpine environment (**Guglielmin et al., 2003; Cannone et al., 2006; Guglielmin et al., 2008; Cannone and Guglielmin, 2009**).

Concerning the potential influence of vegetation (point a), the influence of the plant community on the different GST_GS between sites was relative, because an increase in GST_GS from Site 1 to Site 2 was detected in both CC and SH.

Concerning the effect of snow there are two key parameters: snow cover thickness and duration (point b).

Concerning snow cover thickness, the snow depth from the beginning of May to the snowmelt date of three years of study (excluding year 2014 when only Site 1 was investigated) showed significant differences at year (F=23.7; p<0.01), plant community (F=15.1; p<0.01), site (F=7.1; p=0.01) and year*site (F=3.6; p=0.03) levels, as tested by Factorial ANOVA.

Among the study sites, Site 1 (36 ± 45 cm) had a significantly thicker snow cover than Site 2 (53 cm ± 57 ; p<0.01). In alpine grasslands (CC: Site 1: 36 cm ± 45 ; Site 2: 34 cm ± 43) the difference was not significant, whereas in snowbeds (SH: Site 1: 36 cm ± 45 ; Site 2: 72 cm ± 63) the difference was significant, as tested by Tukey post hoc test (**Fig.II.4.B.1; Fig.II.4.B.2**).

Differences between sites could be ascribed to micro-topographical features and/or to wind effect, those increased snow thickness and delayed snowmelt in Site 2, especially in snowbeds community where the differences were more evident.

Indeed, if plots on grasslands were located on ridges or top areas in both sites, plots in snowbed communities were on ridge in Site 1 and in trough in Site 2, where the snow accumulation was able to increase. This difference between snowbed communities was particularly evident in the OTC experiment, in both warming and control plots.



]	Mean si	nowdepth	[cm] ·	- CC						
		All	years			2014			2015			2016			2017	
Sites	Ν	Mean	SD	Diff	Mean	SD	Diff	Mean	SD	Diff	Mean	SD	Diff	Mean	SD	Diff
all	252	41.5	52.0		72.3	72.4		38.5	43.1		47.8	50.7		8.6	15.6	
1	160	46.1	56.0	12.6	72.3	72.4	mull	36.2	41.0	5.2	53.7	54.3	12 /	10.2	17.2	27
2	92	33.5	43.1	-12.0			nun	41.5	46.0	5.5	40.4	45.6	-13.4	6.5	13.5	-3.7

Fig. II.4.B.1- Comparison of mean snow depth [cm] pattern between Site 1 and Site 2 within Caricetum curvulae association (CC) for each year of study (2014, 2015, 2016, 2017) measured in the field on un-manipulated plots from 7 May (DoY 127) to 3 July (DoY 184). Dots = means, whiskers = ± 0.95 Conf. Interval.



						10	icali si	owacpin	loui	- 511						
		All y	vears			2014			2015			2016			2017	
Sites	Ν	Mean	SD	Diff	Mean	SD	Diff	Mean	SD	Diff	Mean	SD	Diff	Mean	SD	Diff
all	252	57.2	60.3		81.4	71.4		54.8	58.1		68.7	61.4		22.0	27.3	
1	160	48.9	57.3	22.7	81.4	71.4	mu11	31.1	37.5	52.2	58.0	57.5	24.1	16.8	25.0	110
2	92	71.6	63.0	22.1			nun	84.4	65.7	55.5	82.1	64.4	2 4 .1	28.6	29.4	11.0

Fig. II.4.B.2 - Comparison of mean snow depth [cm] pattern between Site 1 and Site 2 within Salicetum herbaceae association (SH) for each year of study (2014, 2015, 2016, 2017) measured in the field on un-manipulated plots from 7 May (DoY 127) to 3 July (DoY 184). Dots = means, whiskers = ± 0.95 Conf. Interval.

Among plant communities, snowbeds showed a 17 cm thicker snow cover compared to grasslands (CC: 35 cm \pm 44; SH: 52 cm \pm 56; p<0.01; Tukey post hoc test), difference that was maintained in both study sites and for all the years of study.

Within the same plant community, the maximum difference between study sites was reported in 2016 for grasslands (13 cm; Site 1: 54 cm; Site 2: 40 cm; p=0.94) and in 2015 for snowbeds (53 cm; Site 1: 31 cm; Site 2: 84 cm; p<0.01).

Among the years of study, 2016 had the thicker snow cover (58 cm \pm 57) both at Site 1 (56 cm) and Site 2 (61 cm), whereas year 2017 had the shallower snow cover (15 cm), both in Site 1 (14 cm) and Site 2 (18 cm).

In terms of date of snowmelt onset (excluding year 2014 when only Site 1 was investigated), the most significant differences were found at year (F=1524; p<0.01), site (F=322; p<0.01) and site*plant community (F=367; p<0.01) levels, as tested by Factorial ANOVA.

Among the study sites, Site 1 (DoY 164) had a significantly advanced snowmelt of 3 days than Site 2 (DoY 167; p<0.01). Alpine grasslands didn't show any difference among study sites (CC: Site 1 and Site 2: DoY 164), with low intra-annual variability (from 0 to 2 days of difference), whereas snowbeds had a significant delay of 8 days in Site 2 (SH: Site 1: DoY 161; Site 2: DoY 172; p<0.01), reaching value up to 13 days of difference (year 2015) (**Fig.II.4.B.3**).

Therefore, snowmelt between study sites was comparable, with difference that occured only within the snowbeds community and was related to differences in local micro-topographical features. The two study sites resulted eventually comparable and could be considered as true replicates.

Among plant communities, snowbeds had a delay of 3 days compared to grasslands (CC: DoY 164; SH: DoY 167; p<0.01; Tukey post hoc test). This difference was not maintained in Site 1, where the snowmelt occurred at DoY 164 in both plant communities, whereas in Site 2 a significant difference of 8 days was maintained (Site 2: CC: DoY 164; SH: DoY 172; p<0.05).

Within the same plant community, in grasslands the maximum difference between study sites occurred in 2015 (+2 days of delay; p<0.05) and 2017 (-2 day of advance; p<0.01), whereas in snowbeds occurred in 2015 (+13.5 days of delay; p<0.001).

Among the years of study, Site 1 had the largest delay of snowmelt in 2014 (DoY 180), both in CC (DoY 178) and SH (DoY 181), whereas Site 2 had it in 2016 (DoY 175), both in CC (DoY 173) and SH (DoY 179).

Concerning the water content (point c), from year 2015 to 2017 the above ground biomass (AGB) was collected in all control plots, at different phenological and production stages (early-July, early-August, mid-September) within each growing season. Above ground biomass were clipped to ground level in one 20x20 cm area within a subplot of 100x50 cm next to the control, dried at 60 °C, and weighing, in order to assess the AGB water content [% of weight].

AGB water content showed a significant differences at year (F=27.1, p=0.01) and plant community (F=7.0, p=0.01) levels, without any differences among study sites.



				S	now N	Melt (SM)	[DoY]				
			CC			· · · ·			S	ЯH		
Year	Site	Ν	Mean	SD	Diff	р	Site	Ν	Mean	SD	Diff	р
2014	1	58	178	3.5			1	54	181	2.6		
2015	1	53	158	2.4	17	<0.05	1	54	156	2.0	12.5	<0.001
2015	2	50	160	0.0	1./	<0.05	2	36	170	6.9	15.5	<0.001
2016	1	50	173	0.0	0.0	1.00	1	54	173	0.0	57	<0.001
2016	2	50	173	0.0	0.0	1.00	2	36	179	4.4	5.7	<0.001
2017	1	45	161	2.0	25	<0.001	1	53	162	1.5	5 2	<0.001
2017	2	47	159	0.0	-2.3	<0.001	2	33	168	3.8	3.2	<0.001
all years	1	148	164	6.7	0.1	<0.001	1	161	164	7.2	0 7	<0.001
all years	2	147	164	6.4	-0.1	~0.001	2	105	172	7.1	0.2	~0.001

Fig. II.4.B.3- Intra-annual and intra-community snowmelt (SM) onset (DoY) of the four years (2014, 2015, 2016, 2017) study period. Dots = means, whiskers = ± 0.95 Conf. Interval. Diff = difference in SM between Site 1 and Site 2, within the same plant community type. p = significant differences (p < 0.05, Tukey post hoc test) compared to control.

However, in general Site 2 showed higher values than Site 1 both at inter-community (Site 1: 60.5% \pm 12.5; Site 2: 63.4% \pm 11.6) and intra-community levels (CC: Site 1: 58.6% \pm 10.7; Site 2: 59.9% \pm 11.8; SH: Site 1: 62.4% \pm 14.1; Site 2: 66.9% \pm 10.4). An exception was CC in 2017, when Site 2 had a lower water content.

Snowbeds had a higher water content compared to grasslands (CC: $59.2\% \pm 11.2$; SH: $64.6\% \pm 12.5$), in both study sites (Site 1: CC: $58.6\% \pm 10.7$; SH: $62.4\% \pm 14.1$; Site 2: CC: $59.9\% \pm 11.8$; SH: $66.9\% \pm 10.4$).

At site and community levels, year 2017 showed the lowest AGB water content (49.6% \pm 8.0) and it was significant different to 2015 (64.8% \pm 8.3) and 2016 (67.2% \pm 11.9) those had comparable values. AGB water content is a parameter that could be used as a proxy of soil moisture, and therefore as an independent factor for the understanding of the differences in GST among sites and communities. However, as the AGB water content may be influenced by other environmental variables (e.g.: species composition, day of collection), it would be more suitable to analyze the soil water content, from soil samples or from soil moisture sensors.

For this reason, two soil trenches were dag in each site, one for each plant community. Soil depth ranged between 90 cm (SH) and 120 cm (CC) at Site 1 and between 35 cm (CC) and 37 cm (SH) at Site 2.

The main characteristics of the horizon O and A of the soils underneath each plant community in both the study sites are reported in **Fig.II.4.B.4** and in **Tab.II.4.B.1**.



Fig. II.4.B.4 – Left panel: soil trenches at the study sites underneath the two studied plant communities; a) Site 1, CC; b) Site 1, SH; c) Site 2, CC; d) Site 2, SH. Right panel: water content [% of weight] of horizon O and A of the soil trenches at the study sites underneath the two studied plant communities.

			Sit	e 1			Sit	e 2	
plant comm	horizon	depth [cm]	water [%]	fine fraction [%]	pН	depth [cm]	water [%]	fine fraction [%]	pН
CC	0	0-6	41.9	68.3	3.6	 0-6	35.5	71.9	3.9
tt	А	6-20	31.0	47.6	3.3	6-11	25.4	57.5	4.3
CII	0	0-7	26.1	46.8	4.1	 0-9	40.9	77.5	3.9
бп	А	7-23	29.8	62.9	4.1	9-14	24.9	74.5	4.5

Fig. II.4.B.1 – Depth, water content, fine fraction ($\leq 2mm$) and pH of horizon O and A of the soil trenches at the study sites underneath the two studied plant communities.

In horizon O (thickness: 6-9 cm) was found most of the roots biomass, despite for some grasslands species (e.g. *Carex curvula*) roots went much deeper in the soil.

At inter-community level, the difference in water content between study sites was low (32.2 vs 31.7 %). At intra-community level, alpine grassland (CC) shows in Site 1 an higher water content than Site 2, in both horizons O (41.9 vs 35.5%) and A (31.0 vs 25.4 %). In snowbed community, the water content in Site 1 was higher for horizon A (28.9 vs 24.9 %), but not in horizon O (26.1 vs 40.9 %). Therefore, Site 1 had higher water content than Site 2 for both the shallower soil horizons (O and A) in each plant community, except for horizon O underneath SH.

Concerning the micro topography (point d), the sites didn't differ from each other, with plots located on an homogeneous surface, without rock outcrops or shading effect from the vicinity of morphological features, as well as far from water gully or streams. The only morphological difference related with snowbeds in Site 2, where the occurrence of a concavity showed an influence on the snow accumulation at the end of spring season and on the snowmelt time compared to snowbeds of Site 1.

In synthesis, the differences in GST among study sites related to micro-topographical features and, more in detail, to a prolonged permanence of snow in snowbeds at Site 2 as well as to a difference in general exposition. For this reason, the difference among sites was maintained for all the study period and therefore, the two sites were considered as true replicates within the experiment design.

II.4.C. Species selection and natural variability in plat phenology

For the understanding of the biological answers to the manipulation experiments, 7 of the 14 monitored species were selected for further analysis: *Alchemilla pentaphyllea* L., *Carex curvula* All. s.l., *Gnaphalium supinum* L., *Leontodon helveticus* Mérat emend. Widder, *Poa alpina* L. subsp. *alpina*, *Salix herbacea* L., *Soldanella pusilla* Baumg. subsp. *alpicola* (F.K. Mey.) Chrtek.

These species were chosen because are representative of the selected vegetation communities (grasslands and snowbeds) and occurred in more than 80% of the monitored plots for each year of study, in order to have the maximum level of replication.

To better evaluate the effect of the different treatment on plant phenology of the 7 selected species, the first analysis concerns the understanding of the natural variability (control plots only) in growth and reproductive stages of the species, among and within sites and plant communities. Detailed information on the natural variability of the two study sites and plant communities are reported in **Fig.II.4.C.1** and **Fig.II.4.C.2**.

The whole growing season (GS) could be divided in four main phenological period: greening (FS, NL, MS), flowering (FB, FF, FSt, FA, MF_beg, MF, WF), seeds (SD, Sdisp), senescence (LS).

Concerning the medians of the beginning of each phenological stages, within the study area and for the whole study period (2014-2017), a wide natural variance has been detected (Fig. 10).

Greening started really soon after the snowmelt (SM), that occurred between DoY 155 and 183. First shoot (FS) appearance ranged between DoY 159 and 216 (57 days), new leaves (NL) between 159 and 212 (53 days) and main shoot (MS) between 159 and 225 (66 days).

Except for some early-flowering species (such as *Soldanella pusilla*), flowering usually follows the greening stages. Flower bud (FB) and first flower (FF) ranged, respectively, between DoY 159 and 212 (53 days) and between DoY 159 and 235 (76 days). The begin of main flowering (MF_beg; that almost overlaps with FSt and FA) started between DoY 159 and 243 (84 days), the full flowering (MF) from DoY 163 and 249 (86 days) and the flower senescence (WF) from DoY 163 to 245 (82

days). The total flowers number (flowers_n) per plots ranged between 0 to 125, with a mean value of 11. The number of individuals in anthesis at the peak of the full flowering was on average 9, but with a maximum value of 120.

Concerning seed development and dispersal, seed development (SD) ranged between DoY 173 and 267 (94 days; the stage with the widest natural amplitude, together with LS) and seed dispersal (Sdisp) from 204 and 280 (76 days), with a reproductive success spanning from 0 to 100%, with a mean value of 51.9%. Leaves senescence (LS) ranged between DoY 178 and 272 (94 days). It usually started before the seed dispersal and in most cases before the seed development.

At inter-annual level, differences in the medians were detected between the two study sites in all the phenological stages, except for FB and MF. The maximum difference were found in FS (Site 1: 177; Site 2: 173; 4 days), FF (184 vs 188; 4 days), SD (204 vs 208; 4 days) and Sdisp (247 vs 243; 4 days) (**Fig.II.4.C.1, Fig.II.4.C.2**).

A one-way ANOVA repeated for each phenological stages (dependent variable) were used to verify the significant differences among sites (categorical predictor) for each plant communities (**Tab. II.4.C.1**).



			Site	e 1 - T	ornante -	CC					Sit	e 2 - S	corluzzo -	- CC		
	Ν	Mean	Min	25%	Median	75%	Max	SD	Ν	Mean	Min	25%	Median	75%	Max	SD
SM	124	168	155	160	168	174	182	8.4	81	164	159	159	160	173	173	6.4
FS	122	174	159	163	177	182	204	10.2	81	172	159	165	165	179	198	9.1
NL	124	178	160	168	179	184	208	10.5	81	176	159	165	175	184	208	11.3
MS	12	172	163	164	171	179	184	8.8	12	168	159	163	165	179	179	8.2
FB	68	180	159	168	180	188	208	11.9	54	181	159	173	182	190	208	12.3
FF	65	185	160	173	184	193	216	13.9	67	186	159	175	188	195	221	15.1
FSt	84	192	160	179	192	200	243	17.8	67	190	159	180	193	201	221	15.5
FA	83	193	163	180	191	202	243	17.1	64	191	159	180	194	201	221	15.2
MF beg	84	193	163	181	192	202	243	16.8	67	191	159	180	194	201	221	14.7
MF	84	197	163	184	197	208	249	18.3	67	194	163	184	195	203	230	15.1
WF	68	197	168	187	197	208	225	15.0	65	198	163	188	198	208	235	15.8
SD	86	205	173	191	204	215	263	18.2	60	206	173	197	207	219	235	15.7
Sdisp	73	245	204	234	249	256	280	18.7	43	237	214	223	240	245	280	16.1
LS	122	221	184	202	214	245	272	22.0	80	213	178	198	209	221	260	17.0

Fig. II.4.C.1- Descriptive statistics of phenological stages in un-manipulated plots for Site 1 and Site 2 (period: 2015-2017) within the alpine grassland community (Caricetum curvulae - CC). Thick horizontal lines = means, boxes = 25% and 75% percentiles, whiskers = minimum and maximum. Phenological stages: Snow melt (SM), First Shoot (FS), New Leaves (NL), Main Shoot (MS), Flower buds (FB), First Flowering (FF), First Anthers (FA), First Stigma (FSt), Main Flowering (MF), Wilting Flower (WF), Seed Development (SD), Seed dispersal (SDisp), Leaf Senescence (LS).



Fig. II.4.C.2- Non-parametric statistics of phenological stages in un-manipulated plots for Site 1 and Site 2 (period: 2015-2017) within the snowbed community (Salicetum herbaceae - SH). Thick horizontal lines = means, boxes = 25% and 75% percentiles, whiskers = minimum and maximum. Phenological stages: Snow melt (SM), First Shoot (FS), New Leaves (NL), Main Shoot (MS), Flower buds (FB), First Flowering (FF), First Anthers (FA), First Stigma (FSt), Main Flowering (MF), Wilting Flower (WF), Seed Development (SD), Seed dispersal (SDisp), Leaf Senescence (LS).

Difference between study sites resulted statistically significant (p<0.01) for snowbeds vegetation only between the means of the onset day of most phenological stages, except for main shoot (MS), first stigma (Fst), first anthers, (FA), begin of main flowering (MF_beg) and senescence (LS).

In snowbed, the significant difference in the first stages (FS, NL) can be attributed to the shift of snowmelt date, that was described in the previous paragraph (II.4.B). Otherwise, the absence of any significant difference in the main shoot (MS) was mainly related to the low occurrence of *S. herbacea* within plots of Site 2, especially in those in the topographical concavity, where *A. pentaphyllea* dominates with coverage up to 80-90%.

F-values	SM	FS	NL	MS	FB	FF	FSt	FA	MF_beg	MF	WF	SD	Sdisp	LS
all communities	10.5	6.0	2.1	0.2	7.8	2.7	2.2	2.1	2.0	2.9	1.7	7.7	1.6	0.9
CC	0.1	0.1	0.2	0.0	3.1	0.7	0.6	0.4	0.3	0.1	0.2	1.1	0.5	0.3
SH	46.9	19.0	11.8	1.1	11.3	4.7	3.7	3.6	3.6	7.4	4.1	11.9	9.1	0.1

Tab. II.4.C.1 - F-values of the one-way ANOVA repeated for each phenological stages (dependent variable) among study sites (categorical predictor) for each plant communities (CC, SH). Significant values (p<0.05) in bold character.

The not-significant difference in the onset of flower anthesis (FSt, FA, MF_beg) and of leaves senescence (LS) means that other factor (e.g. photoperiod), rather than micro-climate only, could influence the appearance of these key stages.

II.4.D. Effect of manipulation deployment on environmental data

II.4.D.a. Open Top Chambers (OTCs)

In the following paragraphs, the impact of passive warming chambers on the ground surface temperature of the growing season (GST_GS; 2 cm depth; °C) will be presented.



Fig. II.4.D.1 - Intra-annual, inter-site and intra-community ground surface temperature (2 cm depth) of the four years (2014, 2015, 2016, 2017) growing season (GST_GS; °C). N = number of daily means. Dots = means (different letters represent statistical significant difference between means, as tested by Tukey post-hoc test); whiskers = ± 0.95 Conf. Interval. Sensor = position of the dataloggers sensor (edge = inside OTC, 10 cm far from the polycarbonate panels; middle = inside OTC, middle; control = control plot, middle). Diff = difference in GST_GS between manipulated (edge, middle) and control (control) plots.

At inter-annual and inter-site levels, over the study period (2014-2017) the OTCs significantly (p<0.05) increased the mean ground surface temperature of the growing season of +0.4°C at the edge and +0.5°C in the middle of the chambers, compared to control plots ($9.5^{\circ}C \pm 3.8$), without any significant difference among each other (**Fig. II.4.D.1**). The maximum increase in soil temperature was recorded in 2014 (edge: +0.5°C; middle: +0.7°C) whereas the lowest in 2015 (edge: +0.5°C; middle: +0.5°C). Interestingly, 2014 and 2015 were, respectively, the years with the lowest and the highest natural GST recorded in control plots only (2014: 7.2°C; 2015: 10.7°C).

In alpine grasslands (CC) the OTCs warming ranged between +0.4 (2017) and +0.9°C (2015) at the edge of the chamber and between +0.6 (2016) and +0.9°C (2015) in the middle, with the relative control plots spanning from +7.7 (2014) to +10.9°C (2015). Differences between edge and middle OTC and control plots were statistically significant for all the investigated years, as tested by Tukey post-hoc test (p<0.05).

In snowbeds (SH) the OTCs warming ranged between +0.2 (2015, 2017) and +0.5°C (2014) at the edge of the chamber and between +0.1 (2015) and +0.7°C (2014, 2017) in the middle, with the relative



	IVI	ean GS	51 - grov	ving	season	[C] - S	ne i		
			OTC-0	CC			OTC-S	SH	
Year	Sensor	Ν	Mean	SD	Diff	Ν	Mean	SD	Diff
	edge	1304	10.3 a	3.6	0.7	1239	9.2 a	3.6	0.7
all yrs	middle	1295	10.4 a	3.9	0.8	1220	9.1 a	3.8	0.6
	control	1212	9.6 b	3.9		1159	8.5 b	4.0	
	edge	356	8.3 a	2.8	0.9	327	7.4 a	2.8	1.4
2014	middle	358	8.2 a	2.9	0.9	327	7.2 a	2.9	1.2
	control	261	7.4 b	2.4		261	6.0 b	2.6	
	edge	326	11.7 a	4.0	1.1	306	10.8 a	4.0	0.6
2015	middle	320	11.8 a	4.1	1.3	300	10.6 a	4.0	0.4
	control	330	10.5 b	4.5		282	10.2 a	4.2	
	edge	318	10.4 a	3.3	0.7	304	9.0 a	3.3	0.6
2016	middle	318	10.5 a	3.6	0.8	299	8.8 b	3.4	0.4
	control	319	9.6 b	3.6		314	8.4 b	3.7	
	edge	304	11.1 a	3.5	0.6	302	9.9 a	3.5	0.7
2017	middle	299	11.3 a	3.7	0.9	294	10.0 b	3.6	0.8
	control	302	10.5 b	3.8		302	9.2 b	3.9	

Fig. II.4.D.2 - Intra-annual and intracommunity ground surface temperature (2 cm depth; °C) of the four years (2014, 2015, 2016, 2017) growing season (GST GS; °C) at Site 1. N = number of daily means. Dots = means (different letters represent statistical significant difference between means, as tested by Tukey post-hoc test); whiskers = ± 0.95 Conf. Interval. Sensor = position of the dataloggers sensor (edge = inside OTC, 10 cm far from the polycarbonate panels; middle = inside OTC, middle; control = control plot, middle). Diff = difference in GST GS between manipulated (edge, middle) and control (control) plots.

control plots spanning from +6.8 (2014) to +10.5°C (2015). Differences between edge and middle and control plots were statistically significant for 2014 and 2016, as tested by Tukey post-hoc test (p<0.05). In 2015, was not detected any significant difference, whereas in 2017 the mean GST in the middle of warming plots was no statistically different compared to controls.

The less evident warming effect in snowbeds could be related to the occurrence of data gaps, in dataloggers mainly located at Site 2 between 2015 and 2017 (see below).

In Site 1, warming effect was on average $+0.7^{\circ}$ C and $+0.8^{\circ}$ C at the edge and middle of the chambers, compared to control plot (9.6° C ± 3.9), in alpine grasslands, and $+0.7^{\circ}$ C and $+0.6^{\circ}$ C compared to control plot (8.5° C ± 4.0) in snowbeds (**Fig. II.4.D.2**).

In Site 2, the OTC deployment on ground surface temperature was affected by several data gaps due to the long lasting snow cover over the dataloggers those were subjected to high frequency damages because of the high amount of melt water and seepage (despite datalogger cases and position were modified every years, in order to avoid this problem). For this reason, the unexpected cooling effect reported in 2014, 2015 and 2017 in snowbeds and the not well visible warming in alpine grasslands at Site 2 was an artefact, due to the problem mentioned above (**Fig. II.4.D.3**).



	М	ean GS	ST - gro	wing	season	[°C] - S	Site 2		
	Mean GST - growing season [°C] - Site 2OTC-CCOTC-SHYearSensorNMeanSDDiffNMeanSDDiffedge91310.8 a3.70.410469.7 a3.40.01 yrsmiddle119910.9 a3.60.510769.9 a3.40.3control119610.3 b3.510589.6 a3.40.3edge1558.1 a2.20.12157.2 a1.8-0.5control2558.6 b2.30.52558.0 b2.20.3control2558.0 a2.12557.7 c2.1edge22512.0 a4.00.728410.6 a3.9-0.2control31711.3 b3.927610.9 a4.1edge31110.7 a3.60.229210.0 a2.80.4control31910.5 a3.33119.3 b2.92.9edge22211.4 a3.50.325510.4 a3.4-0.5								
Year	Sensor	Ν	Mean	SD	Diff	Ν	Mean	SD	Diff
	edge	913	10.8 a	3.7	0.4	1046	9.7 a	3.4	0.0
all yrs	middle	1199	10.9 a	3.6	0.5	1076	9.9 a	3.4	0.3
	control	1196	10.3 b	3.5		1058	9.6 a	3.4	
	edge	155	8.1 a	2.2	0.1	215	7.2 a	1.8	-0.5*
2014	middle	255	8.6 b	2.3	0.5	255	8.0 b	2.2	0.3
	control	255	8.0 a	2.1		255	7.7 c	2.1	
	edge	225	12.0 a	4.0	0.7	284	10.6 a	3.9	-0.3*
2015	middle	323	11.7 a	4.1	0.5	274	10.6 a	3.9	-0.2*
	control	317	11.3 b	3.9		276	10.9 a	4.1	
	edge	311	10.7 a	3.6	0.2	292	10.0 a	2.8	0.7
2016	middle	319	10.8 a	3.4	0.3	292	9.7 b	2.8	0.4
	control	319	10.5 a	3.3		311	9.3 b	2.9	
	edge	222	11.4 a	3.5	0.3	255	10.4 a	3.4	-0.5*
2017	middle	302	11.9 b	3.4	0.8	255	11.3 b	3.6	0.4
	control	305	11.1 a	3.6		216	10.9 a	3.1	



*= data gaps



Fig. II.4.D.4 – Differential ice formation (a) during freezing events inside the OTC (b) and in control plot (c); date: 07/09/2017. Red dashed line = area with ice formation; black solid line = plot limits. Bottom panel (d): wind speed (m/s) variation in different position within an OTC on snowbed community; 1 = windward side-out OTC; 2 = windward side-in OTC; 3 = leeward side-in OTC; 4 = windward side-out OTC.

Further details on warming by OTCs across sites and plant communities is reported in **Fig. II.4.D.2** and **Fig. II.4.D.3**.

From the data mentioned above, we can confirm the occurrence of a warming effect during the growing season due to the OTC deployment, compared to control plots. Moreover, despite not statistically significant (mainly because of data gaps), we often confirm the occurrence of an "edge effect" within the chambers, with higher GST at the edge of the OTC compared to the middle and control plot as well. These findings are underline also by the differential ice formation observed over the vegetation at the edge and middle of the chambers and in the control plots, during frost events at the end of the growing season (**Fig. II.4.D.4**).

The warming generated by the chambers has been well examined since the begin of ITEX protocol (e.g. Marion et al., 1997; Jagerbrand et al., 2009; Bokhorst et al., 2011). However, only few study have into account, at least in part, their influence on wind speed and therefore on sensible and latent heat fluxes (e.g. Zhou et al., 2013).

The effect of OTC on wind speed was confirmed by measurements recorded on a windy day inside and outside the chambers, at different height (**Fig. II.4.D.4**). Between 150 cm 50 cm from the ground, wind speed decreased with similar pattern in all the investigated position. Below 50 cm (the height of OTC), it showed an abrupt decrease in speed inside of the chamber, both on the windward and leeward side. Outside the chambers, the speed decreased gradually in the windward side whereas in the leeward side showed an abrupt decrease, comparable to that inside the OTC.

II.4.D.b. Precipitation shields (PS)

At inter-annual and inter-site levels, over the study period (2014-2017), the ground surface temperature during the PS field deployment (GST_PS; 2 cm depth; °C) was significantly increased by +0.8°C underneath the shield compared to control plots (9.7°C \pm 3.6), with the warming that ranged between +0.6°C (2014) and +1.1°C (2015), as tested by Tukey post-hoc test.

The remaining PS treatment (PSv1, PSv2) didn't have any significant difference to controls, with an effect on GST_PS that ranged between -0.1°C (2016) and +0.2°C (2015) for PSv1 and between +0.2°C (2016) and +0.6°C (2017) in PSv2 (**Fig. II.4.D.5**).

Alpine grasslands (CC) showed the most evident increase in GST_PS, ranging between +0.7°C (2016) and +1.5°C (2015), significant in 2014, 2015 and 2017 but not in 2016. The remaining plots downslope to the shield (PSv1, PSv2) didn't have any significant warming or cooling effect compared

				Mea	an GST	- growin	g sea	son wi	th	PS in pl	ace (GST I	PS;	°C) – all	sites				
		а	ll years			2	2014		_	2	015			2	016		2	2017	
Ass	Treat	Ν	Mean	SD	Diff	Mean	SD	Diff	-	Mean	SD	Diff	-	Mean	SD	Diff	Mean	SD	Diff
	PS	1015	11.0 a	3.7	1.1	9.2 a	2.2	1.1		12.8 a	4.0	1.5		10.7 a	3.3	0.7	11.3 a	3.8	1.2
00	PSv1	1015	10.0 b	3.8	0.1	8.1 b	2.4	0.1		11.5 b	4.3	0.2		9.9 b	3.5	-0.1	10.2 b	4.0	0.1
CC	PSv2	1015	10.2 b	3.6	0.3	8.3 b	2.3	0.3		11.6 b	4.0	0.3		10.1 ab	3.4	0.2	10.7 ab	3.7	0.6
	PSc	1015	9.9 b	3.7		8.1 b	2.4			11.3 b	4.0			9.9 ab	3.5		10.1 b	3.7	
	PS	942	10.0 a	3.6	0.5	8.2 a	2.2	0.4	-	11.7 a	4.1	0.8	_	9.9 b	3.3	0.5	10.3 a	3.5	0.4
CII	PSv1	1015	9.8 ab	3.6	0.2	7.9 a	2.4	0.2		11.0 a	3.9	0.1		10.2 a	3.6	0.8	9.6 a	3.5	-0.3
бП	PSv2	1015	9.5 b	3.5	0.0	7.7 a	2.3	0.0		10.8 a	3.9	-0.1		9.3 b	3.1	-0.1	9.8 a	3.5	-0.1
	PSc	942	9.5 b	3.6		7.7 a	2.3			10.9 a	4.1			9.4 b	3.4		9.9 a	3.5	
	PS	1957	10.6 a	3.6	0.8	8.7 a	2.2	0.8		12.2 a	4.1	1.1		10.3 a	3.3	0.6	10.9 a	3.7	0.8
.11	PSv1	2030	9.9 b	3.7	0.1	8.0 b	2.4	0.1		11.2 b	4.1	0.1		10.0 a	3.5	0.4	9.9 b	3.8	-0.1
all	PSv2	2030	9.9 b	3.6	0.1	8.0 b	2.3	0.1		11.2 b	3.9	0.1		9.7 ab	3.3	0.0	10.2 ab	3.6	0.2
	PSc	1957	9.7 b	3.6		7.9 b	2.4			11.1 b	4.1			9.7 ab	3.4		10.0 b	3.6	

Fig. II.4.D.5 – Intra-annual, inter-site and intra-community ground surface temperature (2 cm depth) of the four years (2014, 2015, 2016, 2017) growing season with the precipitation shield in place (GST_PS; °C) in the PS experiment. N = number of daily means. Dots = means (different letters represent statistical significant difference between means, as tested by Tukey post-hoc test); whiskers = ± 0.95 Conf. Interval. Diff = difference in GST_GS between manipulated (PS, PSv1, PSv2) and control (PSc) plots.

to controls, but in 2016 and 2017 they showed similarities with rain exclusion plot (PS) (Fig. II.4.D.5).

The warming effect in snowbeds (SH) was comprised between +0.4°C (2014, 2017) and +0.8°C (2015), with a mean of +0.5°C, even if it is never resulted significant, as tested by Tukey post-hoc test. All the other plots didn't have significant difference in GST_PS, except for year 2016 when PSv1 had +0.8°C warming effect (p<0.05).

Therefore, the warming effect was more evident in alpine grasslands than in snowbeds.

Analyzing separately the two sites, in Site 1, in alpine grasslands the warmest GST_PS was always recorded underneath the precipitation shield cover compared to controls, with a more evident effect between year 2014 and 2016 (**Fig. II.4.D.6**). In snowbeds it was maintained the same pattern found in alpine grassland, with the exception of year 2016 when the most intense warming (+1.1°C) was recorded in plot PSv1 instead of PS.

Fig. II.4.D.6 – Intra-annual and intra-community ground surface temperature (2 cm depth) of the four years (2014, 2015, 2016, 2017) growing season with the precipitation shield in place (GST_PS; °C) in the PS experiment at Site 1. N = number of daily means. Dots = means (different letters represent statistical significant difference between means, as tested by Tukey post-hoc test); whiskers = ± 0.95 Conf. Interval. Diff = difference in GST_PS between manipulated (PS, PSv1, PSv2) and control (PSc) plots.

In Site 2, alpine grasslands showed a clear thermal gradient between PS (11.4°C), PSv1 (10.7°C), PSv2 (10.5°C) and control (10.3°C), despite only the difference between PS and control was statistically significant (p<0.05, Tukey post-hoc test). This gradient was maintained in all years of study, except for the 2016 growing season (**Fig. II.4.D.7**).

In snowbeds, the differences in GST_PS among manipulated and control plots were less evident and none of those resulted significant to Tukey post-hoc test.

Further details on warming effect by PS deployment across sites and plant communities are reported in **Fig. II.4.D.6** and **Fig. II.4.D.7**.

	Mean GST - growing season with PS in place (GST_PS;°C) – Site 2																	
		i	all years			2	2014			2015		2	2016		2	2017		
Ass	Treat	Ν	Mean	SD	Diff	Mean	Mean SD Diff			SD	Diff	Mean	SD	Diff	Mean	SD	Diff	
	PS	327	11.4 a	3.6	1.1	9.4 a	1.8	1.2	12.9 a	3.9	1.5	11.0 a	3.2	0.4	11.6 a	4.0	1.2	
CC	PSv1	327	10.7 b	3.5	0.4	8.7 ab	1.8	0.5	11.9 ab	3.8	0.5	10.8 a	3.2	0.2	10.8 a	3.8	0.4	
cc	PSv2	327	10.5 b	3.2	0.3	8.5 b	1.6	0.3	11.4 b	3.4	0.1	11.2 a	3.2	0.6	10.5 a	3.3	0.1	
	PSc	327	10.3 b	3.3		8.2 b	1.7		11.4 b	3.5		10.6 a	3.1		10.4 a	3.5		
	PS	327	10.4 a	3.0	0.5	8.6 a	1.5	0.6	11.3 a	3.2	0.6	10.7 a	2.8	0.4	10.6 a	3.3	0.5	
сп	PSv1	327	10.0 a	3.2	0.1	8.0 a	1.6	0.0	11.0 a	3.4	0.3	10.4 a	3.1	0.1	10.0 a	3.4	-0.1	
511	PSv2	327	10.1 a	3.1	0.2	8.4 a	1.5	0.4	11.2 a	3.4	0.6	10.0 a	2.8	-0.3	10.4 a	3.4	0.3	
	PSc	327	9.9 a	3.1		8.0 a	1.7		10.7 a	3.2		10.3 a	3.1		10.1 a	3.2		
	PS	654	10.9 a	3.3	0.8	9.0 a	1.7	0.9	12.1 a	3.6	1.0	10.9 a	3.0	0.4	11.1 a	3.7	0.9	
.11	PSv1	654	10.3 b	3.3	0.2	8.3 b	1.7	0.3	11.4 ab	3.6	0.4	10.6 a	3.2	0.2	10.4 a	3.6	0.2	
an	PSv2	654	10.3 b	3.2	0.2	8.4 b	1.6	0.3	11.3 ab	3.4	0.3	10.6 a	3.1	0.1	10.4 a	3.3	0.2	
	PSc	654	10.1 b	3.2		8.1 b	1.7		11.0 b	3.4		10.5 a	3.1		10.3 a	3.4		

Fig. II.4.D.7 - Intra-annual and intra-community ground surface temperature (2 cm depth) of the four years (2014, 2015, 2016, 2017) growing season with the precipitation shield in place (GST_PS; $^{\circ}$ C) in the PS experiment at Site 2. N = number of daily means. Dots = means (different letters represent statistical significant difference between means, as tested by Tukey post-hoc test); whiskers = ±0.95 Conf. Interval. Diff = difference in GST_PS between manipulated (PS, PSv1, PSv2) and control (PSc) plots.

Along to the ground surface temperature, soil moisture, measured as volumetric water content (VWC; m^3/m^3) at 2 cm of depth, was recorded since growing season 2015 in two precipitation exclusion experiment on alpine grasslands, one for each study site.

Rain gauges were used in the study sites from growing season 2015 to growing season 2017 to assess the amount of summer precipitation and evaluate the quantity of rain intercepted by the precipitation

shields. In Site 1 the amounts of rain during the period with the polycarbonate cover deployment were 384.6 mm in 2015, 321.8 mm in 2016, 424.4 mm in 2017, while in Site 2 were 350.8 mm in 2015 and 497 mm in 2017; in 2016 we had data gap (**Fig. II.4.D.8**).

In the intention of the experimental design, these were the amounts of rain excluded from the plot underneath the shields (PS) and converged to the down-slope plots (PSv1, PSv2), especially in the one on the vertical edge of the polycarbonate cover (PSv1).

Fig. II.4.D.8 - Total liquid precipitation recorded at Site 1 and Site 2 during the whole growing season (left panel) and during the period with precipitation shields deployment only (right panel). * = year with data gaps.

The expected influence of the PS on soil moisture was a decrease of VWC underneath the precipitation shield (PS plots) and an increase in VWC in the down-slope plots (PSv1, PSv2), with a larger influence in the more adjacent (PSv1), located on the vertical projection of the polycarbonate cover that intercepts the rain events. Hereafter, we'll show the results of the VWC monitoring.

Soil moisture in the un-manipulated control plots changed between years (F=11.1, p<0.01, as tested by one-way ANOVA). At inter-site level, year 2017 (36%) had a significantly higher soil moisture than 2015 and 2016, both having comparable values (30%).

Within each study site, mean soil moisture ranged between 18% (2016) and 26% (2017) in Site 1 and between 39% (2015) and 45% (2017) in Site 2, with significant differences between all years of study, as tested by Tukey post-hoc test.

During the period with the precipitation shield cover in place, soil moisture showed differences in means that differed within site and treatment. **Fig. II.4.D.9** reports the mean soil moisture for each of the monitored plots within the study period.

	Soil volumetric water content with PS in place (VWC_PS; % m3/m3)															
			all years	5	_		2015			2016			2017			
Site	Treat	Ν	Mean	SD	Diff	Mean	SD	Diff	Mean	SD	Diff	Mean	SD	Diff		
	PS	263	0.22 a	0.07	0.00	0.19 a	0.07	-0.02	0.24 a	0.05	0.06	0.23 a	0.07	-0.03		
1	PSv1	263	0.27 b	0.05	0.05	0.24 b	0.06	0.03	0.29 ab	0.02	0.11	0.28 c	0.05	0.02		
1	PSv2	263	0.28 b	0.06	0.06	0.24 b	0.07	0.03	0.29 ab	0.05	0.11	0.31 b	0.04	0.05		
	PSc	263	0.22 a	0.06		0.21 a	0.06		0.18 c	0.03		0.26 c	0.06			
	PS	260	0.39 a	0.07	-0.03	0.39 a	0.07	-0.01	0.34 a	0.03	-0.08	0.46 a	0.06	0.00		
C	PSv1	218	0.31 b	0.07	-0.11	0.29 b	0.06	-0.11	0.30 b	0.04	-0.13	0.40 b	0.06	-0.06		
Z	PSv2	257	0.36 c	0.06	-0.06	0.32 c	0.04	-0.07	0.36 c	0.03	-0.06	0.41 b	0.06	-0.05		
	PSc	260	0.43 d	0.08		0.40 a	0.10		0.43 d	0.06		0.46 a	0.08			
	PS	523	0.31 a	0.11	-0.01	0.29 a	0.12	-0.02	0.29 a	0.07	-0.01	0.35 a	0.13	-0.01		
.11	PSv1	481	0.29 b	0.06	-0.03	0.26 ab	0.06	-0.04	0.29 a	0.03	-0.01	0.32 ab	0.08	-0.04		
all	PSv2	520	0.32 a	0.07	0.00	0.28 a	0.07	-0.02	0.33 b	0.06	0.02	0.36 ac	0.07	0.00		
	PSc	523	0.32 a	0.13		0.30 ac	0.13		0.30 a	0.13		0.36 ac	0.12			

Fig. II.4.D.9 – Intra-annual, inter-site (left panel) and intra-site (right panel) soil volumetric water content (VWC; $\% \text{ m}^3/\text{m}^3$) of three years (2015, 2016, 2017) growing season with the precipitation shield in place (VWC_PS) for each PS and control plots at Site 1 and 2 and SH. Sensors are located in CC in both sites. N = number of daily means. Dots = means (different letters represent statistical significant difference between means, as tested by Tukey post-hoc test); whiskers = ±0.95 Conf. Interval. Diff = difference in VWC_PS between manipulated (PS, PSv1, PSv2) and control (PSc) plots.

Considering all the two rain exclusion experiments equipped with sensors, the occurrence of the whole expected pattern of soil moisture was not confirmed. Both at inter- and intra-years levels the PS plots were not the driest, even if they always show a lower VWC than control plots (despite this difference was not statistically significant). These unexpected values of soil moisture, probably related to the occurrence of not-vertical precipitation events due to wind, were not maintained in both the study sites.

Fig. II.4.D.10 - Evidence of precipitation shield (PS) deployment on a late-summer snowfall (28/09/217)

In particular, in Site 1 we could confirm the influence of the precipitation shield on the soil moisture pattern in years 2015 and 2017, when PS (underneath the shield) was respectively -2% (not significant) and -3% dryer (p<0.05) than controls. PSv1 and PSv2 didn't differ from each other in 2015, with an increase of +3% than controls; in 2017 only PSv2 had a significant soil moisture, with an increase of +5% (p<0.05). However, both 2015 and 2017 were characterized by poor rain precipitation and a severe drought in particular during the peak of the growing season.

In 2016, we recorded the largest increase in mean soil moisture in PSv1 (+11%; p<0.05) and PSv2 (+11%; p<0.05) compared to control plot, as expected. Similarly, also PS showed an higher soil moisture than control (+6%; p<0.05), but still not as high as PSv1 and PSv2 (not significant).

In summary, considering the monitoring of both ground surface temperature (GST_PS) and soil volumetric water content (VWC_PS) during the deployment of the precipitation shields we were able to confirm the occurrence of a warming effect underneath the polycarbonate covers of the shields (PS), comparable or even more intense than the warming effect generated by the Open Top Chambers.

Ground surface temperature in all the other manipulated plots (PSv1, PSv1) didn't show any statistically significant difference compared to controls and allowed us to confirm the absence of any soil warming related to the precipitation shield deployment.

For what concerns the rain exclusion, we confirmed that the period of deployment of the shied in each of the growing season intercepted almost more than the 95% of the rain events. However, because of the surface morphology and of the occurrence of winds (often with moderate speeds) during the rain events, from the soil moisture data (VWC_PS) we were not able to find the following expected gradient in soil moisture: PS (dry) \rightarrow PSv2 (mesic) \rightarrow PSv1 (wet).

In Site 1 this gradient was found in year 2015 and 2017, when we were able to confirm the effect of the PS on rain exclusion and redistribution and its consequences on soil moisture in PS, PSv1 and PSv2 plots. In Site 2, none of the investigated years showed the expected values of soil moisture

II.4.D.c. Snow Fence (SF)

Snow cover thickness at Site 1 was assessed since November 2013 with the data collected through the two snowcameras (on plots not influenced by snowfence) when the site was undisturbed and in order to quantify the natural trends of snow cover accumulation and melting in the study site.

When data gaps occurred, it was reconstructed through the snow depth gradient between Valdisotto-Oga S.Colombano AWS (2295 m asl; ARPA Lombardia; station ID 848) and Zaufenkofel-Madriccio AWS (2825m asl; Bolzano Province; station ID 0609), located respectively below and above our study sites and both significantly related to snow depth at our Site 1, as tested by linear regression ($R^2 = 0.67$; $R^2 = 0.88$; p<0.01).

Between October 2013 to September 2017 (four hydrologic years) the mean snow depth was 93 cm, ranging between 67 (2016-2017) and 124 cm (2013-2014). The maximum snow cover thickness ranged between 238 cm (2013-2014) and 143 cm (2014-2015). The mean seasonal and monthly means significantly changed among study years, as tested by one-way ANOVA (**Fig. II.4.D.11**).

Fig. II.4.D.11 – Pattern of mean snow depth (cm) at Site 1 in four hydrologic years (2013-2014, 2014-2015, 2015-2016, 2016-2017). Graph: Dots = means; whiskers = ± 0.95 Conf. Interval. Table: different letters represent statistical significant difference between means, as tested by Tukey post-hoc test); SD = standard deviation; F = F values from one-way ANOVA (reported only if significant), performed for each month (depended variable) among hydrologic years (factorial predictor).

Fig. II.4.D.12 – Mean speed (left panels) and frequency of direction (right panel) of wind for winter (DJF) and spring (MAM) seasons recorded in four hydrologic year (2013-2014; 2014-2015; 2015-2016; 2016-2017) at Zaufenkofel-Madriccio AWS (2825m asl; Bolzano Province; station ID 0609) and at Valdisotto-Oga S.Colombano AWS (2295 m asl; ARPA Lombardia; station ID 848).

A similar pattern, but not comparable amount of snow thickness, could be identified in the hydrologic years 2013-2014 and 2014-2015, with an increase in thickness from October up to March and a further decrease until the snowmelt.

The hydrologic years 2015-2016 and 2016-2017 showed similar patterns and, differently to the previous two, they received a substantial amount of snowfalls at the end of Spring, reaching the maximum snow thickness in May.

Despite the similarity in snow thickness patterns, the begin of snowmelt was comparable between 2014 (DoY 178) and 2016 (DoY 173) and between 2015 (DoY 158) and 2017 (DoY 161).

Wind speed and wind direction of winter (DJF) and spring (MAM) seasons recorded at Valdisotto-Oga S.Colombano AWS (2295 m asl) and at Zaufenkofel-Madriccio AWS (2825m asl) didn't show any appreciable difference among the four years of study (**Fig. II.4.D.12**).

	Snow depth [cm]													
		all years		2013-	2014	2014-2	2015	2015-2	2016	2016	-2017			
Plot	Ν	Mean	SD	Mean	Mean SD		SD	Mean	SD	Mean	SD			
SFN10	416	4.0	20.5	5.3	26.3	5.3	22.3	3.4	18.3	1.2	5.2			
SFN09	416	4.3	22.3	5.5	27.0	6.5	27.3	3.5	18.5	1.0	4.7			
SFN08	416	4.4	22.5	5.1	26.5	6.7	27.4	3.8	20.2	1.0	4.7			
SFN07	416	4.1	21.5	4.9	26.4	6.4	26.0	3.3	17.8	1.0	4.7			
SFN06	952	43.0	55.7	64.7	76.8	22.5	30.5	49.8	48. 7	14.6	24.1			
SFN05	952	41.8	53.4	62.4	73.3	22.4	31.2	48.2	46.4	14.8	24.4			
SFN04	952	44.9	55.4	63.0	73.3	21.5	27.1	55.9	52.0	18.3	29.6			
SFN03	952	49.3	61.5	71.6	82.9	21.9	27.0	61.0	56.5	19.5	31.3			
SFN02	952	56.9	68.5	77.5	87.2	25.6	31.4	73.7	67.0	23.6	38.0			
SFN01	952	59.0	70.8	81.4	90.8	26.5	35.0	74.3	67.3	28.8	46.5			
SFS01	818	46.8	64.4	28.0	53.9	22.7	37.2	59.8	68.5	74.8	76.6			
SFS02	818	43.5	61.7	30.1	55.7	17.4	29.4	58.8	68.6	64.6	69.6			
SFS03	818	40.3	56.4	29.8	57.1	18.3	27.2	55.9	63.8	52.5	58.3			
SFS04	818	36.4	51.6	28.6	55.7	17.0	25.0	50.7	58.2	44.6	51.2			
SFS05	818	35.2	49.9	27.4	55.3	17.4	25.1	48.8	55.7	42.9	49.2			
SFS06	818	33.5	47.8	27.2	55.4	17.3	26.5	44.6	50.8	41.6	48.2			
SFS07	418	3.4	18.7	5.2	27.0	3.8	16.3	2.8	15.8	0.9	4.3			
SFS08	418	3.4	18.6	5.2	27.2	3.8	16.2	2.6	14.8	1.0	4.7			
SFS09	418	4.2	21.1	5.7	27.5	5.0	21.1	3.7	19.9	1.2	5.0			
SFS10	418	4.2	21.3	5.4	27.3	5.1	21.1	3.9	21.0	1.3	5.6			

Fig. II.4.D.13 - Intra-annual snow depth (cm) of the four hydrologic years along the snowfence. The deployment of the SF started in October 2014. Dots = means, whiskers = ± 0.95 Conf. Interval. Dashed grey line = snow fence location. Bolt values are those of plots aimed by the snowcameras

Wind data collected in correspondence of the two stations did not deserve exact wind speed and direction in our study sites, but provided information on their changes in frequency and amplitude. Using the images collected three times a day from the snowcameras, we were able to reconstruct the snow thickness along the snow fence experiment, from SFN06 to SFS06. Mean values are reported in **Fig. II.4.D.13**. Because of the batteries run out during the winter season prior to the installation of a solar panel, we have a complete information on snow depth along the full hydrologic years only for 2015-2016 and for the southern side of snow fence for 2016-2017.

Concerning the sector aimed by snow cameras, and considering only the hydrologic years with complete data at least for one side of the snowfence, it was well visible an increase in mean snow depth in the closest plots to the wooden barrier and a decrease going farther from it.

The effect of the wooden barrier on snow accumulation was confirmed also by the field measurements carried out at the end of spring, in comparable day of years (between DoY 150 and DoY 159; **Fig. II.4.D.14**).

In 2014 (after a winter season without the snow fence deployment) the snow cover on 30/05/2015 (DoY 150) showed a thickness comprised between 140 and 180 cm, with a mean value 165 cm, compared to 172 cm of controls. The maximum snow depth was recorded in N07 (180 cm) and S03 (180 cm).

In 2015, after the first winter of snowfence deployment, the snow cover thickness measured on 01 June (DoY 152) had a minimum, maximum and mean values, respectively, of 60, 120 and 82 cm. The minimum snow depth was recorded in SFN05, N06 and S05, while the maximum in SFN08.

In 2016, the snow depth was recorded on 07 June (DoY 159), with minimum, maximum and mean values of 70, 130 and 101 cm, respectively. The minimum show depth was recorded in SFN05 and N06, while the maximum in SFS01 and S02.

Fig. II.4.D.14 - Snow cover thickness recorded in the field at the end of spring season along the snow fence manipulation experiment plots in 2014 (DoY 150), 2015 (DoY 152), 2016 (DoY 159) and 2017 (DoY 152). Dashed grey line = snow fence location.

In 2017, the snow depth was recorded on 1 June (DoY 152), with minimum, maximum and mean values of 25, 80 and 42 cm, respectively, and showed a comparable pattern with 2016. The minimum snow depth was found in SFS05 and S06, while the maximum in SFN01 and S01.

In 2014 (before the fence deployment begin) the SM along the snow stakes ranged between DoY 173 and DoY 184, with a mean value of DoY 177 (**Fig. II.4.D.15**). The earliest SM was found in SFN05, N04 and N03, while the most delayed in SFS03.

In 2015, after the first winter of snow fence deployment, the SM ranged between DoY 159 and DoY 168, with a mean value of DoY 163. The earliest SM was found in SFS08, while the most delayed in SFS01.

Fig. II.4.D.15 - Snowmelt recorded in the field along the snow fence manipulation experiment plots in 2014 (before SF deployment, used as reference of natural snow melt pattern), 2015, 2016 and 2017 (all used to quantify the SF effect). Dashed grey line = snow fence location.

	SM - Snow melt date [DoY]																			
year	N10	N09	N08	N07	N06	N05	N04	N03	N02	N01	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
2014	176	176	175	174	173	173	173	174	177	177	176	182	184	182	179	179	176	176	176	180
2015	164	167	161	165	162	164	163	164	164	165	168	164	162	159	160	160	160	159	164	164
2016	180	178	178	178	178	178	178	178	183	186	185	185	182	180	179	178	177	178	178	178
2017	162	161	161	161	159	159	161	163	164	165	167	163	162	161	159	157	157	160	162	162
									SM	Idelta										
2017	102	101	101	101	137	137	101	105	SM	Idelta	107	105	102	101	137	137	157	100	102	

									21/1	ueita											
	N10/9	N9/8	N8/7	N7/6	N6/5	N5/4	N4/3	N3/2	N2/1			S1/2	S2/3	S3/4	S4/5	S5/6	S6/7	S7/8	S8/9	S9/10	
2014	0.3	1.0	0.7	0.7	0.3	0.0	-0.7	-3.0	0.0			5.7	2.3	-1.7	-3.7	0.0	-3.0	0.0	0.7	3.3	
2015	-2.7	5.3	-3.3	2.3	-1.3	0.3	-0.3	-0.3	-0.7			-3.7	-2.3	-2.3	0.7	0.0	-0.3	-0.3	4.7	0.0	
2016	1.3	0.0	0.3	0.3	0.0	0.0	-0.7	-5.0	-2.7			0.0	-3.0	-2.0	-0.7	-1.3	-0.3	0.3	0.7	-0.3	
2017	1.3	0.0	0.0	2.0	-0.3	-1.3	-2.3	-1.3	-0.3			-4.3	-1.0	-1.3	-1.7	-1.7	-0.3	3.0	2.0	-0.3	
treat	treat Topographic effect					Control plots				Snow	fence	ence effect				Control plots			Topographic effect		
code	SFtN	SFtN	SFtN	SFtN	SFc	SFc	SFc	SF+	SF+	SF+	SF+	SF+	SF+	0/(+)	SFc	SFc	SFc	SFtS	SFtS	SFtS	

Tab.II.4.D.1 - Snowmelt date (SM) recorded in the field along the snow fence manipulation experiment plots in 2014 (before SF deployment, used as reference of natural snow melt pattern), 2015, 2016 and 2017 (all used to quantify the SF effect). SMdelta indicates the differences in SM date between adjacent snow stakes, at intra-annual level: the occurrence of high differences between adjacent plots since 2015 onward, compared to 2014, indicate the influence of snow fence deployment on snow redistribution and on SM date. SFtN = plots with snow melt difference due to natural condition (topographic influence, north side of the fence); SFtS = plots with snow melt difference due to natural condition (topographic influence, north side of the fence); SF+ = plots with influence of snow fence; SFc = plots without changes, used as controls.

Fig. II.4.D.16—Evidence of snow fence (SF) deployment on snow cover permanence and snow melt time on the north (left, in the picture) and south (right, in the picture) side of the fence on 28 June 2016.

In 2016, the SM ranged between DoY 177 and DoY 186, with a mean value of 180. The earlies SM was found in SFS07, while the most delayed in SFN01. However, in this year was well visible a delay in SM in the first 5-6 m from the fence, on both its sides (**Fig. II.4.D.16**).

In 2017, the SM ranged between DoY 157 and DoY 167, with a mean value of 166. The earliest SM was found in SFS06 and S07, while the most delayed in SFS01.

Starting from the SM date information and using year 2014 as reference of natural snow melt without the influence of the snow fence, we were able to identify the plots most influenced by the fence and those less influenced, further used as control plots (**Tab.II.4.D.1**; see also chapter II.3. Materials and methods).

The parameter used for this evaluation was the SMdelta. Starting from plot SFN10, it was calculated as the difference between the SM date of each plot and its neighbours, going toward the snow fence that is located at the top of the moraine ridge. Plots with changes in SMdelta between year pre- and post- snow fence deployment is considered influenced by the fence; otherwise, plots without changes, are considered as control plots.

In this evaluation, we took in consideration the morphology of the terrain on which the fence was installed and which the three lines of 20 snow stakes each (60 total) were located.

In plots N03, N02, N01, S01, S02 and S03, in years 2015, 2016 and 2017 the SMdelta increased, indicating a delay in snow melt date. This situation was supported by field survey and digital images (**Fig. II.4.D.16**), those showed how the snow cover was still present on these plots while all the surrounding areas (not potentially subjects by the fence) were already snow free. We further use plots N03, N02, N01, S01, S02 and S03 as artificially delayed snow melt treatment, induced by the snow fence. Their treatment code hereafter will be "SF+".

In plots SFN06, N05 and N04 the SMdelta remained almost stable, indicating a substantial unchanged snow melt. Therefore, we use these plots as control plots for the snow fence manipulation experiment; their treatment code hereafter will be "SFc".

Because the snow stake lines cross perpendicularly the Holocene moraine ridge on which all the experiments in Site 1 were located, in the farthest plots (20 m of distance from the fence) the influence of the structure was overcame by the influence of the topography.

In plots SFN10, N09, N08, N07 (on the north side) and in SFS08, S09 and S10 (on the south side) the SMdelta was relatively high but remained unchanged between pre- and post- snow fence deployment. This indicated the prevalence of terrain morphology rather than SF deployment on the snow melt date of these plots. We further use plots SFN10, N09, N08, N07 (on the north side) and in SFS08, S09 and S10 (on the south side) as naturally delayed snow melt, induced by terrain morphology. Their treatment codes hereafter will be "SFtN" for the north side of the fence and "SFtS" for the south side of the fence.

SFtN SF+ SFtS SFc

162

167

162

161

Fig. II.4.D.17 - Intra-annual snow melt date of the four years (2014, 2015, 2016, 2017) in the SF experiment. Dots = median; boxes = 25%-75%; whiskers = min-max. * = year 2014 was not influenced by the snow fence deployment during winter season.

159

173

159

157

177

182

164

180

176

SFc

178

161

159

176

160

178

With the snow fence deployment, the snowmelt in SF+ treatment was delayed from +3 days (2015) to +5 days (2016, 2017) in mean values compared to SFc; median values showed a delay comprised between +4 days (2015) and +6 days (2016). The delay in maximum values showed its maximal difference in year 2017, with a delay of +7 days in SF+. However, if we compare the earliest SM in SFc with the most delay SM in SF+, difference rose up to +11 days (2017) (Fig. II.4.D.17).

The natural delayed snow melt treatments (SFtN, SFtS) had a lower difference than SF+ compared to control plots and in some years (2016) it had also showed a no difference with SFc in mean values. In SFtN the SM was delayed from +2 days (2017) to +3 days (2015) in mean values compared to control plots, but with no change in 2016. Median values showed a delay comprised between +1 day and +4 days. As for SF+, also in this case if we compare the earliest SM in SFc with the most delay SM in SFtN, difference rose up to +8 days (2015).
In SFtS the differences with control plots were slightly less pronounced than in SFtN. The SM was delayed from +1 day (2015) to +2 days (2017) in mean values, but with no change in 2016. Median values showed a delay comprised from +3 days (2017) and +4 days (2015). Here, the maximum difference between SM in SFtS and SFc was +5 days (2015, 2017).

Many similarities between year 2015 and 2017 were found in terms of SM onset among the treatments. The most intense delay was recorded in year 2016 for SF+ treatment.

For the three growing season after the SF deployment, in 2015 and 2017 there were not significant differences (p>0.05) in mean ground surface temperature (GST_GS) among the treatments of the SF experiments (**Fig. II.4.D.18**). Only in 2016 we found significant difference (p<0.05) between SFtN and SF+, as well as between SFtN and SFc, as tested by one-way ANOVA and Tukey post hoc tests.



ŌSFtN ፬ SF+ ፬ SFtS ፬ SFc

Fig. II.4.D.18 - Intra-annual ground surface temperature (2 cm depth) of the four years (2014, 2015, 2016, 2017) growing season in the SF experiment. Dots = means; whiskers = SE.

We could therefore conclude that the influence of the snow fence on the monitored abiotic components was only related to the snow thickness and to the snowmelt time, with only a not significant to negligible influence on the ground surface temperature of the growing season.

II.4.E. Natural changes in plant composition and coverage

In this paragraph, we'll show the natural changes of species richness and of cover of main vegetation layers and growth forms that we recorded between year 2013 and 2017 in all the control plots of the study sites. For this analysis, we excluded controls of the rain exclusion experiment (PSc), because partially influenced by the deployment of the precipitation shield, and we used controls of the warming experiment (OTCc).

Considering the whole period (2013-2017), a gross total of 26 species, one of those (*Primula* sp.) not exactly identified, were recorded among the control plots.

The whole pool of species belonged to 13 different plant families (*Apiaceae* (1), *Asteraceae* (4), *Campanulaceae* (1), *Caryophyllaceae* (2), *Crassulaceae* (1), *Cyperaceae* (3), *Gentianaceae* (1), *Poaceae* (4), *Primulaceae* (2), *Ranunculaceae* (1), *Rosaceae* (3), *Salicaceae* (1), *Scrophulariaceae* (2)), to 5 different growth forms (deciduous dwarf shrub (1), forbs (18), grasses (4), rushes (2), sedges (1); sensu Elmendorf et al.,2012) and 1 cushion plant. All species were well represented in both study sites and in both plant communities.

A list of the identified vascular plant species, is reported in **Tab. S2**.

For what concern the species composition, in 2017 in alpine grasslands (CC) was recorded the ingression of *Avenula versicolor*, *Gentiana punctata* and *Ranunculus montanus* and the disappearance of *Primula sp*. In snowbeds (SH) was recorded the ingression of *Phyteuma hemisphaericum* and the disappearance of *Veronica alpina*.

Alpine grasslands

In alpine grassland, in 2013 the mean species number per plots ranged between 11 and 13, with graminoids comprised from 2 to 4 species and forbs from 8 to 10 species. Shrubs, when recorded, were represented in these control plots only by *Salix herbacea*.

In the re-survey in 2017, the mean species number per plot didn't change.

In terms of vegetation layer coverage, in 2013 total coverage (cov_total_%) ranged between 90 and 100% (mean and median = 95%), vascular plant coverage (cov_vasculars_%) ranged between 60 and 85% (mean=73%, median=75%) and cryptogams layer ranged between 42 and 82% (mean and median=62%) (**Fig. II.4.E.1; Tab.II.4.E.1**).

In 2017, total coverage and vascular layer cover were substantial unchanged, while cryptogams slightly decreased of -5% in mean value, but with a decrease up to -12% in its maximum value.

Within the main growth forms, in 2013 graminoids (cov.graminoids_%) covered an area comprised between 27 and 40%, forbs (cov.forbs_%) between 29 and 63% and shrubs (cov.shrubs_%) between <1 to 7%.

In 2017, graminoid growth form had a statistically significant (p<0.05) increase of +8%, while forbs decreased by -2 and -3% in mean and median values. Shrubs didn't change their cover.

At the species level, in 2013 *A. pentaphyllea* had a coverage that ranged between 4 and 20%, *C. curvula* between 16 and 37% and L. *helveticus* between 15 and 35%; *P. alpina, S. herbacea* and *S. pusilla* had a coverage that was less than 5% each (**Fig. II.4.E.2; Tab.II.4.E.2**).

In 2017, *C. curvula, G. supinum, S. pusilla* and *S. herbacea* didn't show any changes in abundance with reference to 2013. *A. pentaphyllea* slightly decreased its coverage of -2% in mean value, while *C. curvula* increased by +7%.

None of these changes at the species level were statistically significant, as tested by ANOVA and Tukey post-hoc test.



Fig. II.4.E.1 - Coverage of the main vegetation layers (total, vascular plant layer, cryptogamic layer) and of the main growth forms (graminoids, forbs, shrubs) in 2013 and 2017 in control plots (OTCc), for each plant communities. Note the different y-axis scales between plots on the top and on the bottom. Dots = means; whiskers = SE.

			(cov_to	tal_%				co	v_vasc	ulars_	%			cov	_crypt	ogams	_%
year	plant comm	Mean	Min	25%	Med	75%	Max	Mean	Min	25%	Med	75%	Max	Mean	Min	25%	Med	75%
2013	CC	94.9	89.5	92.5	94.9	98.3	99.5	72.7	60.0	60.8	74.5	81.3	84.9	62.1	41.5	45.0	62.0	80.5
2017	CC	94.7	90.0	93.0	94.5	98.0	98.0	73.5	62.0	65.0	75.5	80.0	83.0	57.5	42.0	45.0	59.0	70.0
	Diff	-0.3	+0.5	+0.5	-0.4	-0.3	-1.5	+0.8	+2.0	+4.3	+1.0	-1.3	-1.9	-4.6	+0.5	0.0	-3.0	-10.5
2013	SH	92.2	73.0	91.8	95.6	98.3	99.0	79.1	31.3	74.3	88.8	95.5	96.0	47.9	23.3	38.8	47.8	60.8
2017	SH	92.5	73.0	92.0	96.0	99.0	99.0	78.2	34.0	70.0	86.5	96.0	96.0	52.0	32.0	50.0	51.5	55.0
	Diff	0.3	0.0	+0.3	+0.4	+0.8	0.0	-0.9	+2.8	-4.3	-2.3	+0.5	0.0	+4.1	+8.8	+11.3	+3.8	-5.8
			cov	_gram	inoid_	%		_	(cov_fo	rbs_%	, D				cov.shr	ubs_%	, D
year	plant comm	Mean	Min	25%	Med	75%	Max	Mean	Min	25%	Med	75%	Max	Mean	Min	25%	Med	75%
2013	CC	33.8	26.8	31.3	33.0	38.6	40.0	47.1	29.3	40.4	46.5	56.8	63.4	2.2	0.2	0.4	1.5	2.9
2017	CC	42.1	36.3	36.3	41.3	46.6	50.8	45.0	39.0	40.1	43.9	51.4	51.6	2.4	0.8	1.0	1.8	3.0
	Diff	+8.3	+9.4	+5.1	+8.3	+8.0	+10	-2.2	+9.7	-0.3	-2.6	-5.4	-11.8	+0.2	+0.5	+0.6	+0.3	+0.1
2013	SH	9.3	5.0	5.5	8.3	10.8	18.0	66.9	5.6	48.7	77.8	88.1	103.2	12.6	1.8	1.9	9.6	23.0
2017	SH	10.1	4.3	6.0	8.4	12.3	21.3	67.1	5.9	43.1	75.5	89.3	113.5	11.8	2.5	3.0	11.0	18.0
	Diff	0.8	-0.8	+0.5	+0.1	+1.5	+3.2	0.3	0.3	-5.6	-2.3	1.1	+10.3	-0.9	+0.8	+1.1	+1.4	-5.0

Tab. II.4.E.1- Coverage of the main vegetation layers (total, vascular plant layer, cryptogamic layer) and of the main growth forms (graminoids, forbs, shrubs) in 2013 and 2017 in control plots (OTCc), for each plant communities. Bolt values indicate significant (p<0.05) effect.

Snowbeds

CC

SH

Diff

-3

In snowbed communities, in 2013 the mean species number per plots ranged between 9 and 14, with graminoids comprised from 2 to 4 species and forbs from 4 to 10 species. Shrubs, when recorded, were represented in these control plots only by Salix herbacea, how already shown for alpine grassland.

In the re-survey in 2017, the mean species number per plot didn't change.



Fig. II.4.E.2- Coverage of the six most frequent species (P. alpina is here not reported) in 2013 and 2017 in control plots (OTCc), for each plant communities. Note the different y-axis scales between plots on the top and on the bottom. Dots = means; whiskers = SE.

																			_
				Alc	.pen					Gna	.sup					Car	.cur		
year	plant comm	Mean	Min	25%	Med	75%	Max	Mean	Min	25%	Med	75%	Max	Mean	Min	25%	Med	75%	
2013	CC	12	4	7	11	20	20	2	0	0	1	5	5	26	16	20	25	30	
2017	CC	10	4	7	11	12	13	2	0	0	1	5	5	32	16	21	33	45	
	Diff	-2	0	+1	0	-8	-7	0	0	0	0	0	0	+7	0	+1	+8	+15	
2013	SH	55	22	34	62	68	90	2	0	1	1	3	3	2	1	1	1	3	
2017	SH	55	22	35	60	70	88	1	0	1	1	2	3	2	1	1	2	3	
	Diff	0	0	+1	-2	+2	-2	0	0	0	0	-1	0	0	0	0	0	0	
				Leo	.hel					Sal	her					Sol.	pus		
year	plant comm	Mean	Min	0	Mean	Min	Max	Mean	Min	25%	Med	75%	Max	Mean	Min	0	Med	1	
2013	CC	22	15	16	20	27	35	2	0	0	1	3	7	2	0	1	2	3	

2017	SH	2	1	1	2	3	3	12	3	3	11	18	25	16	2	14	18	22
	Diff	0	0	0	+1	0	0	-1	+1	+1	+1	-5	-5	+2	0	+4	+4	+1
Tab. II. (OTCc) and Tul	4.E.2 - (), for ea	Cover ch pla t hoc t	age of nt con	the sinthe sinthe	ix most ties. No	frequone of	ent spe the difi	cies (P. ferences	<i>alpine</i> in me	a is he an val	ere not lues ar	repor e stiss	ted) in tically s	2013 an significa	nd 201 n, as t	7 in co test wit	ontrol th AN	plots OVA

-1

-1

In terms of vegetation layer coverage, in 2013 total coverage (cov_total_%) ranged between 73 and 99% (mean=92%; median=96%), vascular plant coverage (cov_vasculars_%) ranged between 31 and 96% (mean=79%, median=89%) and cryptogams layer ranged between 23 and 69% (mean and median=48%) **Fig. II.4.E.1; Tab.II.4.E.1**).

In 2017, total coverage and vascular layer cover were substantial unchanged, while cryptogams slightly increased by +4% in mean and median values.

Within the main growth forms, in 2013 graminoids (cov.graminoids_%) covered an area comprised between 5 and 18%, far less than the one reported in grassland. Forbs (cov.forbs_%) ranged between 6 and 106% (meaning an overlay of different species) and shrubs (cov.shrubs_%) between 2 to 30%, about three times the values reported for grassland. **Fig. II.4.E.2; Tab.II.4.E.2**). In 2017, all the growth forms didn't change significantly (less than 2%) their covers.

At the species level, in 2013 in snowbeds *A. pentaphyllea* had more than three times the coverage values than the one reported in grasslands, ranging from 22 and 90% (**Tab.10; Fig.29**). Other two characteristic snowbeds species, *S. pusilla* and *S. herbacea*, showed a coverage between 2 and 26% and between 2 and 30%, respectively. *C. curvula*, *G. supinum* and *P. alpina* had a coverage that was less than 5% each.

In 2017, only *S.pusilla* increased its abundance by +2% (+4 in median value), not statistically significant, as testes by ANOVA and Tukey post-hoc test. All the other species showed an absence or a negligible cover changes.

II.4.F. Effect of the manipulation experiments on vegetation composition

In this paragraph, we'll show the changes in species richness and in coverage of main vegetation layers and growth forms that were recorded between year 2013 and 2017 in the manipulated plots, compared to those in control plots (see paragraph II.4.E).

Considering the whole period (2013-2017), a gross total of 47 species, two of those (*Gentiana* sp., *Primula* sp.) not exactly identified, were recorded among all the manipulated and control plots.

The whole pool of species belonged to 17 different plant families (*Apiaceae* (2), *Asteraceae* (7), *Brassicaceae* (2), *Campanulaceae* (2), *Caryophyllaceae* (4), *Crassulaceae* (1), *Cyperaceae* (3), *Ericaceae* (2), *Fabaceae* (1), *Gentianaceae* (1), *Poaceae* (9), *Polygonaceae* (1), *Primulaceae* (3), *Ranunculaceae* (1), *Rosaceae* (4), *Salicaceae* (1), *Scrophulariaceae* (2)), to 7 different growth forms (deciduous dwarf shrub (1), deciduous shrub (1), evergreen dwarf shrub (1), forbs (31), grasses (9), rushes (2), sedges (1); sensu Elmendorf et al., 2012) and 1 cushion plant. All species were well represented in both study sites and in both plant communities.

A list of the identified vascular plant species is reported in **Tab. S2**.

The investigated alpine grassland (CC) and snowbed (SH) plant communities are representative, respectively, of the associations *Caricetum curvulae* (Kerner,1863) Borckm.-Jer.,1907 and *Salicetum herbaceae* Rübel 1911 with dominance of *Alchemilla pentaphyllea*. Both the communities are widespread in the study area (Giacomini and Pignatti, 1955; Cannone et al., 2007) and well summarize the grasslands and snowbeds of the alpine belt for the whole alpine range, according to the Italian vegetation prodrome (Biondi and Blasi, 2013; Biondi et al., 2014; <u>http://www.prodromovegetazioneitalia.org/introduzione</u>).

Considering all the manipulated plots, between 2013 and 2017 was recorded 1 new grass species (*Agrostis schraderana*) and 1 locally extinct forb species (*Primula minima*).

Here below, we'll show the differences in coverage and species richness between manipulated and control plots for each of the experiments. The percentage values reported are the changes in coverage from year 2013 to year 2017, computed as the difference of relative changes between manipulated and control plots.

II.4.F.a. Open Top Chambers (OTCs)

Alpine grasslands

For what concerns the species composition of the OTCs, in grassland were found for the first time the ingression of *A. schraderana*, *Anthoxanthum odoratum*, *Ranunculus montanus* and the disappearance of 2 *Primula minima* and *V. alpina*.

Between 2013 and 2017 the changes in coverage of the main vegetation layers and of the main growth forms were influenced by different parameters (**Tab. II.4.F.1**).

Compared to changes detected in control plots, total coverage (cov_tot_%) increased by +0.7% in mean relative coverage; vascular plants cover (cov_vascular_%) increased by +1.2%. These changes were mainly influenced by treatment (F=6.94), but not by the temporal predictor (year) (Fig. II.4.F.1).

Compared to changes detected in control plots, graminoids (cov_graminoid_%) and forbs (cov_forbs_%) showed a negligible decrease in percentage cover between 2013 and 2017 (-4.2% and -2.3% in relative coverage), mainly related to year of sampling (F=8.75; p=0.01). Shrubs (cov_shrubs_%) decreased significantly by -50%, but we were not able to perform statistical validation because of their low replication in both the treatment (OTC, OTCc) in alpine grasslands (**Fig. II.4.F.2**).

In grasslands, the cryptogamic layer decreased by -26.1% in mean coverage (reference year: 2013), compared to control. Although the change didn't result significant, this was one of the most evident effect of the OTC deployment on the vegetation structure from 2013 and 2017.

			A	Alpine gra	sslands (CC)					
	cov_to	tal_%	cov_vascu	ilars_%	cov_cryptog	ams_%	cov.gramii	noid_%	cov_fo	rbs_%
	F	р	F	р	F	р	F	р	F	р
year	0.00	0.96	0.03	0.87	2.40	0.14	8.75	0.01	0.58	0.46
treatment_name	0.06	0.81	6.94	0.02	0.10	0.75	0.09	0.77	0.98	0.34
year*treatment_name	0.00	0.96	0.07	0.79	1.89	0.19	0.00	0.99	0.03	0.86
				Snowb	eds (SH)					
	cov_to	tal_%	cov_vascu	ılars_%	cov_cryptog	ams_%	cov.gramii	noid_%	cov_fo	rbs_%
	F	р	F	р	F	р	F	р	F	р
year	0.33	0.61	0.25	0.65	0.77	0.45	0.08	0.79	0.22	0.67
treatment_name	0.64	0.48	0.77	0.45	4.06	0.14	0.25	0.65	2.18	0.24
year*treatment_name	0.40	0.57	0.00	0.95	78.62	0.00	0.03	0.87	0.03	0.87

Tab. II.4.F.1 - Summary of two-way ANOVAs for changes in coverage of the main vegetation layers (total, vascular plant layer, cryptogamic layer) and of the main growth forms (graminoids, forbs) in alpine grasslands (CC) and snowbeds (SH) between 2013 and 2017 in OTC experiment. Bolt values indicate significant (p<0.05) effect.

At the species level, *A. pentaphyllea* (-24.8%), *C. curvula* (-8.6%), *G. supinum* (-3.4%), *S. herbacea* (-50%) and *S. pusilla* (-47.3%) decreased their coverages compared to control plots, while *L. helveticus* (+19.3%) and *P. alpina* (+3.4%) increased. None of this differences resulted statistically significant (p>0.05) (**Fig. II.4.F.3**).

Total plant species richness (sp_n_tot) and intra-growth form species richness (sp_n_graminoids; sp_n_forbs) didn't show any significant change.



Fig. II.4.F.1 – Changes in relative coverage compare to year 2013 of the main vegetation layers (total, vascular plant layer, cryptogamic layer) between 2013 and 2017, computed as difference with the changes found in control plots. Left panels: alpine grasslands (CC); right panels: snowbeds (SH). Dots = means; whiskers = SE.

Snowbeds

For what concerns the species composition of the OTCs, in snowbeds was found for the first time the ingression of *A. odoratum*, *Leontodon helveticus* and *Nardus stricta* in Site 2.

Compared to control plots, total coverage decreased by -9.8% with reference to the value in 2013. Vascular plant cover increased by +10.3%, more than six times the increase in alpine grassland. Graminoids decreased by -3.9%, while forbs increased their abundance by +24.8% and shrubs decreased of -45.5% (Fig. II.4.F.1; Fig. II.4.F.2)

The cryptogamic layer showed a strong decrease of -38.2% and similarly to the outcome in alpine grasslands this is one of the most important changes in vegetation structure detected after 3-4 year of OTC manipulation and it was statistically significant (F=78.62; p<0.001).

In snowbeds, OTC increased the abundance of *A. pentaphyllea* (+28.2%), *L. helveticus* (+48.6%), *C. curvula* (+11.7%), *P. alpina* (+14.7%) and *S. pusilla* (+28.4%) compared to controls, while decreased the abundance of *G. supinum* (-5.5%) and *S. herbacea* (-45.5%). The mean species richness per plots didn't change, as well as for the single growth forms. (**Fig. II.4.F.3**).

II.4.F.b. Precipitation shields (PS)

For what concerns the species composition of the PS experiments (PS, PSv1, PSv2), in 2017 was found for the first time the ingression of *Cardamine resedifolia* (treatment type: PS) in grasslands and of *Sibbaldia procumbens* (PS) in snowbeds of Site 1. *Bistorta vivipara* and *Primula glutinosa* disappeared respectively from grassland of Site 2 (treatment name: PSv2) and snowbed of Site 1 (PS).

Alpine grasslands

In grasslands, between 2013 and 2017 the influence of the different rain exclusion treatments (PS, PSv1, PSv2) compared to control (PSc) on the mean total coverage and on the vascular plant layer coverage are reported in **Tab.II.4.F.2** and in **Fig. II.4.F.1**.

Compared to control plots, total coverage (cov_tot_%) remained almost stable in all the manipulated plots, with a maximum decrease in relative coverage of +1.6% in PSv2. Vascular plants (cov_vasculars_%) increased in all three manipulations, with mean values comprised from +13.6%

				Alpine	grasslands (C	C)				
	cov_to	tal_%	cov_vasci	ılars_%	cov_crypto	gams_%	cov.grami	noid_%	cov_fo	rbs_%
	F	р	F	р	F	р	F	р	F	р
year	0.04	0.85	0.19	0.67	0.05	0.82	1.26	0.28	0.15	0.70
treatment	0.24	0.87	1.49	0.26	3.60	0.04	4.60	0.02	1.35	0.30
year*treatment	0.00	1.00	0.21	0.89	0.38	0.77	0.32	0.81	0.08	0.97
				Sno	wbeds (SH)					
	cov_to	tal_%	cov_vascu	ılars_%	cov_crypto	gams_%	cov.grami	noid_%	cov_fo	rbs_%
	F	р	F	р	F	р	F	р	F	р
year	0.09	0.77	0.94	0.35	0.21	0.65	0.77	0.40	1.68	0.22
treatment	0.55	0.66	0.45	0.72	0.43	0.73	0.36	0.78	0.60	0.62
year*treatment	0.01	1.00	0.24	0.87	0.04	0.99	0.63	0.61	0.03	0.99

Tab. II.4.F.2 - Summary of two-way ANOVAs for changes in coverage of the main vegetation layers (total, vascular plant layer, cryptogamic layer) and of the main growth forms (graminoids, forbs) in alpine grasslands (CC) and snowbeds (SH) between 2013 and 2017 in PS experiment. Bolt values indicate significant (p<0.05) effect.

and +20.0%, while cryptogams decreased by -4.5% underneath the shield (PS) and they increased in all other treatments, especially in PSv1 (+21.9% in relative coverage).

Graminoids (cov_graminoids_%) decreased in PS (-5.7% in relative coverage) and increases in PSv1 (+30.6 %) and PSv2 (+3.2%). Forbs were most influenced by the rain exclusion, with an important decrease of -15.5% in relative coverage. Shrubs increased in all treatments, but with less magnitude in PS (+17.0%), therefore showing a possible soil moisture limitation similar to that of forbs (**Fig. II.4.F.2**)

Of these effects, only the dynamics found for cryptogams and graminoids are statistically related to treatment type (F=3.60 and F=4.60, respectively; p<0.05).

Among species, *A.pentaphyllea* decreased in mean coverage in all the treatments from -8.2% (PSv2) to -14.9% (PS). Differently, *C. curvula* statistically increased in mean coverage in all the treatment from +25.7% (PSv1) to +7.2% (PS) (F=7.23; p<0.001), as well as *S. herbacea* that showed changes comprised from +17% (PS) and +48.9% (PSv2) (F=9.82; p<0.001).

Despite the directions of change were not the same for these three species, the absence of any significant difference in the answer between the treatments could mean that other major factors had influenced the vegetation dynamic from 2013 and 2017. These factors could be due a not well function of the precipitation shields that did not avoid the rain to reach the surface underneath the polycarbonate panels; otherwise could be related to local disturbance due to the summer trampling during the phenological monitoring.

L. helveticus was negatively affected by the warm and dry condition (PS), with a decrease of -17.1% in relative coverage, while it was positively influenced by an increase in water availability (PSv1), with an increase of +17.4%. In PSv2, the change was less evident (+4.9%).

Similarly to *L. helveticus*, also *G. supinum* and *P. alpina* showed a decrease in relative coverage in PS and an increase coverage in PSv1and PSv2, compared to control changes. Differently, *S. pusilla* increased by +17.8% and by +11.0% in PS and PSv1, indicating an absence of any relation in water availability, but decreased by -5.8% in PSv2 (**Fig. II.4.F.3**).

Snowbeds

Concerning the snowbed community, (**Tab.II.4.F.2; Fig. II.4.F.1**), compared to control plots, total coverage decreased from -0.5% to -1.6% in mean value, without any relevant differences between treatments. Vascular plants increased from +11.7 (PS) to +16.3% (PSv1) in relative coverage, while cryptogams decreased from -4.7% (PSv2) to -11.8% (PS). In both cases, we didn't detect any differences among plots.

Within growth forms, in graminoids we found an important increase of +96.8% relative cover in PSv1, while forbs decreased by -5.6% in PS, indicating in both cases a sensibility to soil moisture but more influencing on forbs. Indeed, while in PS plots forbs decreased in coverage, shrubs showed a slightly increase indicating the absence of a drought limiting factor. Shrubs showed an increment in all the treatments, comprised between +45.1% in PSv2 and +83.6%, indicating their enhanced growth in moist conditions (Fig. II.4.F.2).

Among the selected species, in PSv1 we found an important decrease of *A. pentaphyllea* (-17.9% in relative coverage) and an increase of all the other species, up to a +104% in relative coverage in *P. alpina*. In PS, compared to the other treatments, we detected a decrease of *C. curvula* (-27.1%), *A. pentaphyllea* (-17.9%) and *L. helveticus* (-8.6%). (Fig. II.4.F.3).

S. herbacea showed an important increment in all the treatments, but mainly related to the starting condition of the experiment (F=3.89; p<0.05) and not to its temporal change. *G. supinum* remained almost stable in absolute coverage, despite in relative coverage compared to 2013 we found an increase in all the treatments, but without any difference between warm and dry plot (+28.5%) and moist plot (+28.4%).

Changes in species richness and density were extremely limited in all the treatment among the two plant communities.



Fig. II.4.F.2 – Relative cover changes compared to year 2014 of the main growth forms (graminoids, forbs, shrubs) between 2013 and 2017, computed as difference with the changes found in control plots. Left panels: alpine grasslands (CC); right panels: snowbeds (SH). Dots = means; whiskers = SE. Note the different y-axis values between panels.

II.4.F.c. Snowfence (SF)

The snow depth and snowmelt manipulation experiment (SF) was conducted on grasslands in Site 1 only. Changes in species composition were found over the study period. In 2017 were found 3 new species: *A. schraderana* (plot SFN10), *A. odoratum* (SFS06), *A. versicolor* (SFN09, SFN07, SFS09). However, because the influence of the fence was mostly localized to the first three plots for each sides (see paragraph II.4.D.c), more detailed information on the effect on species composition must be looked at the SF+ treatment. Indeed in 2017 in this zone where snow depth was increased and snowmelt delayed, were found *Arenaria biflora*, *Arenaria ciliata*, *Bistorta vivipara*, *Sibbaldia procumbens*, all species well adapted to prolonged snow cover.

Vegetation structure didn't show any difference, as tested by two-way ANOVA (Tab.II.4.F.3).

Compared to the changes found in control plots, total coverage (cov_tot_%) didn't have any appreciable difference in mean values in any of the snow fence (SF) treatments, with a decrease of less than 1% in area with artificially delayed snow melt (SF+) and with natural delayed snow melt on the north side and south side of the fence (SFtN, SFtS).

Vascular plants layer (cov_vasculars_%) decreased by -3.0 % in SF+, while in SFtN and SFtS didn't show any significant change.

Cryptogams (cov_cryptogams_%) showed an increase of +2.3% in SF+, +4.8% in SFtN and +0.8% in SFtS, compared to control plots, with 2013 as reference year.

Among the growth forms, shrubs decreased in all the treatments, with a more intense response in SFfN (-42.6%), while forbs slightly increased in the SF+ (+0.53%) and in SFtS (+1.2%) but decreased in SFtN (-4.3%). Graminoids decreased in SF+ (-4.3%) and SFtN (-1.3%) while increased in SFtS (+8.6%) (**Fig. II.4.F.2**).

In the area with artificially delayed snow melt (SF+), most of the species considered in this analysis decreased their 2013 cover; this species are *C. curvula* (-6.3%), *L. helveticus* (-2.0%), *P. alpina* (-4.6%), *S. herbacea* (-13.9%), *S. pusilla* (-15.4%). Conversely, *A. pentaphyllea* (+0.3%) didn't change in cover, while *G. supinum* (+19.2%) increased (**Fig. II.4.F.3**).

Despite the significant influence of the treatment on *C. curvula* (F=3.92; p<0.05), *S. herbacea* (F=5.13; p<0.001) and *S. pusilla* (F=3.08; p<0.05) cover changes, none of those changes resulted different to control plot, as tested by Tukey post-hoc test. However, within forbs, we found differences among species. The artificially delayed snowmelt, indeed, enhanced the relative coverage of *G. supinum*, but decreased those of all the other species.

The relative coverage of this species increased also in all the other treatments with delayed snow covers, thus confirming its sensitivity to the snow cover permanence.

				Alpine	grasslands (C	C)								
	<u>cov_total_%</u> cov_vasculars_% cov_cryptogams_% cov.graminoid_% cov_for													
	F	р	F	р	F	р	F	р	F	р				
year	0.04	0.85	0.04	0.85	0.51	0.48	2.27	0.14	0.01	0.92				
treatment	3.92	0.01	3.14	0.03	3.05	0.03	2.66	0.05	0.77	0.51				
year*treatment	0.18	0.91	0.14	0.94	0.18	0.91	0.29	0.84	0.16	0.92				

Tab. II.4.F.3- Summary of two-way ANOVAs for changes in coverage of the main vegetation layers (total, vascular plant layer, cryptogamic layer) and of the main growth forms (graminoids, forbs) in alpine grasslands (CC) between 2013 and 2017 in SF experiment. Bolt values indicate significant (p<0.05) effect.



Fig. II.4.F.3 - Changes in relative coverage compare to year 2013 of the species selected for the phenological analyses, computed as difference with the changes found in control plots. Left panels: alpine grasslands (CC); right panels: snowbeds (SH). Dots = means; whiskers = SE. Note the differences in y-axis values. Note the different scales in y-axis values.

II.4.G. Effect of the manipulation experiments on plant phenology

Here are shown the main effects of the manipulative experiments (OTC, PS and SF) on the phenological stages of 4 selected target species, with a particular focus on *L. helveticus* (forb) and *C. curvula* (graminoid). All the analyses are shown at inter-annual and intra-annual level and separately for:

- a. snowmelt and greening: DoY of snowmelt (SM), first shoot (FS) and new leaves (NL);
- b. flowering: DoY of flower bud (FB), peak of main flowering (MF);
- c. seed development and leaf senescence: DoY of seed development (SD), seed dispersal (Sdisp), leaf senescence (LS);
- d. plant height: height (in mm) and DoY of occurrence of the maximal mean and maximum plant height (Hmean, Hmean_DoY; Hmax, Hmax_DoY);
- e. reproductive investment and success: number of reproductive individuals (flowers_n) and reproductive success (rep_succ).

For all the phenological stages, the answer of the treatments compared to control is presented as intratreatment values, as well as differences between manipulated and control plots. For each species, the results from the various experimental approaches (OTCs, PSs, SF) are summarized and compared for all the selected phenological stages.

Concerning the precipitation exclusion experiment (PS), according to the results referring to the abiotic gradients (with special reference to soil moisture) (see **Chaper II.4.D.b**), we selected the two plots with clear and statistically significant differences of soil moisture response and therefore more useful to disentangle the effects of the PS manipulation on plant phenology, in particular PS and PSv1.

As the abiotic results indicated that the polycarbonate cover deployment induced a significant increase in GST in PS (up to $+0.8^{\circ}$ C compared to controls), both in alpine grasslands and in snowbeds, the PS manipulation represents a combined treatment of temperature increase and liquid precipitation/moisture decrease, while PSv1 an increase of liquid precipitation/moisture without any change in GST. Hence, the comparison between phenological answer between OTC and PS will give information on the different impacts of warm (OTC) and of dry and warm (PS) conditions on the phenology of the selected species, allowing to prove or not our first hypothesis (*i*).

According to the results of the abiotic data, the following plots were selected to assess and compare the effect of the different manipulation experiments on plant phenology: PS (dry and warm conditions), PSv1 (moist conditions), OTCc (control plots of OTC, representative of the morphological conditions of PS and PSv1 plots, but not influenced by the precipitation shields vicinity), SF+ (artificially delayed snow melt, due to snow fence); SFtN (naturally delayed snow melt, due to surface topography on the North side of the fence) and SFtS (naturally delayed snow melt, due to surface topography on the South side of the fence).

For the snow fence experiment, the phenological results are considered since the 2015 growing season, the first affected by the winter deployment of the snow fence.

II.4.G.a. Forbs – L. helveticus

II.4.G.a.1. Snowmelt and greening

As the precipitation shields were set every year after the onset of SM, FS and NL, these stages are not shown in the snowmelt and greening part.

Open Top Chambers (OTCs)

At inter-annual level, snowmelt (SM) in control plots (OTCc) ranged between DoY 155 and 182 (median = 163). Among plant communities, SM ranged between 155 and 182 (median: 160) in CC as well as in SH (**Tab. II.4.G.a.1.1**). Overall, there wasn't any difference in SM between OTC and control plots. Among plant communities, differences were found only in SH, with delay of +1 day in CC and of +5 days in SH (**Fig. II.4.G.a.1.1**). The wider delay of OTC in SH was due to the snowmelt in year 2016.



Fig. II.4.G.a.1.1 - Descriptive statistics of snow melt (SM) in warming (OTC) and control (OTCc) plots for the whole study period (2014-2017) *for L. helveticus* at intra-annual level for both the plant communities (CC = alpine grassland; SH = snowbed). Black squares = medians, boxes = 25%-75%, whiskers = min-max.



Fig. II.4.G.a.1.2– View of an Open Top Chambers after an early summer snow fall (year 2017)

				SM					FS					NL		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Mir	25%	Med	75%	Max
all	OTC	155	159	163	173	182	159	163	168	179	184	15	9 163	171	179	188
all	OTCc	155	159	163	173	182	159	163	168	179	184	16) 165	168	179	184
CC	OTC	155	159	160	173	182	159	163	165	179	179	15	163	165	179	184
CC	OTCc	155	159	160	173	182	159	163	165	179	179	16) 163	168	179	184
SH	OTC	155	159	173	179	182	160	165	174	183	184	16) 165	177	183	188
SH	OTCc	155	160	168	174	182	160	163	173	179	184	16) 168	173	179	184

Tab. II.4.G.a.1.1 - Descriptive statistics of snow melt (SM), first shoot (FS) and new leaves (NL) computed in warming (OTC) and control (OTCc) plots of *Leontodon helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

First shoot (FS) in control plots ranged between DoY 159 and 184 (median=168) and NL between 160 and 184 (median=168). Among plant communities, FS ranged between 159 and 179 (median=165) in alpine grasslands (CC) and between 160 and 184 (median=173) in snowbeds (SH). New leaves (NL) ranged between 160 and 184 (median=165) in CC and between 160 and 184 (median=173).



Fig. II.4.G.a.1.3 - Descriptive statistics of first shoot (FS) and new leaves in warming (OTC) and control (OTCc) plots for the whole study period (2014-2017) for *L. helveticus* at intra-annual level for both the plant communities (CC = alpine grassland; SH = snowbed). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

Also for FS, like for SM, there wasn't any difference between OTC and control plots. Among plant communities, in OTC we recorded a delay of +1 day in SH, while in CC it didn't change. In NL we found a +3 days delay in median values, with different responses among plant communities. In CC we found a -3 days advance in median values, while in SH we found a delay of +4 days (**Fig. II.4.G.a.1.3**).

However, none of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test (**Tab. II.G.a.1.1**).



Fig. II.4.G.a.1.4 – Examples of first shoots (FS) of L. helveticus (09/06/2016; DoY 161).

Snow fence (SF)

At inter-annual level, for the period 2015-2017 the snowmelt (SM) in control plots (SFc) ranged between DoY 155 and 173 (median = 160) (**Tab. II.4.G.a.1.2**).

Compared to control, *L. helveticus* had a delay in the snowmelt of +5 days in SF+ and SFtN and of +3 days in SFtS (**Fig. II.4.G.a.1.5**).



Fig. II.4.G.a.1.5 - Descriptive statistics of snow melt (SM) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *L. helveticus*, at intra-annual level. Coloured square = medians, boxes = 25%-75%, whiskers = min-max.

FS and NL in control plots ranged between DoY 160 and 179 (median=168). In all treatments we found a delay of FS onset of +1 day in SF+ and SFtN and of +7 days in SFtS. With the NL, in the artificially delayed treatments (SF+) we still found the highest delay of +5 days, while in SFtN it was only of +1 day and in SFtS we already reported an advance of -3 days compared to controls (**Fig. II.4.G.a.1.6**).



Fig. II.4.G.a.1.6 - Descriptive statistics of first shoot (FS) and new leaves (NL) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *L. helveticus*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

				SM					FS					NL		
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
2015-2017	SFtN	159	159	165	173	173	163	165	169	179	179	163	168	169	179	179
2015-2017	SF+	160	160	165	179	183	163	165	169	183	188	165	168	173	183	188
2015-2017	SFtS	155	160	163	173	173	160	163	175	179	179	160	163	165	179	179
2015-2017	SFc	155	155	160	173	173	160	163	168	179	179	160	163	168	179	179

Tab. II.4.G.a.1.2 - Descriptive statistics of snow melt (SM), first shoot (FS) and new leaves (NL) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *L. helveticus*, at inter-annual level since the growing season 2015.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test.

Comparison of manipulation effects on snowmelt and greening – L. helveticus synthesis

The experiments deployment over the period 2014-2017 induced different responses in the phenological stages of the snowmelt and greening period of *L. helveticus*.

Snowmelt (SM) showed a high inter-annual variability that copied the natural variability in snowfalls reported over the study period, with a natural delay SM in 2014 and 2016 and an early ones in 2015 and 2017. Within this wide variability, our data show that the OTC induced only a marginal or a null effect on the SM. Only an occasional delay in OTC in snowbeds was found in year 2016. For this reason, all the subsequent influences of OTC on phenological stages can be considered due to the

warming only. Unlike the OTC, the snow fence (SF) deployment induced important differences compared to control plots both on the snowmelt as well as on the greening stages.

As the OTC did not influence SM, the first shoot (FS) followed strictly the natural snowmelt pattern. It was related to the natural variability of snow falls and snowmelt dates, as confirmed by the generally low differences in the first shoot appearance between warming and control plots and by the permanence of the natural differences already found in SM, both at inter- and intra-community levels. Therefore, OTC (warming) apparently did not affect this phenological stage. On the contrary, the SF experiment induced a delay in FS in all treatments (SF+, SFtS, SFtN).

Warming exerted different effects on new leaves (NL) due to the prevalence of the species-specific ecological requirements over warming alone on the early stages, with an advance in the alpine grasslands and a delay in the snowbeds. The SF treatment shown the importance of topography and aspect in the maintaining or recovering the differences in the greening stages after a delayed snowmelt. Indeed, on the south-exposed side of the stakes line, *L. helveticus* showed an advance compared to control plots, while the north-exposed side still had a delay, as well as the artificially delayed plots.

The maximum delays in FS and NL were found in SF+ treatment, indicating a lower ability of this species to cope with an artificial (SF+) rather than natural delay (SFtN, SFtS) of snowmelt.

II.4.G.a.2. Flowering

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the day of first appearance of flower bud (FB) of *L*. *helveticus* in control plots ranged between DoY 165 and 204 (median=182). In alpine grasslands (CC) it ranged between 165 and 196 (median=178), while in snowbeds (SH) between 169 and 204 (median=179) (**Tab. II.4.G.a.2.1**).

Overall, in OTC we found a -9 days advance in median values of FB. Among plant communities, the advance of *L. helveticus* was more evident in CC than in SH, with a shift of -10 and -6 days respectively (**Fig. II.4.G.a.2.1**). At the intra-annual level, FB advanced in all the years of study in SH, whereas it advanced only in warm and dry years (2015, 2017) in CC.

The peak of the flowering (MF) in control plots was registered between DoY 197 and 240 (median=240). In CC it spanned from DoY 197 to 240 (median=206), in SH from DoY 197 to 240 (median=213) (**Tab. II.G.a.2.1**).

Overall, in OTC we recorded an earlier MF of -12 days in median values, with a different response among plant communities, with an advance of -8 days in CC, and a delay of +3 days in SH (**Fig. II.4.G.a.2.1**).

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test, because of the wide differences between years of study, especially in CC, due the wide climatic variability reported in the study period. Indeed in years with general advance of snowmelt (2015, 2017), even if the medians of FB were the same in OTC and OTCc, part of the OTCs population responded earlier compared to control. This featured was not found in the further phenological stage (MF), except for 2017 in CC and SH and for 2015 for CC, but with reduced differences between OTC and control compared to those found here in FB.



Fig. II.4.G.a.2.1 - Descriptive statistics of flower bud (FB) and peak of flowering (MF) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max. Note the different scales in y-axis values.

				FB					MF		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	OTC	160	165	173	193	204	190	197	198	221	235
all	OTCc	165	169	182	193	204	197	201	210	225	240
CC	OTC	160	164	168	193	204	195	197	198	221	235
CC	OTCc	165	168	178	193	196	197	201	206	225	240
SH	OTC	160	165	179	188	196	190	195	216	230	235
SH	OTCc	169	173	185	194	204	197	202	213	228	240

Tab. II.4.G.a.2.1 - Descriptive statistics of flower bud (FB) and peak flowering (MF) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Precipitation Shields (PSs)

During the four years of study (2014-2017), the day of first appearance of flower bud (FB) in control plots ranged between DoY 165 and 193 (median=173). In alpine grasslands (CC) it ranged between 165 and 193 (median=173), while in snowbeds (SH) between 165 and 193 (median=173) (**Tab. II.4.G.a.2.2**).

The data shown a general delay in FB compared to control plots, but with differences between plant communities. The most evident effect was in PS where the FB medians showed a delay of +11 days, a pattern evident for both CC (PS: +10 days) and SH (PS: +14 days). However, in the former most of the signal was due to the important delay in PS in year 2017, while in the latter we found a constant delay (more pronounced in year 2014). PSv1 didn't have appreciable differences compared to controls in CC, while it delayed as well in SH (+5 days), despite no differences were found in 2014 and 2016 (**Fig. II.G.a.2.2**). Overall, under these experimental drivers, FB of *L. helveticus* was more sensitive to treatment in snowbeds than in alpine grasslands.



Fig. II.4.G.a.2.2- Descriptive statistics of flower bud (FB) and peak of flowering (MF) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *L. helveticus* at intra-annual and intraplant communities levels (CC = alpine grassland; SH = snowbed). Coloured square = medians, boxes = 25%-75%, whiskers = min-max. Note the different scales in y-axis values.

The peak of the flowering (MF) in control plots was registered between DoY 197 and 240 (median=210). In CC it spanned from DoY 197 to 235 (median=210), in SH from DoY 197 to 240 (median=210).

Comparing treatments to control plots, an advance of -2 days in MF was found in PS, while a delay of +1 was found in PSv1. Among plant communities, in PS the MF was delayed by +1 day in CC but advanced by -2 days in SH, underlying how the same species answers differently in different plant communities. In plots PSv1 we found ad advance of -4 days in CC, while a delay up to +11 days was detected in SH (**Fig. II.4.G.a.2.2**).

				FB						MF		
plant	treat	Min	25%	Med	75%	Max	•	Min	25%	Med	75%	Max
all	PS	160	173	184	193	204	_	197	201	208	228	235
all	PSv1	160	173	176	188	193		197	206	211	230	245
all	OTCc	165	169	173	188	193		197	204	210	221	240
CC	PS	165	168	183	188	193		197	197	211	230	235
CC	PSv1	165	165	173	193	193		197	204	206	225	240
CC	OTCc	165	165	173	193	193		197	201	210	221	235
SH	PS	160	173	187	196	204		197	201	208	221	235
SH	PSv1	160	173	178	188	193		197	206	221	230	245
SH	OTCc	165	169	173	188	193		197	204	210	230	240

Tab. II.4.G.a.2.2 - Descriptive statistics of flower bud (FB) and peak of flowering (MF) computed *in dry+warm (PS), moist (PSv1) and control (OTCc) plots* of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test.

Snow fence (SF)

Between the growing seasons 2015 and 2017, the day of first appearance of flower bud (FB) in control plots ranged between DoY 160 and 188 (median=175) (**Tab. II.4.G.a.2.3**). The species didn't show any difference between control and treatments (**Fig. II.4.G.a.2.3**), indicating a complete recovery



Fig. II.4.G.a.2.3 - Descriptive statistics of flower bud (FB) and peak of flowering (MF) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *L. helveticus*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max. Note the different scales in y-axis values.

after the delayed snowmelt or a flower bud pre-formation, as documented for many arctic and alpine species.

However, except for 2016 (when all the treatments showed the same median values), a wide interannual variability was found between treatments, related to the different snowmelt patterns.

The peak of the flowering (MF) in control plots was registered between DoY 197 and 221 (median=202). SF+ was the only treatment that still presented a delay compared to control plots (+4 days), showing how the influence of an artificially induced delayed snowmelt was extended up to the main flowering of this species (**Fig. II.4.G.a.2.3**). This was particularly evident in years 2015 and 2017, characterized by a general early snowmelt and by a warm and dry summer months (July in 2015; August in 2017).

None of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test.

				FB						MF		
year	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
2015-2017	SFtN	165	173	175	188	198	-	197	201	202	216	221
2015-2017	SF+	165	169	175	188	193		197	201	206	221	225
2015-2017	SFtS	163	168	175	188	193		197	201	202	221	221
2015-2017	SFc	160	168	175	183	188		197	197	202	212	221

Tab. II.4.G.a.2.3 - Descriptive statistics flower bud (FB) and peak of flowering (MF) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *L. helveticus*, at inter-annual level since the growing season 2015.

Comparison of manipulation effects on flowering – L. helveticus synthesis

Over the study period we found a wide natural variability also in the flowering phenological stages of *L. helveticus*, indicating a strong effect of the inter-annual variability of the climatic conditions, how already reported for the greening stages.

The OTC deployment induced a general advance of the phenological stages of the flowering period, much more evident than on the greening stages. Differences among plant communities were still detected, with a clear advance of flower bud (FB) and main flowering (MF) in both plant communities, but more evident in the alpine grasslands. The only exception was a delay (+3 days) in MF observed in snowbeds, confirming the influence of the vegetation community on this stage.

While the warming alone (OTC) induced an advance in flowering stages, the combination of heat and drought (PS) induced a delay of FB formation in both plant communities (up to +14 days in snowbeds), showing a drought sensitivity on this stage. On the contrary, the PS treatment induced only a smaller change in the MF, differently from the OTC.

The increased soil moisture (PSv1) generally induced a delay in FB onset, but with differences between plants communities: a delay in snowbeds (+5 days) and a no change in alpine grasslands. Then, in the alpine grasslands this species advanced the MF in moist but not in dry and warm conditions, while in snowbeds it did the opposite, underlining the occurrence also of other driving factors (such as starting moisture conditions, which are higher in the snowbeds than in grasslands, species composition and resources competition).

The earlier onset of FB and MF (and later up to the seed development stages) in OTC rather than in PS, showed how warming (OTC) was able to advance more than warming plus drought (PS) the flowering stages of this species, further emphasizing the influence of soil moisture on these stages.

The influence of the snow fence (SF) deployment found in the greening stages (FS, NL) was lost in the early flower bud (FB). Indeed, in all the treatments with natural (SFtN, SFtS) or artificial delayed snowmelt (SF+) the flower bud onset was not delayed compared to controls, indicating the possible influences of photoperiod or of other genotypic features, as well as the occurrence of flower bud preformation.

In the snow fence experiment, the possible influence of an enhanced soil moisture seemed to be overcame by the delay in the begin of growing season. Indeed, if for FB we found an agreement of response between moist (PSv1) and delayed snowmelt conditions (SF+, SFtN, SFtS), for MF the delay in snowmelt induced a no change (in SFtN and SFtS) or a delay as well (in SF+), while in moist plots (PSv1) it advanced. These findings confirmed how after the complete snowmelt an increase of water availability (due to the precipitation shields) could be a driver to regulate the main flowering time of *L. helveticus*.

In summary, FB and MF were more affected by warming and only secondarily by soil moisture, despite in snowbeds the MF pattern in the PS experiment needs further years of monitoring to better understand the influence of moisture on this species. The snowmelt affected more MF than FB, but only in SF+.



Fig. II.4.G.a.2.4 - Main flowering (MF) of *Leontodon helveticus*, western cirque of Mt. Scorluzzo; on the background: Mt. Radisca (2967 m asl), Mt. Braulio (2979 m asl) and Bocchetta di Forcola (2768 masl) on the hydrographic right side of the Braulio Valley.

II.4.G.a.3 - Seed development and leaf senescence

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the first day of seed development (SD) in control plots ranged between DoY 201 and 247 (median=219). In alpine grassland (CC) it spanned from DoY 201 to 247 (median=214), in snowbeds (SH) from DoY 201 to 247 (median=225) (**Tab. II.4.G.a.3.1**).

Overall, in OTC we recorded an earlier SD of -13 days in median values; among plant communities a strong advance of -12 days in CC and a delay of +1 day in SH. However, in contrast to the interannual level, at the intra-annual level SD advanced in all years of study (with enhanced magnitude in the snowbeds), except for 2015 where OTC and OTCc had comparable values; moreover, in alpine grassland the species reported an unexpected delay in 2014, an year characterized by a particularly cold summer (**Fig. II.4.G.a.3.1**).

Seed dispersal (Sdisp) in control plots ranged between DoY 208 and 272 (median=234). In CC it spanned from DoY 214 to 267 (median=234), in SH from DoY 223 to 272 (median=247) (**Tab. II.G.a.3.1**).

Overall, in OTC we did not record any difference in median values. Similarly to what we found in SD, *L. helveticus* showed diverse responses among plant communities: a strong advance of -10 days median values in CC and a slight delay of +2 days in SH (**Fig. II.4.G.a.3.1**). However, looking at the intra-annual level it was visible a clear advance of Sdisp in OTC, except for years 2014 and 2017 when in CC and SH, respectively, the OTCs had a much wider variance than control plots, due to the lower number of individuals of this stage for those years and communities, underlining the negative effect of both cool year (2014) as well as warm and dry year (2017) on SD.



Fig. II.4.G.a.3.1 - Descriptive statistics of seed development (SD) and seed dispersal (Sdisp) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

Leaf senescence (LS) in control plots ranged between DoY 187 to 247 (median=212). In CC it spanned from DoY 187 to 247 (median=218), in SH from DoY 208 to 247 (median=222) (**Tab. II.4.G.a.3.1**).



Fig. II.4.G.a.3.2 - Descriptive statistics of leaf senescence (LS) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max. Note the different scales in y-axis values.

Differently to all the previous phenological stages, in OTC we recorded a delay of leaf senescence (LS) of +1 day in median values. Among plant communities, CC didn't show any difference in median values compared to controls, while in SH the shift was stronger, up to +4 days (**Fig. II.4.G.a.3.2**), indicating the positive influence of the warming on the length of the growing season, particularly evident in both communities in year 2014. However, within the study period, also advances of LS were reported for 2015 (extreme year; warm and dry summer) and 2017 (warm) in CC and 2016 and 2017 in SH, underlining once more the occurrence of a variability between years and plant communities. The earlier LS in warm and dry years (2015, 2017) in CC indicated a conservative strategy of the species, similar to the one found in woodlands species.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test (**Tab. II.4.G.a.3.1**).

				SD					Sdisp					LS		
plant	treat	Min	25%	Med	75%	Max	Mir	25%	Med	75%	Max	Mi	n 25%	Med	75%	Max
all	OTC	198	201	206	230	243	208	216	234	253	272	18	7 204	213	230	263
all	OTCc	201	204	219	234	247	214	223	234	260	272	18	7 210	212	225	247
CC	OTC	198	201	202	230	243	208	214	224	253	272	18	7 203	211	223	263
CC	OTCc	201	201	214	234	247	214	223	234	260	267	18	7 203	211	218	247
SH	OTC	201	201	225	234	243	214	223	249	255	263	19	8 211	226	247	255
SH	OTCc	201	209	225	241	247	223	225	247	266	272	20	3 211	222	239	247

Tab. II.4.G.a.3.4 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaves senescence (LS) cmputed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Precipitation Shields (PSs)

During the four years of study (2014-2017), the first day of seed development (SD) in control plots ranged between DoY 201 and 247 (median=219). In alpine grasslands (CC) it spanned from DoY 201 to 243 (median=219), in snowbeds (SH) from DoY 201 to 247 (median=222) (**Tab. II.4.G.a.3.2**). Overall, SD of *L. helveticus* shifted of -1 day in PSv1 only. The species didn't show a clear pattern, with contrasting effect on SD between the two communities. In CC, at the inter-annual level it delayed by +3 days in PS and advanced by -5 days in PSv1; however, looking at the intra-annual patterns, there was a delay in both PS and PSv1, especially in years 2014 and 2016, characterized by late snowmelt and cool and wet summers. Otherwise in SH it advanced by -3 days in PS but delayed by +5 days in PSv1 (**Fig. II.G.a.3.3**), with intra-annual patterns complementary to grasslands, except for year 2017.

Seed dispersal (Sdisp) in control plots ranged between DoY 218 and 273 (median=239). In CC it spanned from DoY 218 to 263 (median=234), in SH from DoY 218 to 273 (median=244). Overall, Sdisp didn't change compared to control plots in PS while it delayed by +5 days in PSv1.



Fig. II.4.G.a.3.3 - Descriptive statistics of seed development (SD) and seed dispersal (Sdisp) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *L. helveticus* at intra-annual and intraplant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

Among plant communities, we found a strong delay in all treatments in CC, pattern confirmed for all

the years of study except 2015 and 2017, when in PS advanced. Differently, we found an earlier Sdisp in all treatments in SH (**Fig. II.4.G.a.3.3**), but mainly related to a particular earlier value in PS in year 2016. Interestingly, in 2017 no differences and neither variance were found between and within treatments, probably because of the lower replication (3 of 6 replicates available in snowbeds for this year) of the species in this year characterized by warm and dry August.

Leaf senescence (LS) in control plots ranged between DoY 183 to 247 (median=212). In CC it spanned from DoY 195 to 247 (median=218), in SH from DoY 183 to 247 (median=211). Compared to control plots, this phenophase was delayed by +2 days in PS and by +4 days in PSv1. Among plant communities, in CC we saw an advance in all the plots: -4 days in PS and -7 days in PSv1. In SH we found a delay of +1 day in PS and of +5 days in PSv1 (**Fig. II.4.G.a.3.4**). However, differences at intra-annual level were reported for each of the treatments. In alpine grasslands LS was particularly delayed (see 75-perc) in PSv1 in years 2014 and 2016, while in snowbeds it was only in 2016, in both PSv1 and control plots.



Fig. II.4.G.a.3.4 - Descriptive statistics of leaf senescence (LS) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *L. helveticus* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

		SD							Sdisp			LS					
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	
all	PS	201	204	219	239	247	214	226	239	260	272	198	208	213	219	247	
all	PSv1	201	214	218	239	247	214	229	244	267	273	198	208	215	240	272	
all	OTCc	201	208	219	232	247	218	227	239	254	273	183	206	212	225	247	
CC	PS	201	204	222	239	247	214	223	244	260	272	198	206	214	221	247	
CC	PSv1	201	201	214	239	247	214	223	249	267	273	198	202	211	240	272	
CC	OTCc	201	204	219	230	243	218	223	234	249	263	195	202	218	225	247	
SH	PS	201	204	219	230	247	218	234	239	260	272	202	208	212	218	247	
SH	PSv1	201	214	227	240	247	218	234	239	267	273	206	208	216	245	255	
SH	OTCc	201	211	222	240	247	218	230	244	263	273	183	206	211	240	247	

Tab. II.4.G.a.3.2 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaf senescence (LS) computed in drought (PS), moist (PSv1), mesic (PSv2) and control (PSc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test. *Snow fence (SF)* During the three years of snow fence deployment (2015-2017), the first day of seed development (SD) of *L.helveticus* in control plots ranged between DoY 201 and 230 (median=206). SD was delayed in all the treatments of +4 days, compared to controls (**Tab. II.4.G.a.3.4**), but with different magnitude and directions within the years of study.



Fig. II.4.G.a.3.5 - Descriptive statistics of seed development (SD) and seed dispersal (Sdisp) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *L. helveticus*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

Seed dispersal (Sdisp) in control plots ranged between DoY 211 and 260 (median=229). Unlike SD, Sdisp didn't show differences with control plots in all of the treatment types. For *L. helveticus* this was the first stage for which we reported the recovery of the starting delay of the snow melt time (**Fig. II.4.G.a.3.5**), but it must be noted, as reported for the previous stage, how also in Sdisp some differences were visible within the same treatment between years of study.



Fig. II.4.G.a.3.6 – View of from south-west of Mt. Scorluzzo at the end of growing season (03/10/2014; DoY 277)



Fig. II.4.G.a.3.7 - Descriptive statistics of leaf senescence (LS) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *L. helveticus*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

Leaf senescence (LS) in control plots ranged between DoY 198 to 243 (median=214). Compared to controls, the treatments showed an advanced LS of -2 days in SFtN and of -3 days in SF+ and SFtS, indicating an advanced LS as a consequence of a longer snow cover (**Fig. II.4.G.a.3.7**), but without any difference between natural and induced delays. The advance of LS was particularly visible in year 2017 and with a less extent in 2015. It must be also noted the very late and simultaneous LS in 2014 (without the snow fence deployment) and the advanced but more variable LS between treatment in 2017.

	SD								Sdisp			LS					
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	
2015-2017	SFtN	201	204	210	225	230	218	223	229	245	253	187	198	212	214	226	
2015-2017	SF+	201	204	210	225	230	214	218	229	253	253	198	203	211	216	221	
2015-2017	SFtS	201	202	210	225	230	211	219	229	245	253	191	198	211	216	221	
2015-2017	SFc	201	204	206	225	230	211	218	229	245	260	198	211	214	218	243	

Tab. II.4.G.a.3.4 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaf senescence (LS) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *L. helveticus*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test.

Comparison of manipulation effects on seed development and leaf senescence -L. helveticus synthesis

Over the study period in *L. helveticus* we found a wide natural variability in seed development and leaf senescence due the inter-annual climatic variability, that led to a lack of statistically significant differences in any of the experiments respect to the control plots.

Despite the results at the inter-annual level and despite some unexpected responses, the OTC deployment advanced seed development (SD) and seed dispersal (Sdisp) in both alpine grasslands and snowbeds.

Soil moisture or drought had a further influences, with different magnitude and direction between plant communities. In alpine grasslands the additional drought over warming (PS) led to a delay of both SD and Sdisp, while the enhanced soil moisture (PSv1) led to an advance of SD and to a delay of Sdisp, indicating a prolonged period of seed ripening. In snowbeds, the response patterns were complementary to grasslands for SD, while for Sdisp the answer of the species was less affected by the drought/moist gradient and probably more related to other local conditions, such as the lower water limitation in snowbeds, irrespective of the treatment types.

The snow fence treatments (SF+, SFtN, SFtS) all showed a delay in SD compared to controls whereas, despite the intra-annual variability, Sdisp didn't change compared to control plots. This showed how a later begin of growing season affected the reproduction of *L. helveticus* up to the seed development and how only at the seed dispersal the species was able to recover the delay, indicating here the influences of other major factors, such as genotypic or phenotypic adaptations or photoperiod.

For the seeds phenological stages (SD, Sdisp) of *L. helveticus* we could therefore highlight how warmer conditions were able to accelerate the conclusion of the reproductive cycle of the species and how drought got more influence only in the more water limited community (grasslands), where both SD and Sdisp were delayed. Without any of these external drivers, the snowmelt was still able to influence SD while other environmental or species-specific factors mainly regulated Sdisp, as found in the snow fence experiment.

At the inter-annual level, leaf senescence (LS) under the OTC deployment was delayed in snowbeds and it didn't change in grasslands, indicating an advantage of individuals those grow in cooler and moister condition (such the snowbeds). In the typical plant community of *L. helveticus*, alpine grasslands, the species showed differential responses between warm and dry summers (2015, 2017: advances) and cool summers (2014, 2016: delay), underlining the negative effect of an excessive warming in extreme years on LS and, therefore, on the end of growing season. In snowbeds, the influences of extreme years were less visible; however, in agreement with alpine grasslands, it was still evident the delay in OTC in year 2014 (cool and wet), indicating a prolonged growing season under warming condition in years without any water shrinkage.

The negative influence of a reduced soil moisture on LS was found also in the precipitation shields experiment. Here, in alpine grasslands the species advanced its leaf senescence in PS (dry and warm) in all years, except for 2017, and delayed in PSv1 (moist) confirming the influence of soil moisture on this phenological stage and showing a conservative strategy similar to the one found for trees species. In snowbeds the influence was less visible, probably because of the higher soil water content usually found in this community compared to grasslands, that allow to go less frequently under moisture shortage.

In the snow fence experiment we found an advance of LS in all the treatments (SF+, SFtN, SFtS) compared to control plots, despite differences between treatments were particularly visible in 2017. However, further years of monitoring are needed to disentangle the influence of snowmelt on the leaf senescence of this species.

We could conclude how under a warmer but no water limited scenario the leaf senescence (LS) of *L*. *helveticus* delays while the opposite occurs when water limitation occurs. In particularly warm and dry summers, the soil moisture gains more importance in regulate this phenophase, with differential responses between plant communities as well. Also the delayed snowmelt is able to influence the end of the growing season of *L. helveticus*, but further years of monitoring are necessary to clarify this point. It must be also noted how LS started earlier than Sdisp and occasionally (year 2017) even before SD, irrespectively to the experiments or treatments.

II.4.G.a.4. Plant height

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the mean plants height (Hmean) of *L. helveticus* in control plots ranged between 10 and 45 mm (median=29). In alpine grasslands (CC) it spanned from 15 to 40 mm (median=30), in snowbeds (SH) from 10 to 45 mm (median=26) (**Tab. II.4.G.a.4.1**). Overall, in OTC we recorded an increase in mean height of +6 mm (21%) in median values compared to control plots. Among plant communities, in terms of median value the increment was of +5 mm (17%) in CC and +11 mm (37%) in SH (**Fig. II.4.G.a.4.1**). The responses were consistent and well visible in all the years, with the most intense increase in 2017 (+43% in CC; +83% in SH).

The maximum plant height (Hmax) in control plots ranged between 70 and 130 mm (median=70). In CC it spanned from 29 to 115 mm (median=70), in SH from 29 to 181 mm (median=85) (**Tab. II.4.G.a.4.1**).

In agreement with Hmean, overall in OTC we recorded an increase in maximum height (Hmax) of +40 mm (+57%) in median values compared to control plots. Among plant communities, mean values



Fig. II.4.G.a.4.1 - Descriptive statistics of the mean height (Hmean) and of the day of achievement of the maximal mean height (Hmean_DoY) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

reported an increase of +30 mm (+43%) in CC and of +35 mm (+41%) in SH (**Fig. II.4.G.a.4.2**). In alpine grasslands, and only to a less extent in snowbeds, was also visible an intra-annual variability, with enhanced maximum height in warm years (2015, 2017), indicating the occurrence of a natural variability over the treatment variability.



Fig. II.4.G.a.4.2 - Descriptive statistics of the maximum height (Hmax) and of the day of achievement of the maximum height (Hmax_DoY) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

				Hmean	Hmax							
plant	treat	Min	25%	Med	75%	Max		Min	25%	Med	75%	Max
all	OTC	22	30	35	40	75		29	73	110	123	181
all	OTCc	10	22	29	32	45		25	33	70	95	130
CC	OTC	30	30	35	40	75		45	60	100	115	175
CC	OTCc	15	23	30	32	40		29	33	70	90	115
SH	OTC	22	30	35	40	50		29	115	120	130	181
SH	OTCc	10	20	26	31	45		25	33	85	102	130

Tab. II.4.G.a.4.1 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

As tested by Tukey post-hoc test (p < 0.05), the difference in Hmean and Hmax between OTC and controls resulted statistically significant at inter- and intra-community levels.

Precipitation Shields (PSs)

During the four years of study (2014-2017), the mean plants height (Hmean) of *L. helveticus* in control plots ranged between 15 and 37 mm (median=29). In alpine grasslands (CC) it spanned from 15 to 35 mm (median=24), in snowbeds (SH) from 20 to 37 mm (median=34) (**Tab. II.4.G.a.4.2**).

Overall, *L. helveticus* increased its mean height by +2 mm(5%) in PS, while it decreased by -4 mm(-12%) in PSv1. We found a difference between CC and SH; Hmean increased in PS and PSv1 in alpine grasslands but it decreased in snowbeds, meaning a possible prevalence of the local conditions over the treatments forcing (**Fig. II.4.G.a.4.3**). However looking at the intra-annual level the variability was higher in snowbeds and looking at the single years it was visible an increase in PS (in 2014, 2015 and 2016) and a decrease in PSv1 (all years).



Fig. II.4.G.a.4.3 - Descriptive statistics of mean height (Hmean) and of the day of achievement of the maximal mean height (Hmean_DoY) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *L. helveticus* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

The maximum plant height (Hmax) in control plots ranged between 30 and 111 mm (median=88). In CC it spanned from 30 to 105 mm (median=69), in SH from 40 to 111 mm (median=90).

Overall, Hmax increased by +10 mm (+11%) in PS and by +3 mm (+3%) in PSv1. Among plant communities, all the treatments in alpine grasslands showed an higher maximum plant height compared to control plots, with the most evident effect reported in PS (+21 mm; 17%). In snowbeds, the differences were of +4 mm (4%) in PS and of -10 mm (-11%) in PSv1 (**Fig. II.4.G.a.4.4**), but with an extremely wide variance in 2017 for both the treatments.

As tested by one-way ANOVA and Tukey post-hoc test, only the differences in Hmean reported for alpine grasslands resulted statistically significant (p < 0.05).



Fig. II.4.G.a.4.4 - Descriptive statistics of maximal height (Hmax) and of the day of achievement of the maximum height (Hmax_DoY) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *L. helveticus* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

				Hmean			Hmax							
plant	treat	Min	25%	Med	75%	Max		Min	25%	Med	75%	Max		
all	PS	15	25	30	35	54		20	40	97	120	150		
all	PSv1	11	20	25	35	45		21	60	90	109	155		
all	OTCc	15	20	29	35	37		30	55	88	100	111		
CC	PS	15	25	28	35	36		35	40	100	120	150		
CC	PSv1	11	20	30	36	45		55	60	90	110	121		
CC	OTCc	15	20	24	29	35		30	55	69	92	105		
SH	PS	15	20	30	42	54		20	36	94	120	135		
SH	PSv1	16	20	25	30	42		21	35	80	109	155		
SH	OTCc	20	28	34	35	37		40	55	90	110	111		

Tab. II.4.G.a.4.2 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), the mean plants height (Hmean) of *L*. *helveticus* in control plots ranged between 11 and 40 mm (median=28). Hmean increased of +2 (5%) mm in SFtN and of +3 mm (11%) in SFtS, while in SF+ it decreased of -3 mm (-11%) (**Tab. II.4.G.a.4.3**).

In areas with natural snow accumulation (SFtN, SFtS) this species grew more and faster than in areas with artificial snow cover increase (SF+), where it took more time to reach a smaller size than in controls, confirming the influence of the topography on the size of the species (**Fig. II.4.G.a.4.5**).

The maximum plant height (Hmax) in control plots ranged between 26 and 130 mm (median=95). It decreased of -8 mm (-8%) in SFtN and of -19 mm in SF+ (-20%), while it increased of +7 mm (7%) in SFtS (**Fig. II.4.G.a.4.5**). Here the inter-annual variability was less visible than for Hmean, but for all the treatments was reported a relative low maximum height in year 2016, more pronounced in SF+.

Comparing Hmax and Hmax_DoY, it is interesting to note how in SF+ the species grew less in size and needed more time to reach that size, indicating a negative influence of a delayed snowmelt on the growth rate of this species or the overcoming effect of the terrain topography on the climatic drivers.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test.

				Hmean	Hmax							
year	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
2015-2017	SFtN	10	20	30	35	40	-	40	55	88	102	105
2015-2017	SF+	18	22	25	36	57		30	32	76	110	130
2015-2017	SFtS	23	30	31	34	40		40	42	102	109	130
2015-2017	SFc	11	19	28	30	40		26	65	95	110	130

Tab. II.4.G.a.4.3 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *L. helveticus*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).



Fig. II.4.G.a.4.5 - Descriptive statistics of mean height (Hmean), maximum height (Hmax) and day of achievement of the maximal mean height (Hmean_DoY) and maximum height (Hmax_DoY) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *L. helveticus*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

Comparison of manipulation effects on plant height – L. helveticus synthesis

The OTC deployment induced evident increases in mean height (Hmean) and maximum height (Hmax) of *L. helveticus*, especially in the snowbeds. Overall, in the OTC we could identify an enhanced and faster growth in CC and an enhanced and prolonged growth in the SH, that both could lead to an increase in size and to an enhanced species performance.

In the precipitation shield experiment, in alpine grasslands (CC) Hmean and Hmax of *L. helveticus* increased in all the treatments. Despite they showed the same direction, here we found a statistically significant lower Hmean in PS (comparable to the value found in OTCs) than in PSv1, showing how a higher moisture in the typical community could support an enhanced growth compared to warming or drought plus warming.

In PSv1 (moist) was observed a prolonged growth with higher soil moisture availability than in PS (dry and warm).
Differently to alpine grasslands, in snowbeds (SH) the Hmean and Hmax decreased in PSv1 plots, underlining how in this community (not the typical for *L. helveticus*) an excessive moisture was not favorable to the growth of the species.

However, compared to controls, the growth was characterized by a lower Hmean in both the treatments (PS, PSv1), with a reduced and shorter growth under an excess of soil moisture. Differently, for what concerns Hmax, the species grew taller (+Hmax) and faster in PS, while it grew shorter (-Hmax) and slower in PSv1.

In the snow manipulation experiment (SF), we found how the species was able to cope with the natural delayed snowmelt, growing more and faster than in artificially delayed snowmelt. Moreover, in the concavity on north and south side of the moraine (SFtN, SFtS), the species grew more than on the windswept ridge, indicating a co-occurrence of a topographic control on the mean plant size. For what concern the maximal plant size, in SFtN the maximum height was lower (-Hmax) and reached earlier compared to control plots, while in SFtS it was higher (+Hmax) and reached later. In agreement with the mean height, in SF+ the species showed also a lower maximum height that was reached later in the season, indicating once again a slow and limited growth capacity under those conditions.

In summary, under a warmer scenario the size (Hmean, Hmax) and the rate of growth (Hmean_DoY, Hmax_DoY) of *L. helveticus* increased, as long as an excess of soil moisture (especially in snowbeds) or of soil moisture shrinkage (especially in alpine grasslands) do not buffer or outcompete this trend. The influence of the snowfence experiment on plant heights seemed to be, at least to our experiment, due to the local topography rather than due to snowmelt. Indeed, under similar snowmelt date, *L. helveticus* grew less and slower in the more exposed plots (SF+; moraine ridge), than in the more sheltered ones (SFtN; SFtS).

II.4.G.a.5. Reproductive investment and success

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the number of reproductive individuals (flowers_n) of *L.helveticus* in control plots was on average 7 (median=3), with a maximum value up to 40. Alpine grasslands (CC) had a mean value of 10 (median=5) and snowbeds (SH) of 3 (median=2), with a maximum value of 40 and 7 individuals, respectively (**Tab. II.4.G.a.5.1**). Because *L. helveticus* is a typical species of the alpine grasslands, its flowers_n was more elevated in CC than in SH, with a maximum value reached in 2015. In 2014 was reported the lowest value of flowers_n, due to the extreme climatic conditions (late snowmelt, cold summer) that characterized that summer.

Overall, in OTC we found a higher number of reproductive individuals (median: +5; +150%) compared to control plots. Among plant communities, in CC the flowers_n increased by +5 (+100%) in median values, while in SH it didn't change (**Fig. II.4.G.a.5.1; Fig.II.4.G.a.5.2**). However, at the intra-annual level, also in SH it was visible an increment of flowers_n, more pronounced in 2016. Moreover, it is interesting to note how in 2015 (warm and dry year) the flowers_n in OTCs was lower than in control plots in both plant communities, indicating a negative effect of an excessive warming in extreme climatic conditions.



Fig. II.4.G.a.5.1 - Descriptive statistics of the number of reproductive individuals (flowers_n) and of the reproductive success (rep_succ) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

The reproductive success (rep_succ) in control plots was on average 92% (median=100), with a maximum of 100%. On average, in CC the rep_succ was 68% (median=83), with a maximum value of 100%, and in SH it was 86% (median=100), with a maximum value of 100%, in SH (**Tab. II.4.G.a.5.1**).

Overall, in OTC the reproductive success increased by +15% in median value compared to control plots. Among plant communities, in CC the increment was of +18%, while in SH it didn't change in median value. This highlights the different reproductive ability of *L. helveticus* within the two main plant communities in a climate warming scenario (**Fig. II.4.G.a.5.1**).

However, none of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test (**Tab. II.G.a.5.1**).

				flowers_1	n				rep_succ		
plant	treat	Min	25%	Med	75%	Max	 Min	25%	Med	75%	Max
all	OTC	1	2	8	13	41	40	94	100	100	100
all	OTCc	1	2	3	7	40	0	64	85	100	100
CC	OTC	2	7	10	17	41	40	88	100	100	100
CC	OTCc	1	3	5	12	40	0	50	83	100	100
SH	OTC	1	2	2	4	13	75	100	100	100	100
SH	OTCc	1	2	2	4	7	43	67	100	100	100

Tab. II.4.G.a.5.1 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).



Fig. II.4.G.a.5.2 – Examples of flowers_n at the peak of flowering in OTC (a) and OTCc (b) in an alpine grassland. Plot size: 1x1 m (red frame).

Precipitation Shields (PSs)

During the four years of study (2014-2017), the number of reproductive individuals (flowers_n) in control plots was on average 13 (median=6), with a maximum value up to 63. Alpine grasslands (CC) had a mean value of 16 (median=11) and snowbeds (SH) of 10 (median=5) as well, with a maximum value of 63 and 36 individuals, respectively (**Tab. II.4.G.a.5.2**). As already reported for the OTC

				flowers_1	1				rep_succ		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	1	2	6	9	34	0	50	100	100	100
all	PSv1	1	2	5	9	30	0	50	100	100	100
all	OTCc	1	3	6	15	63	0	50	76	100	100
CC	PS	1	2	4	8	34	0	50	100	100	100
CC	PSv1	1	1	5	9	16	25	78	100	100	100
CC	OTCc	1	2	11	16	63	0	33	76	100	100
SH	PS	1	2	6	10	32	0	50	100	100	100
SH	PSv1	1	2	5	5	30	0	43	70	100	100
SH	OTCc	2	4	5	15	36	0	50	75	100	100

Tab. II.4.G.a.5.2 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

experiment, in year 2015 we found the highest number of flowers within the study period for all the treatments investigated, while in 2014 we found the lowest value.

Overall, the flowers_n of *L. helveticus* decreased by -9% (-1 n) in PSv1, while no change were found in PS. In alpine grasslands, flowers_n decreased in PS (-64%; -7 n) and PSv1 (-55%; -6 n), while in snowbeds it decreased in PSv1 (-10%; -1 n) and increased in PS (+20%; +1 n) (**Fig. II.4.G.a.5.3**). At the intra-annual level, from 2015 to 2017 in CC and from 2015 and 2016 in SH flowers_n was lower in both PS and PSv1 compared to control plots, underlining the need of further years of study to understand the soil moisture effects on this quantitative variable.

The reproductive success (rep_succ) in control plots was on average 70% (median=76), with a maximum of 100%. On average, in CC the rep_succ was 71% (median=76), with a maximum value of 100%, and in SH it was 70% (median=75), with a maximum value of 100%, in SH.

Compared to control plots, rep_succ increased in all treatments at the inter-communities level and within alpine grasslands, where it showed an increment of more than 20% in both the treatments (+24%). In snowbeds, it increased by +25% in PS, but it slightly decreased by -5% in PSv1 (**Fig. II.4.G.a.5.3**). However, it was not possible to identify similar responses between treatments at the



Fig. II.4.G.a.5.3 - Descriptive statistics of the number of reproductive individuals (flowers_n) and of the reproductive success (rep_succ) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *L. helveticus* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

intra-annual level, indicating how the rep_succ was more affected by the natural climatic and environmental drivers rather than by the experimental treatments.

As tested by one-way ANOVA and Tukey post-hoc test, the differences of flowers_n in alpine grasslands and of rep_succ in snowbeds resulted significantly affected by treatment type (p < 0.05).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), the number of reproductive individuals (flowers_n) of *L. helveticus* in control plots was on average 16 (median=18), with a maximum value up to 32 (**Tab. II.4.G.a.5.3**). Within the years of study, 2015 was the one with the highest flowers_n value in all the snow fence treatments, as already seen for OTC and PS experiments. Compared to control plots, the number decreased of -22% (-4 n) in SFtN and in SF+, while increased of +56% (+10 n) in SFtS (**Fig. II.4.G.a.5.4**). The pattern found at the inter-annual level was maintained for all the years of the snow fence deployment, except in 2016 (year characterized by the latest snowmelt).



Fig. II.4.G.a.5.4 - Descriptive statistics of the number of reproductive individuals (flowers_n) and of the reproductive success (rep_succ) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *L. helveticus*, at intra-annual level. Coloured square = medians, boxes = 25%-75%, whiskers = min-max.

				flowers_	n				rep_succ		
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
2015-2017	SFtN	7	10	14	17	31	57	71	89	97	100
2015-2017	SF+	5	11	14	17	45	79	88	100	100	100
2015-2017	SFtS	5	10	28	30	42	71	100	100	100	100
2015-2017	SFc	3	9	18	22	32	86	100	100	100	100

Tab. II.4.G.a.5.3 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *L. helveticus*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

The reproductive success (rep_succ) in control plots was really high, on average 99% (median=100), with a maximum of 100%. Compared to controls, only SFtN showed a decrease of -11% (**Fig. II.4.G.a.5.4**), visible for all the years of snow fence deployment and in particular for 2016.

As tested by one-way ANOVA and Tukey post-hoc test, only the differences in rep_succ resulted significantly affected by treatment type (p<0.05), with rep_succ in SFtN statistically lower than the others treatments and control.

Comparison of manipulation effects on reproductive investment and success – L. helveticus synthesis

Over the study period, in all the three experiments we were able to find an inter-annual variability in both the number of reproductive individuals (flowers_n) and the reproductive success (rep_succ).

The OTC deployment increased the flowers_n of *L. helveticus* in both alpine grasslands and snowbeds, with the exception of year 2015 (warm and dry), indicating a negative effect of an excessive warming in extreme climatic conditions. OTC increased also the rep_succ that, differently to the previous one, was not negatively affected by the extreme year.

The influence of dry and warm (PS) and moist (PSv1) conditions on flowers_n and rep_succ was not clear and often overcame by other environmental and site-specific factors, which didn't allow to find differences among treatments at the inter-communities level.

In alpine grasslands, we didn't find any treatments effect on these quantitative variables and the absence of any moisture influence. In snowbeds, was reported a different response of flowers_n and rep_succ between treatments, but mainly related to the forcing year 2016 that showed a wide variability compared to the others. Overall, in snowbeds, more data are needed to clarify the role of soil moisture in the reproductive effort and success of *L. helveticus*.

Also the snow manipulation experiment influenced the reproductive investment and success of *L*. *helveticus*. The flowers_n increased where snow accumulated naturally on the south side of the moraine ridge (SFtS) but decreased on the north side (SFtN) and in the artificially delayed snowmelt, indicating a lower attitude and plasticity of this species to a prolonged snow cover and to less exposed sites.

For what concerns the reproductive success (rep_succ), the species was unaffected by the longer snow cover of SF+ and SFtS, but it showed a decrease in SFtN; this underline once more the importance of the topography on other environmental factors and the sensitivity (negative) of *L. helveticus* to less exposed sites for a successful reproduction.

In summary, temperature was the first limiting factor in the reproductive investment and success of *L. helveticus*, with topography and soil moisture as further drivers (especially on the numbers of reproductive individuals). However, in extremely years (such as 2015), under warming condition (OTC) some threshold could be passed, with consecutive negative effects on the two phenological data.

II.4.G.b – Graminoids – C. curvula

II.4.G.b.1. Snowmelt and greening – C. curvula

As the precipitation shields were set every year after the onset of SM, FS and NL, these stages are not shown in the snowmelt and greening part.

Open Top Chambers (OTCs)

At inter-annual level, snowmelt (SM) in control plots ranged between DoY 155 and 183 (median = 173), showing a wide inter-annual variability (**Tab. II.4.G.b.1.1**). Warming plots didn't show any difference compared to control (**Fig. II.4.G.b.1.1**). As reported for *L. helveticus*, also for this species it was evident the inter-annual variability, with a later SM in 2014 and 2016 compared to earlier ones in 2015 and 2017, independently by treatments or community types.



Fig. II.4.G.b.1.1 - Descriptive statistics of snow melt (SM) in warming (OTC) and control (OTCc) plots for the whole study period (2014-2017) for *C. curvula* at intra-annual level for both the plant communities (CC = alpine grassland; SH = snowbed). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

First shoot (FS) in control plots ranged between 159 and 216 (median=176) and new leaves (NL) between 160 and 198 (median=186). Among plant communities, it ranged between 159 and 188 (median=168) in alpine grasslands (CC) and between 168 and 216 (median=183) in snowbeds (SH). Both the greening stages followed the natural snowmelt patters, with later onsets in 2014 and 2016 and earlier onsets in 2015 and 2017 (**Fig. II.4.G.b.1.2**).

At the inter-communities level, warming showed an advance in median values of -3 days in FS and -7 in NL. The observed advances were different among the two plant communities; in CC FS didn't show any difference, while in SH it advanced of -3 days.

NL formation were advanced of -6 days in CC and of -7 days in SH, in agreement with the response of the greening stages of *C. curvula* to warming among plant communities (**Fig. II.4.G.b.1.2**). The earlier NL in OTC was found in all the years of study except for 2016, when in SH it was slightly delayed compared to control plots.

However, none of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test (**Tab. II.4.G.b.1.1**).

Also in this case it was appreciable the large inter-annual climatic variability (well evident for SM), with impacts on the onset dates of both FS and NL, which strictly followed the patterns of SM.



Fig. II.4.G.b.1.2 - Descriptive statistics of first shoot (FS) and new leaves in warming (OTC) and control (OTCc) plots for the whole study period (2014-2017) for *C. curvula* at intra-annual level for both the plant communities (CC = alpine grassland; SH = snowbed). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

				SM					FS			_			NL		
plant	treat	Min	25%	Med	75%	Max	Mir	25%	Med	75%	Max		Min	25%	Med	75%	Max
all	OTC	155	160	171	175	183	159	165	173	180	212		160	168	179	187	198
all	OTCc	155	160	173	175	183	159	165	176	183	216		160	173	186	188	198
CC	OTC	155	159	160	173	182	159	162	168	179	212		160	165	178	184	188
CC	OTCc	155	159	160	173	182	159	165	168	182	188		160	169	184	188	193
SH	OTC	160	168	175	179	183	165	168	180	184	193		165	173	180	188	198
SH	OTCc	160	168	173	179	183	168	173	183	184	216	_	173	180	187	188	198

Tab. II.4.G.b.1.1 - Descriptive statistics of snow melt (SM), first shoot (FS) and new leaves (NL) computed in warming (OTC) and control (OTCc) plots of *Carex curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

At the inter-annual level, for the period 2015-2017 the snow melt (SM) in control plots (SFc) ranged between DoY 155 and 173 (median = 160) (**Tab. II.4.G.b.1.2**).

In all the treatments of the SF experiment (SF+, SFtN, SFtS) we reported a delay compared to controls, of +5 days (SF+, SFtN) and +3 days (SFtS) (**Fig. II.4.G.b.1.3**), the same values already found for *L. helveticus*.



Fig. II.4.G.b.1.3 - Descriptive statistics of snow melt (SM) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *C. curvula*, at intra-annual level. Coloured square = medians, boxes = 25%-75%, whiskers = min-max.

First shoot (FS) in control plots ranged between 160 and 188 (median=168) and new leaves (NL) between 160 and 198 (median=178). Similarly to *L. helveticus*, in this species the FS was delayed by +1 day in SFtN and SF+, but it remained unchanged in SFtS compared to control plots. Since the NL, the delays reported in FS were lost; indeed SFtS didn't have differences compared to control, while in plots with artificially delayed SM (SF+) and in the low area of the north side with natural



Fig. II.4.G.b.1.4 - Descriptive statistics of first shoot (FS) and new leaves (NL) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *C. curvula*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

delayed SM (SFtN) the NL appeared -3 days earlier than in control (**Fig. II.4.G.b.1.4**). However, at the intra-annual level these responses were not verified; indeed only in 2017 was found an earlier NL in SF+, while in 2016 and 2015 it was delayed and comparable to SFc, respectively. Therefore, the influence of the inter-annual variability was higher than the manipulation induced by the treatments.

				SM					FS					NL		
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
2015-2017	SFtN	159	159	165	173	173	165	168	169	183	183	165	173	175	188	193
2015-2017	SF+	160	160	165	179	183	163	165	169	183	188	165	169	175	188	198
2015-2017	SFtS	155	160	163	173	173	160	163	168	179	188	160	173	178	188	193
2015-2017	SFc	155	155	160	173	173	160	163	168	179	188	160	173	178	183	198

Tab. II.4.G.b.1.2. - Descriptive statistics of snowmelt (SM), first shoot (FS) and new leaves (NL) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *C. curvula*, at inter-annual level since the growing season 2015.

However, none of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

Comparison of the manipulation effects on snowmelt and greening – C. curvula synthesis

In agreement with the other species, snowmelt (SM) for *C. curvula* showed a wide inter-annual variability that followed the natural variability in snowfalls; in this frame, the OTCs treatment induced only a marginal or null effect. Also the snow fence (SF) deployment showed the same inter-annual variability, but here the influence of the experiment was clearly visible, with the strongest delays found in SF+, even more pronounced than the ones in SFtN and SFtS.

The greening stages followed the natural variability as well. On this pattern, we found some experiment induced responses. The first shoot (FS) in OTC showed only a slightly advance in both alpine grasslands and snowbeds, while new leaves (NL) was more affected (advanced), especially in 2017. Otherwise, snow fence (SF) deployment seemed to not affect FS and NL, because the responses of these stages didn't follow the SM pattern, differently to what found for *L. helveticus*. Indeed, for *C. curvula* in SF+ and SFtN treatments showed an advance in NL (despite the delayed SM), indicating a fast recovery or otherwise a tissues pre-formation before the snowmelt, as reported for many alpine species.

In summary, the greening stages of *C. curvula* were more affected by warming rather than by snowmelt time, indicating the ability of this species to cope with an artificial (SF+) rather than natural delay (SFtN, SFtS).

II.4.G.b.2. Flowering – C. curvula

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the day of first appearance of flower bud (FB) in control plots ranged between DoY 159 and 193 (median=178). In alpine grasslands (CC) it ranged between 159 and 188 (median=165), while in snowbeds (SH) between 165 and 193 (median=183) (**Tab. II.4.G.b.2.1**), indicating a general delay in SH, independently by the treatment.

Overall, in OTC we didn't find difference in median values, while among plant communities the OTCs showed a +8 days delay in CC and, conversely, a -2 days advance in SH (**Fig. II.4.G.b.2.1**). However, these values were the results of the inter-annual variability that characterized the study period. Otherwise, looking at the intra-annual level it was evident the earlier FB in OTC, particularly visible in SH in 2017.

The peak of the flowering (MF) in control plots was registered between DoY 168 and 219 (median=188). In CC it spanned from DoY 168 to 212 (median=184), in SH from DoY 187 to 219 (median=194) (**Tab. II.4.G.b.2.1**), indicating also for MF a delay in SH (as already reported for FB). At the inter-annual level, in OTC we recorded an earlier MF of only -1 day in median values. Among plant communities, the warming effect was more evident in SH where MF advanced of -7 days, compared to the advance of -2 days in CC. The strongest effect was found in 2017, characterized by an early snowmelt date, and especially in SH. These findings highlighted the importance for the flowering stages of *C. curvula* of warmer conditions in early growing season, when still frost events could occur and damage the reproductive organs.



Fig. II.4.G.b.2.1 - Descriptive statistics of flower bud (FB) and peak of flowering (MF) computed in warming (OTC) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max. Note the different scales in y-axis values.

				FB						MF		
plant	treat	Min	25%	Med	75%	Max		Min	25%	Med	75%	Max
all	OTC	159	163	178	182	188	-	168	178	187	193	212
all	OTCc	159	165	178	184	193		168	180	188	203	219
CC	OTC	159	160	171	179	184		168	180	182	188	212
CC	OTCc	159	163	165	183	188		168	173	184	193	212
SH	OTC	163	168	182	184	188		173	178	187	193	212
SH	OTCc	165	178	183	184	193		187	187	194	206	219

Tab. II.4.G.b.2.1 - Descriptive statistics of flower bud (FB), begin of peak flowering (MF_beg), peak flowering (MF) computed in warming (OTC) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

However, none of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

Precipitation Shields (PSs)

During the four years of study (2014-2017), the day of first appearance of flower bud (FB) in control plots ranged between DoY 163 and 184 (median=168). In alpine grasslands (CC) it ranged between 163 and 184 (median=168), while in snowbeds (SH) between 163 and 184 (median=176) (**Tab. II.4.G.b.2.2**), showing a similar response range between communities.

At the inter-community level we recorded a delay in FB that was of +1 day in PS and +9 days in PSv1 (**Fig. II.4.G.b.2.3**), but different results came from the intra-annual level. Indeed, except for the general delays in 2014 and 2016 and the advances in 2015 and 2017 (related to the snowmelt variability) it was not visible a clear treatments pattern, neither in CC nor in SH. It must be also noted the lack of flowering individuals in PSv1 of snowbeds in 2017 (as reported by field surveys), that could have affected the responses found at the inter-annual level. Overall, for the FB it was well visible an inter-annual variability, much wider and clear than the effect generated by the PS deployment (**Fig. II.4.G.b.2.3**).



Fig. II.4.G.b.2.2 – Example of *Caricetum curvulae*; in the backgroung the Stelvio pass (2757 m asl) and Mt. Ortles (3905 masl)



Fig. II.4.G.b.2.3- Descriptive statistics of flower bud (FB) and peak of flowering (MF) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *C. curvula* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured square = medians, boxes = 25%-75%, whiskers = min-max. Note the different scales in y-axis values

The peak of the flowering (MF) in control plots was registered between DoY 173 and 212 (median=186). As well, in CC it spanned from DoY 173 to 212 (median=187), in SH from DoY 173 to 212 (median=184).

At the inter-communities level, we recorded a similar pattern of FB: in PS the phenopase advanced by -2 days while in PSv1 it delayed by +5 days, but similarly to what found for FB, the results at the intra-annual level did not highlight any clear response among treatments (**Fig. II.4.G.b.2.2**), especially in years 2017 (CC) and 2016 (SH) when all the treatments showed the same MF values.

None of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

				FB					MF		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	160	163	169	183	188	173	175	184	193	212
all	PSv1	160	165	177	183	184	173	184	190	193	212
all	OTCc	163	165	168	183	184	173	178	186	193	212
CC	PS	163	163	165	183	184	173	173	184	193	212
CC	PSv1	163	163	169	183	184	173	173	184	193	208
CC	OTCc	163	165	168	179	184	173	173	187	193	212
SH	PS	160	164	171	186	188	173	178	184	193	212
SH	PSv1	160	170	181	184	184	184	187	193	201	212
SH	OTCc	163	165	176	183	184	173	178	184	193	212

Tab. II.4.G.b.2.2 - Descriptive statistics of flower bud (FB), begin of peak flowering (MF_beg), peak flowering (MF) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

In *C. curvula* the day of first appearance of flower bud (FB) in control plots ranged between DoY 160 and 183 (median=172), with later onsets in 2014 and 2016 and earlier in 2015 and 2017 (**Tab. II.4.G.b.2.3**). Moving in north-south direction, FB exhibited differences compared to control plots. Indeed, FB advanced by -3 days in SFtN, while it delayed by +1 day in SF+ and by +4 days in SFtS (**Fig. II.4.G.b.2.4**). The pattern was not maintained at the intra-annual level, where a more pronounced influence of SF+ was detected, with a delayed FB particularly appreciable with a later begin of the growing season (2016).

The peak of the flowering (MF) in control plots was registered between DoY 173 and 212 (mean=188; median=184). For this stage, we found a complete recovery in SFtN that didn't show any difference of MF compared to controls. Moreover, in SF+ and SFtS we found an advance in MF, indicating here the overcoming influence of the micro-topography over the treatment types to reach the main flowering. In the most exposed side (SFtS) and on the top of the moraine ridge (SF+) we registered the advance of MF, while in the less exposed side (SFtN) we reported a no change compared to control (**Fig. II.4.G.b.2.4**).



Fig. II.4.G.b.2.4 - Descriptive statistics of flower bud (FB) and peak of flowering (MF) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *C. curvula*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max. Note the different scales in y-axis values.

In 2016, the absence of variance of MF in all treatments SFtN, SFtS, SF+ and SFc, indicated by the same median values, was an evidence that the main flowering occurred contemporary, despite the differences in snow melt at the begin of the season. Other factors (such as photoperiod) could have led the time of the main flowering in this year (2016). Therefore *C. curvula* was able to recover the delayed snowmelt at the peak of flowering.

None of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

				FB						MF		
year	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
2015-2017	SFtN	168	168	169	179	179	-	173	173	184	193	193
2015-2017	SF+	163	167	172	183	188		173	178	182	193	193
2015-2017	SFtS	165	165	175	179	179		168	178	180	193	193
2015-2017	SFc	160	163	172	179	183		168	178	184	193	193

Tab. II.4.G.b.2.3 - Descriptive statistics flower bud (FB), begin of peak flowering (MF_beg) and main flowering (MF) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *C. curvula*, at interannual level since the growing season 2015.

Comparison of manipulation effects on flowering – C. curvula synthesis

The OTC deployment induced an advance of both flowers bud (FB) and peak of flowering (MF). These changes were particularly visible in year with early snowmelt (such the 2017), underlining the sensitivity of the reproductive organs of the species to the early summer cold temperatures and frost events.

On the contrary, the PS deployment did not generate any differential responses in FB and MF between treatments, in none of the studied plant communities. However, comparing with OTCs, from our results we can suppose the negative influence of drought on the flowering stages onset (especially FB), as in PS (dry and warm) we did not detect the advancement found in OTC (warm only). At the same time, the increased soil moisture availability without any warming did not produce any changes, as confirmed by the similar dates of MF in PSv1 and control plots.

The effect of the snow fence (SF) deployment was visible on FB (especially in 2017) but not on MF, in disagreement with what found for *L. helveticus*, indicating a weakening influence of the snowmelt date on the flowering stages of this graminoid and the extended influence of other factors, such as photoperiod or terrain morphology. Indeed, the best exposed plots (south side of the moraine ridge) recovered the delay faster than the worst exposed plots (north side of the moraine ridge), underlining the increased influence of the topography along the growing season.

In summary within the natural inter-annual variability, the flowering stages of *C. curvula* were mainly affected by warming, rather than soil moisture or snowmelt time, but also with an increased influence of the topography.

II.4.G.b.3 - Seed development and leaf senescence – C. curvula

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the first day of seed development (SD) in control plots ranged between DoY 178 and 247 (median=200). In alpine grassland (CC) it spanned from DoY 178 to 208 (median=197), in snowbeds (SH) from DoY 190 to 247 (median=203) (**Tab. II.4.G.b.3.1**). This indicated a wide natural variability, found also in the further seed dispersal and particularly visible in snowbeds, that followed the inter-annual climatic variability: later SD with delayed snow melt seasons (2014, 2016), earlier SD with advanced snowmelt seasons (2015, 2017). Overall, in OTC we recorded an earlier SD of -6 days in median values. Among plant communities, advancement was of -7 days and -6 days in CC and SH, respectively, despite the comparable values found in OTC and control plots at the intra-annual level (year 2014).

Seed dispersal (Sdisp) in control plots ranged between DoY 234 and 280 (median=247). In CC it spanned from DoY 234 to 280 (median=247), in SH from DoY 243 to 280 (median=272) (**Tab. II.4.G.b.3.1**). Here it is important to note the lack of this stage in OTCc of SH in year 2017, despite the occurrence of the previous flowering stages and of SD.



Fig. II.4.G.b.3.1 - Descriptive statistics of seed development (SD) and seed dispersal (Sdisp) computed in warming (OTC) and control (OTCc) plots of *L. helveticus* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max. Note the different scales in y-axis values.

Overall, in OTC we recorded a delay of Sdisp of +3 days, but with effects less marked than on SD. Among plant communities, in CC we found a delay of +3 days in median values, despite the diverse answers reported for 2014-2015 (advance in OTC) and for 2016-2017 (delay). Differently, in SH there was an advance of -22 days of Sdisp. However, this latter value at the inter-annual level was an artifact due to the lack of Sdisp in OTCc but not in OTC reported for year 2017. This situation highlighted the importance of warming to conclude the sexual reproduction of *C. curvula* in snowbeds and, therefore, confirmed the advancement of Sdisp in OTC compared to control plots found in the other years.

Therefore, this species showed a different response, in terms of magnitude, between the two plant communities (**Fig. II.4.G.b.3.1**), with the most intense advance of Sdisp in snowbeds (not the typical vegetation). In alpine grasslands, the influence of warming was less marked and diverse between years of study.



Fig. II.4.G.b.3.2 – Example of flower senescence and begin of seed development (SD) in *C. curvula* (11/07/2016; DoY 193)

Leaf senescence (LS) in control plots ranged between DoY 187 to 272 (median=214). In CC it spanned from DoY 187 to 272 (median=214), in SH from DoY 210 to 247 (median=223) (**Tab. II.4.G.b.3.1**).

Differently to all the previous phenological stages and how already showed for *L. helveticus*, in OTC we recorded a delayed LS in median values of +5 days. Among plant communities, we didn't find any important differences (+5 days in CC; +6 days in SH), but within the natural variability, the delay clearly persisted in three of four years of study (2014, 2016, 2017) in SH, whereas it did only in two years (2015, 2016) in CC (**Fig. II.4.G.b.3.3**). The delay was more evident in SH.



Fig. II.4.G.b.3.3 - Descriptive statistics of leaf senescence (LS) computed in warming (OTC) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

				SD						Sdisp					LS		
plant	treat	Min	25%	Med	75%	Max	M	in	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	OTC	178	187	194	208	234	23	34	238	250	273	280	 187	211	224	240	263
all	OTCc	178	191	200	208	247	23	34	243	247	272	280	187	211	219	230	272
CC	OTC	178	187	190	203	208	23	34	238	250	273	280	187	208	219	235	263
CC	OTCc	178	187	197	208	208	23	34	243	247	272	280	187	204	214	225	272
SH	OTC	183	190	197	208	234	23	88	238	250	267	280	211	214	229	240	263
SH	OTCc	190	191	203	212	247	24	3	243	272	273	280	210	214	223	230	247

Tab. II.4.G.b.3.1 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaves senescence (LS) cmputed in warming (OTC) and control (OTCc) plots of C. curvula at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

As tested by one-way ANOVA and Tukey post-hoc test, none of these effects resulted statistically significant compared to control (p>0.05).

Precipitation Shields (PSs)

During the four years of study (2014-2017), the first day of seed development (SD) in control plots ranged between DoY 178 and 215 (median=195). In alpine grassland (CC) it spanned from DoY 178 to 215 (median=190), in snowbeds (SH) from DoY 183 to 215 (median=198) (**Tab. II.4.G.b.3.2**). Overall, we found a delay of +3 days in SD of *C. curvula* in PS and an advance of -1 day in PSv1. Among plant communities, in CC we found a delay of +8 days in PS only, while PSv1 didn't change. Differently, in SH this species showed and advance of SD in all treatments: -5 days in PS, -2 days in PSv1 (**Fig. II.4.G.b.3.4**). However, the inter-annual variability exceeded the experiment forcing on SD, that did not show any persistent pattern between years of study among the treatments, except for a general delay in PS of CC.

Seed dispersal (Sdisp) in control plots ranged between DoY 230 and 280 (median=256). In CC it spanned from DoY 234 to 280 (median=256), in SH from DoY 230 to 280 (median=256). It must be noted, as already seen in OTC, how the species did not reach the Sdisp stages in PSv1 in both communities in 2017 and in PS in SH.

Overall, we found a delay in Sdisp in PS (+5 days) and an advance in PSv1 (-1 day), pattern maintained also among plant communities: in PS *C. curvula* showed a delay of +2 days in CC and of +7 days in SH; in PSv1 the species showed an advance of -1 day in CC and of -9 days in SH. As for SD, the patter of response was not maintained between years of study and, therefore, other factors could have influenced the Sdisp of the species, rather than drought or water availability. However, we could suppose that drought and warm delayed the Sdisp, whereas an increased moisture advanced it, in particular in the snowbeds where the soil water availability was already higher than in the grasslands.



Fig. II.4.G.b.3.4 - Descriptive statistics of seed development (SD) and seed dispersal (Sdisp) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *C. curvula* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

Leaf senescence (LS) in control plots ranged between DoY 190 to 255 (median=220). In CC it spanned from DoY 190 to 255 (median=214), in SH from DoY 194 to 255 (median=224). Overall, LS occurred later in years with delayed snowmelt and with cool summers (2014, 2016) and earlier in

years with advanced snowmelt and with hot summers (2015, 2017), indicating a linkage with the natural climatic conditions.

Looking at the difference in median values between treatments and controls, LS was advanced by -1 day in PS and by -4 days in PSv1. Among plant communities, in CC the LS was delayed by +4 days in PSv1, but remained unchanged in PS. Otherwise, in SH the most evident effect was an advance of -10 days in PSv1, while in PS the LS delayed by +1 day (**Fig. II.4.G.b.3.5**).

Despite the results just mentioned, the inter-annual natural variability still exceeded the experiment influence, but the slight advanced LS reported in warm and dry summers (2015, 2016) in PS indicated a negative influence of the co-occurrence of warming and drought conditions, especially if compared to the delay in LS found in the OTCs. More data are needed to clarify the influence of soil moisture on LS of *C. curvula*.



Fig. II.4.G.b.3.5 - Descriptive statistics of leaf senescence (LS) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *C. curvula* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

				SD					Sdisp					LS		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	178	187	197	203	215	238	256	261	280	280	190	201	219	245	272
all	PSv1	173	187	194	204	215	238	238	255	267	280	194	201	217	240	255
all	OTCc	178	189	195	201	215	230	243	256	273	280	190	211	220	240	255
CC	PS	178	187	198	214	215	238	256	258	280	280	190	201	214	245	272
CC	PSv1	173	183	190	203	215	238	238	255	274	280	194	201	219	240	255
CC	OTCc	178	183	190	198	215	234	241	256	273	280	190	197	214	240	255
SH	PS	183	187	194	201	215	252	258	263	267	280	197	197	225	249	255
SH	PSv1	183	190	196	208	212	238	243	247	255	280	197	201	214	245	255
SH	OTCc	183	190	198	203	215	230	247	256	280	280	194	211	224	240	255

Tab. II.4.G.b.3.2 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaves senescence (LS) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

As tested by one-way ANOVA and Tukey post-hoc test, none of these effects resulted statistically significant compared to control (p>0.05).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), the first day of seed development (SD) in control plots ranged between DoY 183 and 238 (median=190) (**Tab. II.4.G.b.3.3**). At the inter-annual level, *C. curvula* showed an advance of -3 days in both the topographically lows area (SFtN, SFtS), while in SF+ it didn't show any appreciable difference (**Fig. II.4.G.b.3.6**). However, a delay in SF+ and SFtN were found in 2016 and 2017, whereas SFtS showed a comparable or slightly advanced SD.

Seed dispersal (Sdisp) in control plots ranged between DoY 234 and 280 (median=238). Differently from the previous species, at the inter-annual level *C. curvula* showed an advance of -4 days in SFtN and a strong delay of +18 days in SF+ and of +5 days in SFtS (**Fig. II.4.G.b.3.5**).

However, that was the consequence of the wide natural variability reported between years. Otherwise, looking at the intra-annual level it was visible, especially in 2016 and 2017, the increasing influence of the topography; indeed, as for the flowering (MF), in the south-exposed plots (SFtS) the Sdisp advanced compared to the north-exposed (SFtN) or to the moraine ridge (SF+).



Fig. II.4.G.b.3.6 - Descriptive statistics of seed development (SD) and seed dispersal (Sdisp) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *C. curvula*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

Leaf senescence (LS) in control plots ranged between DoY 187 to 230 (median=218). The LS was delayed by +3 days in SFtN and advanced by -4 days in SF+ and SFtS (**Fig. II.4.G.b.4.6**). Despite the annual variability, the most consistent intra-annual pattern was a delay of LS in SFtN, without any other relative difference between the treatments. Moreover, it must be noted the extreme difference in LS between years 2014 and 2016 (cold and wet) and year 2015 and 2017 (warm and dry), underlining once more the stronger linkage of this stage with the climatic condition rather than with the experimental forcing (snowmelt).

As tested by one-way ANOVA and Tukey post-hoc test, none of these effects resulted statistically significant compared to control (p>0.05).



Fig. II.4.G.b.3.6 - Descriptive statistics of leaf senescence (LS) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *C. curvula*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

				SD					Sdisp					LS		
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
2015-2017	SFtN	183	187	187	203	203	226	234	234	256	280	197	201	221	229	245
2015-2017	SF+	183	187	190	198	203	234	243	256	280	280	187	198	214	225	225
2015-2017	SFtS	183	184	187	198	203	226	238	243	267	280	201	206	214	225	229
2015-2017	SFc	183	183	190	198	238	234	234	238	256	280	187	211	218	225	230

Tab. II.4.G.b.3.3 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaf senescence (LS) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *C. curvula*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

Comparison manipulation effects on seeds and leaf senescence – C. curvula synthesis

Over the study period, on seeds (SD, Sdisp) and leaf senescence (LS) of *C. curvula* we found influences due to experiments deployment, climatic variability and terrain morphology.

As reported also in *L. helveticus*, the OTC deployment advanced the seed development (SD) and seed dispersal (Sdisp) of *C. curvula*, with a more intense effect in snowbeds.

The PS deployment didn't affect SD in snowbeds, but generated a delay in alpine grasslands, underlining the possible negative effect of drought on this stage, especially if compared with the earlier onset reported in OTC. Differently, Sdisp didn't show any appreciable changes among treatments, meaning a stronger influence of other factors, rather than soil moisture, on seed dispersal. The snowmelt manipulation, through the SF deployment, did not affect SD and Sdisp those otherwise were more related by the position and topography of the plots (as already found for MF), with the better exposed ones (SFtS) showing an earlier seed development and dispersal.

Overall, seed development (SD) and seed dispersal (Sdisp) are mainly affected by soil warming (advance) and only to a secondary extent by water shortage (delay), and surface morphology (advance in south exposure), whereas snowmelt date do not have any appreciable influence.

In agreement with the results of *L. helveticus*, the OTC deployment delayed the leaf senescence (LS) of *C. curvula*, especially in snowbeds, probably because of the less intense competition, the higher soil water availability or the ability to lengthen the growing season under a fall warming.

The influence of soil moisture on LS was partially confirmed by the PS deployment, where we found an advance of LS in PS (dry and warm) that compared with OTC (warm alone, where LS delayed) allowed to suppose a water limitation on this phenological stage, as already found for *L. helveticus*. However, more data are need to clarify this point.

From the SF deployment and in agreement with SD and Sdisp, LS of *C. curvula* didn't seem to be affected by snowmelt but it was influenced by the surface morphology and the relative edaphic condition. Indeed in the more cool and moist plots (on the north-side of the moraine) the species showed a delayed LS, underlining once more the probable positive influence of enhanced soil moisture (as a consequence of exposure and concavity) on the delay of leaf senescence. Moreover, this confirmed how the lack of any water shortage allowed to get advantage form a fall warming and to lengthen the growing season.

In summary, under a warmer scenario the leaf senescence (LS) of *C. curvula* delays, as found for the other typical alpine grassland species *L. helveticus*, whereas under soil moisture shortage (especially combined with warming) it advances. Soil moisture alone seemed to not affect LS, but more years of monitoring are need to clarify this point. Moreover, the surface morphology (exposure, concavity) and the related edaphic conditions are able to exacerbate or buffer the responses to the climatic drivers.

Also for this species, it must be noted how LS started earlier than Sdisp and occasionally (year 2017) even before SD, irrespectively to the experiments or treatments.



Fig. II.4.G.b.3.7 – Example of leaf senescence (LS; 06/10/2016; DoY 280) (a) and of a late summer frost event (07/09/2017; DoY 251) (b) on *Carex curvula*.

II.4.G.b.4. Plant height – C. curvula

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the mean plants height (Hmean) of *C. curvula* in control plots ranged between 5 and 105 mm (median=50). In alpine grasslands (CC) it spanned from 25 to 80 mm (median=50), in snowbeds (SH) from 5 to 105 mm (median=60) (**Tab. II.4.G.b.4.1**). Hmean showed a particularly low value in year 2015, characterized by early snowmelt and by warm and dry summer.

Overall, in OTC we recorded an important increase in mean height of +20 mm (+40%) median values compared to control plots. Among plant communities, on average the increment was of +11 mm (21%) in CC and +30 mm (50%) in SH, almost three times the increments in snowbeds (Fig. II.4.G.b.4.1).

Irelation with the natural climatic variability, in cool and wet years (2014, 2016) in OTC the Hmean_DoY was delayed in CC and advanced in SH, wheras in warm and dry years (2015, 2017) it was advanced in CC and delayed in SH. Combined with the general shorter size in 2015 (warm and



Fig. II.4.G.b.4.1 - Descriptive statistics of the mean height (Hmean) and of the day of achievement of the maximal mean height (Hmean_DoY) computed in warming (OTC) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

dry), these results meant that under warm and dry conditions the species grew slower in CC than in SH.

The maximum plant height (Hmax) in control plots ranged between 10 and 140 mm (median=90). In CC it spanned from 50 to 130 mm (median=90), in SH from 10 to 140 mm (median=100) (**Tab. II.4.G.b.4.1**). Hmax showed a particularly low value in year 2015, characterized by early snowmelt and by warm and dry summer.

Overall, in OTC we recorded an increase in maximum height of +30 mm (+33%) in median values compared to control plots. Among plant communities, median values reported an increase of +11 mm (+12%) in CC, while in SH it was much more enhanced up to +55 mm (+55%) (Fig. II.4.G.b.4.2).

The responses induced by the OTC deployment on Hmax and Hmax_DoY, brought to the same conclusion of Hmean: slower growth in CC than in SH under warm and dry conditions and faster growth in CC than in SH under cool and wet conditions.

As tested by Tukey post-hoc test (p<0.05), only Hmax difference between OTC and controls resulted statistically significant at inter-community level.



Fig. II.4.G.b.4.2 - Descriptive statistics of the maximum height (Hmax) and of the day of achievement of the maximum height (Hmax_DoY) computed in warming (OTC) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

				Hmean					Hmax		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	OTC	20	60	70	90	130	39	100	120	152	300
all	OTCc	5	39	50	70	105	10	71	90	115	140
CC	OTC	20	46	61	73	90	39	90	101	123	200
CC	OTCc	25	38	50	60	80	50	71	90	110	130
SH	OTC	48	70	90	100	130	115	130	155	160	300
SH	OTCc	5	40	60	80	105	10	75	100	125	140

Tab. II.4.G.b.4.1 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in warming (OTC) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Precipitation Shields (PSs)

During the four years of study (2014-2017), the mean plants height (Hmean) of *C. curvula* in control plots ranged between 20 and 115 mm (median=60). In alpine grasslands (CC) it spanned from 20 to 90 mm (median=60), in snowbeds (SH) from 40 to 115 mm (median=70) (**Tab. II.4.G.b.4.2**), indicating first of all a natural higher size in snowbeds than in alpine grasslands for this experiment. Overall, the mean height didn't change in PS and PSv1. Among plant community, in CC we found an increase of +5 mm (8%) in PS and a decrease of -6 mm (-10%) in PSv1, while in SH we found a decrease of -10 mm (-14%) in PS but no change in PSv1 (**Fig. II.4.G.b.4.3**).

A wide inter-annual and intra-annual variability were found in each of the treatments, without any robust and repeated treatment effect. Moreover, comparing the results of contrasting extreme years (2014 vs 2015) in PS, we found an increment of Hmean in alpine grasslands and a decrease in snowbeds in both years, underlining therefore the prevalence of plant community type over soil moisture on the regulation of this phenological stage. However, it must be noted the general lower Hmean in 2015 and therefore the probable negative effect of extreme warm and drought years on the maximum plant size. Finally, compared to the enhanced Hmean found in OTC, the lack of a clear response in PS meant a negative influence of dry and warm condition on Hmean.

The maximum plant height (Hmax) in control plots ranged between 50 and 168 mm (median=110). In CC it spanned from 50 to 165 mm (median=110), in SH from 60 to 168 mm (median=110), without any important difference between years of study.

Compared to control plots, Hmax increased by +5 mm (5%) in PS and by +3 mm (2%) in PSv1. Among plant communities, we confirmed the occurrence of an increase size in the two treatment, except for PSv1 in SH, were Hmax decreased by -10 mm (-9%) (Fig. II.4.G.b.4.4). At the intraannual level, underneath PS of CC we found an enhanced Hmax compared to control plots and compared to PSv1 (except for 2016). This result, combined with the higher size found in OTC, could mean the absence of any soil moisture influence on Hmax of *C. curvula* or the ability of this species to reach and to use better the available soil water (i.e. deeper root system) than the other species. In SH it was found an higher variability compared to CC that did not allow to find any clear pattern at the intra-annual level.



Fig. II.4.G.b.4.3 - Descriptive statistics of mean height (Hmean) and of the day of achievement of the maximal mean height (Hmean_DoY) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *L. helveticus* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max

The day of achievement of maximum plant height (Hmax_DoY) in control plots ranged between DoY 194 and 272 (median=214). In CC it spanned from DoY 198 and 272 (median=214), in SH from 194 to 255 (median=214).

For the achievement of Hmax, alpine grasslands didn't show any differences in median values compared to control plots, while snowbeds had a general delay comparable among the treatments (PS: + 7 days; PSv1: +9 days), that combined with the effect on Hmax induced an enhanced and prolonged growth in SH (**Fig. II.4.G.b.4.4**).

As tested by one-way ANOVA and Tukey post-hoc test, only the differences in Hmax resulted statistically significant (p<0.05) at inter-communities level and within the snowbeds.



Fig. II.4.G.b.4.4 - Descriptive statistics of maximal height (Hmax) and of the day of achievement of the maximum height (Hmax_DoY) in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *C. curvula* at intra-annual and intra-plant communities levels (CC = alpine grassland; SH = snowbed). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

				Hmean				Hmax							
plant	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max			
all	PS	15	50	60	70	120	-	24	90	115	135	240			
all	PSv1	15	45	60	70	145		55	80	113	156	295			
all	OTCc	20	46	60	75	115		50	90	110	120	168			
CC	PS	30	50	65	70	90		75	90	115	135	215			
CC	PSv1	25	40	54	70	90		65	100	120	140	160			
CC	OTCc	20	46	60	70	90		50	90	110	115	165			
SH	PS	15	45	60	90	120		24	75	113	160	240			
SH	PSv1	15	50	70	90	145		55	80	100	210	295			
SH	OTCc	40	46	70	90	115		60	85	110	140	168			

Tab. II.4.G.b.4.2 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in drought (PS), moist (PSv1), mesic (PSv2) and control (PSc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), the mean plants height (Hmean) of *C. curvula* in control plots ranged between 30 and 80 mm (median=50) (**Tab. II.4.G.b.4.3**). It increased of +50 mm (55%) in SFtN and of +20 mm (20%) in SFtS, while SF+ have a small change compared to controls (**Fig. II.4.G.b.4.5**).

In 2016 and 2017 *C. curvula* in SFtN showed enhanced values of Hmean compared to 2014 and 2015, that led to the overall delay reported for the inter-annual level. Moreover for all the years of SF deployment we found in SFtS an enhanced Hmean compared to control plots, confirming the positive influence of the terrain morphology (southern exposure, concavity) on Hmean. In SF+ we found contrasting effects between years with late snowmelt and cool and wet summers (2016) and years with early snowmelt and warm and dry summers (2015, 2017); indeed if in the former the mean plant height decreased, in the latter it increased. This highlighted the positive influence on Hmean of an extra water supply at the begin of a long, warm and dry season, and its negative influence on a season with an already delayed snowmelt.



Fig. II.4.G.b.4.5 - Descriptive statistics of mean height (Hmean), maximum height (Hmax) and day of achievement of the maximal mean height (Hmean_DoY) and maximum height (Hmax_DoY) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *C. curvula*, at intra-annual level. Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

The maximum plant height (Hmax) in control plots ranged between 80 and 190 mm (median=113). Like for Hmean, also for Hmax we found a more pronounced increased in SFtN (+87 mm; +77%) and SFtS (+17 mm; +15%) than the one found in SF+ (+7 mm; +6%) (**Fig. II.4.G.b.4.5**), results confirmed also at the intra-annual level. These findings highlighted the positive influence of an enhanced snow cover (both artificial and natural) on the maximum height of *C. curvula*.

				Hmean			Hmax							
year	treat	Min	25%	Med	75%	Max		Min	25%	Med	75%	Max		
2015-2017	SFtN	20	40	100	120	130		38	182	200	250	350		
2015-2017	SF+	32	50	50	70	100		60	90	120	180	204		
2015-2017	SFtS	45	60	70	80	90		86	120	130	161	175		
2015-2017	SFc	30	41	50	60	80		80	95	113	150	190		

Tab. II.4.G.b.4.3 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *C. curvula*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

Comparison of manipulation effects on plant height – C. curvula synthesis

Over the study period the OTC deployment induced evident increases in mean height (Hmean) and maximum height (Hmax) of *C. curvula*, especially in the snowbeds, as already reported for *L. helveticus*. Moreover, a slower growth speed was detected in CC under warm and dry conditions.

Comparing the responses of extreme years (2014 vs 2015), the precipitation shield didn't show any difference between treatments suggesting the absence of any experiment induced effect on the species size and the prevalence of other local conditions, such as the plant community types. However, the lower mean size (Hmean) in PS but not in OTC, indicated a negative influence of dry and warm condition on Hmean in snowbeds.

Differently, the maximum plant size (Hmax) in both CC and SH didn't seem to be negatively affected by drought and warming, because of its increase in both OTC and PS independently by the plant community types. This confirmed the ability of *C. curvula* to grow under a warming scenario and even under drought condition, probably because of its ability to reach and use better the available soil moisture.

In the snow manipulation experiment (SF), the plant size increased mainly as a response of the terrain morphology (increase in south exposed plots) and only in year with early snowmelt and warm and dry summers (2015, 2017) as a response of a longer snow cover (SF+), probably as a consequent increase in soil water availability. Because of the disagreement with the results just reported for the PS experiment, the role of soil moisture on *C. cuvula* size need further years of monitoring.

Overall, the heights and the time of achievement of these heights of *C. curvula* in the SF experiment were more affected by the surface morphology and only in early seasons by the prolonger snow cover.

In summary, the mean and maximum size of *C. curvula* are primarly affected by soil warming (especially in the snowbeds), and secondarily by soil moisture and surface morphology (with its related edaphic conditions). In addition, the growth speed to achieve the maximal sizes were mainly affected by soil warming and surface morphology, without any appreciable difference between plant communities.

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the number of reproductive individuals (flowers_n) in control plots was on average 6 (median=5), with a maximum value up to 15. Alpine grasslands (CC) had a mean value of 6 (median=5), snowbeds (SH) of 6 (median=5) as well, with a maximum value of 15 and 11 individuals, respectively (**Tab. II.4.G.b.5.1**). Despite *C. curvula* is a typical species of the alpine grasslands, its flowers_n was comparable in CC and in SH. Within the inter-annual variability, it must be noted a general lower flowers_n in CC and SH (in both OTC and control plots) in 2014, an extreme year characterized by prolonged snow cover and cool and wet summer. In 2015, a further extreme year characterized by shorter snow cover and warm and dry summer, the flowers_n was within the natural variability reported for the other years.

Overall, in OTC we found a higher number of reproductive individuals (median=+3; +50%) compared to control plots. Among plant communities, the number increased by +3 in median values



Fig. II.4.G.b.5.1 - Descriptive statistics of the number of reproductive individuals (flowers_n) and of the reproductive success (rep_succ) computed in warming (OTC) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds). Black squares = medians, boxes = 25%-75%, whiskers = min-max.

in CC (60%), and by + 1 in SH (+20%) (**Fig. II.4.G.b.5.1**). For both the plant communities the OTC effect was detected in all the years, except for 2014 when flowers_n stayed lower and it was not enhanced by the OTC deployment. This unexpected answer could be explained by the counteracting effect of the enhanced soil moisture due to the very abundant and localized summer precipitation in 2014.

The reproductive success (rep_succ) in control plots was on average 40% (median=25), with a maximum of 100%. On average it was 46% (median=45) in CC and 27% (median=5) in SH, but with maximum value up 100% of reproductive success in both plant communities (**Tab. II.4.G.b.5.1**). For this variable, the natural variability of *C. curvula* didn't follow the climatic variability: the rep_succ was higher in the first two years of study and lower in the latter two, and this was found also for all the other experiments, indicating how the sexual reproduction and the seedling establishment was still a rare event, if compared to the clonal growth.

Overall, in OTC the reproductive success increased by 14% in median values. The two plant communities responded differently; in CC we found a decrease of -8% in median values, while in SH we found an increase of +35% (Fig. II.4.G.b.5.1). The difference between plant communities was explained by the intra-annual variability; indeed if in CC an enhanced rep_succ was found in 2015 and 2017 only, in SH it increased in all the years of study, underlining the higher sensitivity of *C. curvula* in the snowbeds.

As tested by Tukey post-hoc test (p<0.05), only flowers_n difference between OTC and controls resulted statistically significant at inter-community level and in alpine grasslands (CC) (**Tab. II.4.G.b.5.1**).

				flowers_1	n	rep_succ								
plant	treat	Min	25%	Med	75%	Max	 Min	25%	Med	75%	Max			
all	OTC	0	2	8	14	21	0	4	39	79	100			
all	OTCc	0	2	5	9	15	0	0	25	75	100			
CC	OTC	0	2	8	13	21	0	7	38	80	100			
CC	OTCc	0	2	5	8	15	0	0	45	88	100			
SH	OTC	1	2	6	14	20	0	0	40	67	100			
SH	OTCc	0	1	5	11	11	0	0	5	64	100			

Tab. II.4.G.b.5.1 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) in warming (OTC) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

As tested by one-way ANOVA and Tukey post-hoc test, differences in Hmax resulted statistically significant influenced by the SF deployment (p<0.05).

Precipitation Shields (PSs)

During the four years of study (2014-2017), the number of reproductive individuals (flowers_n) in control plots was on average 9 (median=10), with a maximum value up to 25. Alpine grasslands (CC) had a mean value of 11 (median=10), snowbeds (SH) of 7 (median=6) as well, with a maximum value of 25 and 18 individuals, respectively (**Tab. II.4.G.b.5.2**). As for the OTC experiment, it must be noted a general lower flowers_n in CC and SH in 2014, an extreme year characterized by prolonged snow cover and cool and wet summer. Moreover, in 2017 the variance in PSv1 of SH was much wider than any other treatments in this plant community.

Overall, the flowers_n of *C. curvula* decreased in all plots compared to controls: -58% (-6 n) in PS and -26% (-3 n) in PSv1. Among plant communities, in alpine grasslands the flowers_n decreased in PS (-40%; -4 n), in particular in 2016, and increased in PSv1 (+20%; +2 n), in particular in 2015. In snowbeds, it decreased in both PS and PSv1 (-67%; -4 n) (**Fig. II.4.G.b.5.2**), indicating a prevalence of other factors, rather than the treatments, on this quantitative stage.

The reproductive success (rep_succ) in control plots was on average 23% (median=14), with a maximum of 100%. On average the rep_succ was 24% (median=17) in CC and 22% (median=7) in SH, but with maximum value up 100% of reproductive success in both plant communities. As for the OTC experiment, the rep_succ was higher in the first two years of study (especially in SH) and lower in the latter two. Therefore other species-specific factors or ecological conditions, rather than the climatic variability, could have affected the rep succ.

Within this natural variability, rep_succ increased by +11% in PS compared to control plots, while in PSv1 it didn't change. The most evident increase was found in snowbeds, with percentage that ranges between +21 (PSv1) to +53% (PS) (**Fig. II.4.G.b.5.2**), indicating for this community the prevalence of heat control over moisture control. However, despite the different magnitude recorded, the reproductive success seemed to be more related to plant communities than to treatments.



Fig. II.4.G.b.5.2 - Descriptive statistics of the number of reproductive individuals (flowers_n) and of the reproductive success (rep_succ) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots for the whole study period (2014-2017) for *C. curvula* at intra-annual and intra-plant communities levels (CC = alpine grasslands; SH = snowbeds). Coloured squares = medians, boxes = 25%-75%, whiskers = min-max.

				flowers_1	1			rep_succ							
plant	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max			
all	PS	0	1	4	9	30	-	0	7	25	100	100			
all	PSv1	0	2	7	14	30		0	0	14	57	100			
all	OTCc	1	4	10	14	25		0	0	14	33	100			
CC	PS	1	1	6	14	30		0	7	17	57	100			
CC	PSv1	1	7	12	18	25		0	0	14	43	100			
CC	OTCc	1	7	10	15	25		0	0	17	33	100			
SH	PS	0	1	2	5	15		0	0	60	100	100			
SH	PSv1	0	1	2	7	30		0	0	29	100	100			
SH	OTCc	1	3	6	11	18		0	0	7	33	100			

Tab. II.4.G.b.5.2 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *C. curvula* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

As tested by one-way ANOVA and Tukey post-hoc test, only the differences in reproductive success whitin snowbeds community resulted statistically significant (p < 0.05).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), the number of reproductive individuals (flowers_n) in control plots was on average 8 (median=7), with a maximum value up to 18 (**Tab.II.4.G.b.5.3**). Also in this case in 2014 (year without the SF deployment) was recorded the lowest flowers_n value.

Compared to control plots, the number increased of +107% (+8 n) in SFtN (the highest increase reported among all the experiments) and of +71% (+5 n) in SFtS, while decreased of -29% (-2 n) in SF+ (**Fig. II.4.G.b.5.3**), as emerged also at the intra-annual level.



Fig. II.4.G.b.5.3 - Descriptive statistics of the number of reproductive individuals (flowers_n) and of the reproductive success (rep_succ) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *C. curvula*, at intra-annual level. Coloured square = medians, boxes = 25%-75%, whiskers = min-max.

The reproductive success (rep_succ) in control plots was on average 41% (median=40), with a decrease of -19% in SF+ and of -15% in SFtS. These results indicated how in *C. curvula*, typical

species of the alpine grasslands, a delayed snow melt leads to a decrease of the reproductive success more intense for instance than in *A. pentaphyllea* (see the further Chapter II.4.G.c.5), typical snowbeds species. Moreover, they indicated that the decrease was enhanced by the north exposed topography of SFtN (**Fig. II.4.G.b.5.3**) and that, at the same time, a longer permanence of snow not due to topography decreased the flowers_n, as well. However, the results at the intra-annual level were in disagreement. Indeed they showed an increase of rep_succ in SF+ and SFtS and a decrease in SFtN compared to control, indicating here the influence of the surface topography.

			:	flowers_	n		rep_succ								
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max				
2015-2017	SFtN	8	12	15	20	20	0	0	7	42	100				
2015-2017	SF+	2	4	5	8	19	0	0	21	40	100				
2015-2017	SFtS	2	5	12	12	17	6	20	25	50	100				
2015-2017	SFc	2	5	7	10	18	0	7	40	60	100				

Tab. II.4.G.b.5.3 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *C. curvula*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

As tested by one-way ANOVA and Tukey post-hoc test, differences in flowers_n resulted statistically significant influenced by the SF deployment (p<0.05).

Comparison of manipulation effects on reproductive investment and success – C. curvula synthesis

Over the study period, in all the three experiment types we were able to find an inter-annual variability in both the number of reproductive individuals (flowers_n) and the reproductive success (rep_succ), with a particular lower flowers_n in 2014 and a reduced rep_succ in 2016 and 2017, independently by the plant community type.

The OTC deployment increased the flowers_n of *C. curvula* in both alpine grasslands and snowbeds, as found for the other typical grasslands species *L. helveticus*, but was counteracted by short, cold and wet summers (such in 2014). OTC increased also the rep_succ that, differently to the previous one, was not negative affected by the extreme year. Therefore, extreme short, cool and wet season affected the number of flowers of *C. curvula*, but not their reproductive success.

In the rain exclusion experiment, the influences on flowers_n and rep_succ were more related to the plant community types, with enhanced values in snowbeds, rather than by drought/moist conditions, speculating here the absence of any water limitations.

The snow manipulation experiment influenced the reproductive investment and success of *C. curvula* in different ways. Indeed the artificially delayed snowmelt (SF+) decreased the flower numbers but increased their reproductive success, compare to control plots. The terrain topography influenced as well, with the south exposed plots showing an increase in both flowers_n and rep_succ.

In summary, temperature was the first limiting factor in the reproductive investment and success of *C. curvula* (in agreement with L. helveticus), with topography as further drivers; soil moisture seems to not affect the quantitative variables for this species. A prolonged and artificially longer snow permanence (SF+) decrease the flowers number but not their reproductive success.

II.4.G.c – Forbs – A. pentaphyllea

II.4.G.c.1. Snowmelt and greening – A. pentaphyllea

As the precipitation shields were set every year after the onset of SM, FS and NL, these stages are not shown in the snowmelt and greening part.

Open Top Chambers (OTCs)

At inter-annual level, snowmelt (SM) in control plots ranged between DoY 155 and 183 (median = 173), showing a wide inter-annual variability, with prolonged snow cover in 2014 and 2016 and an reduced one in 2015 and 2017, but without any effect of OTC deployment on SM (**Fig. S1**, in supplementary materials).

The first shoot (FS) of *A. pentaphyllea* followed the SM inter-annual variability, but with an advancement under the OTC deployment, particularly visible in alpine grasslands (CC) in all years of study and in snowbeds (SH) in 2014 and 2017 (**Fig. S1**). Similarly, the new leaves (NL) within the natural variability showed an earlier onset in all the years, except for 2014 in CC when none change was found with control plots.

		SM								FS			NL					
plant	treat	Min	25%	Med	75%	Max	Ν	/lin	25%	Med	75%	Max	Min	25%	Med	75%	Max	
all	OTC	155	160	173	175	183	1	159	163	168	179	188	160	168	175	183	188	
all	OTCc	155	160	173	175	183	1	159	163	173	179	188	160	169	176	184	193	
CC	OTC	155	159	160	173	182	1	159	160	165	179	179	160	168	169	182	188	
CC	OTCc	155	159	160	173	182	1	159	163	165	179	184	160	168	169	183	188	
SH	OTC	155	168	174	179	183	1	160	168	177	183	188	163	169	180	184	188	
SH	OTCc	155	168	173	179	183	1	160	168	179	183	188	163	173	183	188	193	

Tab. II.4.G.c.1.1- Descriptive statistics of snow melt (SM), first shoot (FS) and new leaves (NL) computed in warming (OTC) and control (OTCc) plots of *Alchemilla pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

However, none of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

Snow fence (SF)

At inter-annual level, for the period 2015-2017 the snow melt (SM) in control plots (SFc) ranged between DoY 155 and 173 (median = 160), with a wide natural variability.

In all the treatments of the SF experiment (SF+, SFtN, SFtS) we reported a delayed snowmelt, of +5 days (SF+, SFtN) and +3 days (SFtS) (**Fig. S2**), the same values already found for *L. helveticus* and *C. curvula*. It must be noted for year 2016 the simultaneous SM for all the treatments, except SF+.

The first shoot (FS) followed strictly the snowmelt patter and in particular it was affected by the delay in SF+, whereas for the new leaves (NL) together with the delay in SF+ we could detect also the advance in SFtN and SFtS. Therefore, the artificially induced delay of SM influenced more A. *pentaphyllea* than the natural delayed snowmelt.

However, none of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.
				SM					FS					NL		
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
2015-2017	SFtN	159	159	165	173	173	163	163	169	179	179	168	169	173	179	188
2015-2017	SF+	160	160	165	179	183	163	165	173	183	188	168	169	175	188	193
2015-2017	SFtS	155	160	163	173	173	160	163	165	179	179	163	168	169	179	188
2015-2017	SFc	155	155	160	173	173	160	163	165	179	179	163	168	175	183	188

Tab. II.4.G.c.1.2 - Descriptive statistics of snow melt (SM), first shoot (FS) and new leaves (NL) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *A. pentaphyllea*, at interannual level since the growing season 2015.

Comparison of manipulation effects on snowmelt and greening – A. pentaphyllea synthesis

In agreement with the other species, snowmelt (SM) for *A. pentaphyllea* showed a wide inter-annual variability that followed the natural variability in snowfalls; in this frame, the OTCs treatment induced only a marginal or null effect while the SF induced an evident delay, especially in SF+.

The greening stages (FS, NL) followed the snowmelt patterns and, within its inter-annual variability, they advanced in the onset dates under the OTC deployment and delayed under a prolonged snow cover (such in SF+).



Fig. II.4.G.c.1.1 – First shoot of *A. pentaphyllea* (01/07/2016; DoY 183).

II.4.G.c.2. Flowering – A. pentaphyllea

Open Top Chambers (OTCs)

For *A. pentahylle*a, we didn't find any difference in flowers bud (FB) onset at inter-annual level (**Tab. II.4.G.c.2.1**), while differences could be detected at intra-annual level and between plant communities. In particular, in alpine grasslands (CC) we could see a delayed onset under the OTC deployment, except for 2017, while in snowbeds (SH) we could appreciate differences between cold and wet years (2014, 2016), when FB didn't change, and warm and dry years (2015, 2017), when FB advanced. Moreover in 2014, flower bud and further flowering and seeds stages were not detected in OTC of CC, indicating the negative impact of such extreme year. In SH the FB was particularly delayed, without any difference between OTC and control, confirming therefore the prevalence of harsh climatic condition (cold and wet) over the OTC deployment.

Within the wide natural variability, the peak of flowering (MF) advanced in both plant communities under the OTC deployment (**Fig. S3**), both at inter-annual and intra-annual levels.

				FB					MF		
plant	treat	Min	25%	Med	75%	Max	 Min	25%	Med	75%	Max
all	OTC	168	173	184	193	204	178	191	195	208	225
all	OTCc	168	175	184	193	204	187	194	201	208	230
CC	OTC	168	173	180	188	193	178	190	194	195	208
CC	OTCc	168	175	180	188	198	187	191	195	203	225
SH	OTC	168	180	188	193	204	191	194	204	212	225
SH	OTCc	168	180	188	193	204	187	195	208	212	230

Tab. II.4.G.a.2.1 - Descriptive statistics of flower bud (FB), begin of peak flowering (MF_beg), peak flowering (MF) computed in warming (OTC) and control (OTCc) plots of *A.pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

As tested by one-way ANOVA and Tukey post-hoc test, the advance of MF in OTCs resulted statistically significant compared to control (p<0.05) at the inter-community level.

Precipitation Shields (PSs)

For what concerns FB, at inter-annual level *A. pentaphyllea* was affected only in alpine grasslands (CC), where it delay the FB by +4 days in PS and by +1 day in PSv1 (**Tab. II.4.G.c.2.2**). In snowbeds (SH), no differences were found. At the intra-annual level it was not possible to identify a clear response patter between treatments. However, in 2014 it must be noted how in CC were not detected reproductive individuals in PSv1 and control plots (**Fig. S4**) and how in SH the response of FB was particularly delayed, as already reported for the OTC experiment.

At the inter-annual level, the effect of treatments on the peak of flowering (MF) was opposite than on FB, but still without any evident influence of enhanced drought (PS) or moisture (PSv1) on this species. Indeed, in CC we found an advance of -7 days in median values in both PS and PSv1, and similarly in SH we found an advance of -8 days and of -4 days in both PS and PSv1. If we exclude the particularly late MF in 2014 (as reported also for FB), it was not possible to identify a clear pattern between treatments and control among years of study (**Fig. S4**).

None of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test.

				FB					MF		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	168	175	180	193	204	187	191	194	212	221
all	PSv1	168	173	180	188	208	191	194	195	212	225
all	OTCc	168	173	180	188	208	187	191	201	208	225
CC	PS	168	173	183	196	198	187	191	194	212	216
CC	PSv1	168	173	180	184	193	191	191	194	208	212
CC	OTCc	168	174	180	188	193	187	191	201	203	208
SH	PS	168	175	180	193	204	187	191	194	216	221
SH	PSv1	168	173	180	188	208	191	194	198	212	225
SH	OTCc	168	173	180	188	208	187	191	202	212	225

Tab. II.4.G.c.2.2 - Descriptive statistics of snow melt (SM), first shoot (FS) and new leaves (NL) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *A. pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

For what concerns the flower bud (FB), at the inter-annual level *A. pentaphyllea* didn't show differences in median values in any of the SF treatments compared to control plots (**Tab. II.4.G.c.2.3**), indicating a complete recovery of the delayed snowmelt date (**Fig. S5**). However, at the intra-annual level it was still present the delay in SF+ in 2016 and 2017,

Compared to control plots, the peak of the flowering (MF) didn't show any difference in median values in SF+ while it had a delay of +4 days in SFtN and an advance of -2 days in SFtS (**Fig. S5**). However, at the intra-annual level it was still visible in each of the treatments a delay in MF compared to controls, less pronounced in SFtS than in SF+ and SFtN, indicating the influence of both microtopography (SFtN, SFtN) and snowmelt (SF+) forcing. Indeed in the most exposed side (SFtS) we registered the lowest advance, whereas in the less exposed side (SFtN) the highest advance.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test.

				FB						MF		
year	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
2015-2017	SFtN	168	180	184	188	193	-	191	195	201	208	212
2015-2017	SF+	168	173	184	188	198		187	195	197	208	212
2015-2017	SFtS	163	168	184	188	193		191	194	195	203	212
2015-2017	SFc	163	173	184	188	193		187	190	197	203	208

Tab. II.4.G.c.2.3 - Descriptive statistics flower bud (FB), begin of peak flowering (MF_beg) and main flowering (MF) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *A. pentaphyllea*, at inter-annual level since the growing season 2015.

Comparison of manipulation effects on flowering – A. pentaphyllea synthesis

Over the study period we found a wide natural variability also in the flowering phenological stages of *A. pentaphyllea*, indicating a strong relationship with the natural variability of climatic condition, how already reported for the greening period.

The OTC deployment affected more the main flowering (MF) than the flower bud (FB) formation, that slightly advanced only in snowbeds and indeed seemed more affected by the natural climatic condition and seemed to get advantage from warming only in its typical community. However, from the PS deployment the flowering stages were not apparently affected by drought or soil moisture availability. The snowmelt still affected FB and to lesser extent MF, that otherwise started to be related also to the surface topography and to its consequence on edaphic condition.



Fig. II.4.G.c.2.1 – Flowers of A. pentaphyllea at the main flowering (MF; 26/07/2016; DoY 208).

II.4.G.c.3 - Seed development and leaf senescence – A. pentaphyllea

Open Top Chambers (OTCs)

During the four years of study (2014-2017), at the inter-annual level in OTC we recorded an earlier seed development (SD) of -5 days in median values (**Tab. II.4.G.c.3.1**); among plant communities, this advancement was of -4 days in alpine grasslands (CC) and of -7 days in snowbeds (SH) (**Fig. S6**).

However, important differences between years and plant communities could be detected at the intraannual level. In CC, it must be noted how in 2016 the species after the main flowering didn't reach the seed development (and therefore neither the further seed dispersal) in OTC. Together with the findings of 2014, when neither the FB was recorded in OTC, we can speculate the negative influence of cool and wet summers (2014, 2016) on the reproductive stages of *A. pentaphyllea* that neither the warming from the OTC deployment was able to counterbalance. Otherwise, in SH the species advanced the SD in OTC in all the years, except in 2017 when the warm and dry August could have led to an excessive warming within the OTC.

Seed dispersal (Sdisp) at the inter-annual level didn't have any difference in median values between OTC and control plots, despite among plant communities the advancement was of -3 days in CC and of -6 days in SH. At the intra-annual level, in CC we didn't find any clear pattern among years, if we exclude the advance in 2017, due to the lack of flowering and seed development reported in cool and wet years (2014, 2016). In SH the influence of OTC deployment was less clear than for SD and more influenced by the climatic variability (**Fig. S6**)..

Leaf senescence (LS) under the OTC deployment showed different responses between plant communities: an advance of -2 days in alpine grasslands (CC), a delay of +4 days in snowbeds (SH), indicating a contrasting response of *A. pentaphyllea* between the two main plant communities, appreciable also at the intra-annual level (**Fig. S6**). These findings highlighted the positive influence of fall warming to delay the LS and to lengthen the growing season in community (such snowbeds) with cooler soil temperature and higher water availability.

As tested by one-way ANOVA and Tukey post-hoc test, none of these effects resulted statistically significant compared to control (p>0.05).

				SD					Sdisp					LS		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	OTC	198	206	208	227	235	234	238	243	255	280	187	197	208	230	255
all	OTCc	198	204	213	230	243	234	238	243	272	280	187	201	206	230	255
CC	OTC	198	202	204	208	208	234	234	236	241	243	187	195	206	230	255
CC	OTCc	198	204	208	230	234	234	238	239	243	273	187	198	208	230	255
SH	OTC	202	206	216	230	235	238	239	247	263	280	194	202	210	230	255
SH	OTCc	202	208	223	235	243	234	243	253	277	280	194	202	206	230	247

Tab. II.4.G.c.3.1 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaf senescence (LS) cmputed in warming (OTC) and control (OTCc) plots of *A. pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Precipitation Shields (PSs)

Overall, we found a delay in seed development (SD) of +3 days in PS, while in PSv1 there wasn't any important difference in median values (**Tab. II.4.G.c.3.2**). Among plant communities, in alpine grasslands (CC) we found an earlier onset of -1 day in PSv1, but no changes in the other treatment (PS). For this community in 2014 it must be noted the lack of data for PSv1 and control plots that were due to the difficulties to detect the stages in the field and not to the lack of the reproductive stages *per se* (indeed Sdisp was present, see further paragraph). In snowbeds (SH) SD was delayed by +3 day in PS only, mainly as a result of the later onset in 2015 and 2017, characterized by warm and dry summers. This underlined the negative effect on SD of an enhanced drought under a warmer scenario, at least in SH.

At the inter-annual level, the seed dispersal (Sdisp) advanced by -7 days both in PS and PSv1 in alpine grasslands (CC) and delayed by +1 days and +4 days in PS and PSv1 in snowbeds (SH), indicating the prevalence of local conditions (vegetation type and related ecological niche) over the experiment forcing. Also at the intra-annual level the experiment didn't show any significative patterns between treatments. However, in years 2015 and 2017 (warm and dry), the advancement of Sdisp found in PS and PSv1 was more pronounced in CC than in SH, indicating shorter seed maturation period in the warmer and drier plant community (CC). However, it must be remember how an earlier seed dispersal under warmer conditions do not always mean ripe seeds or higher viability and germination (**Fig. S7**).

At the inter-annual level, leaf senescence (LS) of *A. pentaphyllea* in alpine grasslands (CC) delayed of +5 days in PS only, while in snowbeds (SH) all the plots indicate an advance of LS compared to control plots, with a more intense effect in PS (-8 days) (**Tab. II.4.G.c.3.2**). However similarities between communities were found at the intra-annual level, with LS delayed in all years of study in PS (dry and warm) in both CC and SH, while in PSv1 (moist) the LS was advanced in CC and didn't showed a clear pattern in SH (**Fig. S7**). In CC, the results in PS were in disagreement with those from the OTC deployment (where the LS advanced), speculating a positive influence of drought on the

delayed LS; differently, in SH the results from OTC and PS were in agreement, indicating both a delayed LS. Further years of study can help to clarify the influence of soil moisture on *A. pentaphyllea* LS.

As tested by one-way ANOVA and Tukey post-hoc test, none of these effects resulted statistically significant compared to control (p>0.05).

				SD					Sdisp					LS		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	202	206	211	230	239	238	238	247	259	272	187	198	213	240	255
all	PSv1	202	204	208	230	239	238	239	250	267	273	187	197	214	230	255
all	OTCc	202	202	208	230	235	234	243	250	263	273	187	201	214	235	255
CC	PS	202	206	208	234	239	238	238	243	263	272	194	198	219	245	255
CC	PSv1	202	203	207	215	230	238	239	243	263	273	187	194	214	225	247
CC	OTCc	202	202	208	225	230	238	243	250	256	267	194	201	214	235	255
SH	PS	202	206	211	230	234	238	239	250	255	267	187	198	206	240	255
SH	PSv1	202	204	208	230	239	238	243	253	273	273	194	201	210	240	255
SH	OTCc	202	208	208	230	235	234	243	249	267	273	187	201	214	235	247

Tab. II.4.G.c.3.2 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaf senescence (LS) computed in drought (PS), moist (PSv1), mesic (PSv2) and control (PSc) plots of *A. pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), at the inter-annual level *A. pentaphyllea* didn't show any difference in seed development (SD) between treatments and control plots (**Tab. II.4.G.c.3.3**), whereas at the intra-annual level was visible a delay in SF+, indicating how SD as well as MF was still affected by the prolonged snow cover (**Fig. S8**).

For what concern the seed dispersal (Sdisp), at the inter-annual level the species didn't show difference compared to control plots in SF+ and SFtN, but it advanced in SFtS (-5 days) in median values. This pattern was confirmed also at the intra-annual level in years 2015 and 2017 (early snowmelts, warm and dry summers), allowing to speculate the influence of both snowmelt (SF+) and topography (especially in the south exposures) on a later or earlier Sdisp (**Fig. S8**).

Leaf senescence (LS) at inter-annual level was delayed by +4 days in SFtN and advanced by -4 days in SF+, while in SFtS it didn't show any difference (**Fig. S8**). Here in SF+ it must be noted how in 2015 and 2017 (early snowmelts, warm and dry summers) the LS was advanced and how in 2016 (late snowmelt, cool and wet summer) it was delayed, indicating an ability of *A. pentaphyllea* to prolong the growing season in consequence of a delayed snowmelt.

As tested by one-way ANOVA and Tukey post-hoc test, none of these effects resulted statistically significant compared to control (p>0.05).

				SD					Sdisp					LS		
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
2015-2017	SFtN	198	202	211	225	225	238	243	243	267	273	201	206	214	225	235
2015-2017	SF+	198	202	211	225	230	234	241	243	259	280	197	197	206	225	235
2015-2017	SFtS	198	202	211	225	230	234	234	238	243	280	197	202	210	216	230
2015-2017	SFc	198	202	211	225	225	234	238	243	256	273	197	204	210	216	243

Tab. II.4.G.c.3.3 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaf senescence (LS) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *A. pentaphyllea*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).



Fig. II.4.G.c.3.1 – Examples of senescent plants of A. pentaphyllea (MF; 29/09/2016; DoY 273).

Comparison manipulation effects on seeds and leaf senescence – A. pentaphyllea synthesis

Over the study period, on seeds (SD, Sdisp) and leaf senescence (LS) of *A. pentaphyllea* we found influences due to experiments deployment, climatic variability and terrain morphology, as reported also for *L. helveticus* and *C. curvula*.

The seed development (SD) of *A. pentaphyllea* advances under warmer condition (OTC), but only in its typical plant community (snowbeds) and only in the absence of any drought forcing, as reported from the PS deployment, whereas a prolonged snow cover was still able to delay this phenologial stage, as already found for MF.

Differently to SD, seed dispersal (Sdisp) of *A. pentaphyllea* seemed more affected by the inter-annual climatic variability (especially with a negative influence on cool and wet summers), snowmelt date and surface topography rather than the by warming or drought conditions. However, further years of study are needed to clarify the influence of soil moisture on this stage.

Also for leaf senescence (LS) the influence of drought or soil moisture availability was not clear, while under a warmer scenario the responses differed between plant communities with a delay in its typical community, the snowbeds (characterized by cool soil temperature and higher water avavilability), and an advance in the alpine grasslands. From these considerations we can here

speculate a positive effect of warming over the growing season length only in vegetation characterized by a lower water limitation, such the snowbeds. In alpine grasslands, the snow fence deployment emphasized also the ability of the species to delay the LS in consequence of a prolonged snow cover, in disagreement with the results reported in OTC where it was mainly advanced.

II.4.G.c.4. Plant height – A. pentaphyllea

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the mean height (Hmean) of *A. pentaphyllea* increased by +10 mm (+50% of the control plot size) in median values compared to control plots in both plant communities (**Tab. II.4.G.c.4.1**). This was clearly visible in all the years of study except for 2014, when we recorded a decrease in Hmean in alpine grasslands (**Fig. S9**).

Moreover, different responses were detected on the growth rates between communities: enhanced and fast growth in alpine grasslands (CC) and an enhanced slower growth in snowbeds (SH).

The maximum plant height (Hmax) in OTC showed the same responses (increase) already seen for Hmean and with general bigger size in its typical plant community, irrespectively to the treatments. Also the growh rate had the same patterns and indicated under warming conditions a faster growth in CC and a slower growth rate in SH (**Tab. II.4.G.c.4.1; Fig. S10**).

As tested by one-way ANOVA and Tukey post-hoc test, Hmean was significantly influenced by OTC deployment both at inter-community and at intra-community level, while Hmean_DoY was achieved with a statistically significant (p<0.05) advance only in grasslands.

				Hmean						Hmax		
plant	treat	Min	25%	Med	75%	Max		Min	25%	Med	75%	Max
all	OTC	15	22	30	35	40	-	22	35	45	50	66
all	OTCc	15	20	20	25	36		21	30	35	45	80
CC	OTC	15	21	30	35	40		27	35	40	45	64
CC	OTCc	15	20	20	25	36		25	30	35	42	66
SH	OTC	20	25	30	35	40		22	40	48	52	66
SH	OTCc	16	20	20	25	32		21	30	35	46	80

Tab. II.4.G.c.4.1 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in warming (OTC) and control (OTCc) plots of *A.pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Precipitation Shields (PSs)

During the four years of study (2014-2017), the mean plants height (Hmean) showed a decrease in mean height of -3 mm (-12%) in PSv1 while no differences were found in PS (**Tab. II.4.G.c.4.2**). There was low agreement in the answer between the plant communities. In alpine grassland (CC) Hmean didn't change in PS compared to control plots, while it decreased by -5 mm (-20%) in PSv1. In snowbeds (SH), we found a decrease in size of -2 mm (-9%) in PS and an increase of +2 mm in PSv1 (+9%) (**Fig. S11**). At the intra-annual level, in SH was found an opposite response in PS and PSv1 in contrasting extreme years; indeed in 2014 and 2016 (cool and wet summers) Hmean decreased in PS and increased in PSv1, while in 2015 and 2017 (warm and dry summers) it did the

opposite, indicating the positive influence of enhanced soil moisture on Hmean in SH. The pattern was not found in CC.

The day of achievement of the maximal mean height (Hmean_DoY) advanced, in both plant communities, without any clear pattern between treatments. However, in CC advanced more than in SH (**Fig. S11**).

The maximum plant height (Hmax) in the PS experiment was more affected by plant communities rather than treatments types. Indeed in snowbeds (SH) we found an increase of +5 mm (+17%) and +7 mm (+23%) in PS and PSv1, while in alpine grasslands (CC) it didn't change in PS and decreased of -5 mm (-14%), indicating the preference of the species to grow in its typical community (SH) where it didn't show any water limitation (**Tab. II.4.G.c.4.1; Fig. S12**).

Concerning the growth rate, among plant communities, in CC we found a delay in PSv1 and an advance in PS, while in SH we found a delay in PS only.

Differently to the mean size, comparing the values of Hmax and its growth rate we could speculate a reduced and limited maximum plant size in CC under warm and dry conditions (PS), in contrast with the results of the OTC deployment where the plant increased the size and the growth rate. Therefore, in the most water limited plant community (alpine grasslands) warming but not warming and drought were able to increase the maximum plant size of *A. pentaphyllea*.

way ANOVA and Tukey post-hoc test.		

None of these effects resulted statistically significant compared to control (p>0.05), as tested by one-

				Hmean					Hmax		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	17	20	25	28	35	24	29	35	38	52
all	PSv1	15	20	22	25	35	25	30	35	40	53
all	OTCc	15	20	25	30	35	21	30	35	37	45
CC	PS	18	24	25	28	35	24	30	35	38	48
CC	PSv1	15	20	20	25	35	25	25	30	36	53
CC	OTCc	15	20	25	30	35	25	33	35	40	45
SH	PS	17	20	21	30	30	25	28	35	40	52
SH	PSv1	15	20	25	25	34	30	33	37	40	50
SH	OTCc	15	20	23	30	33	21	30	30	36	44

Tab. II.4.G.c.4.2 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *A. pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), the mean plant height (Hmean) and maximum plant height (Hmax) of *A. pentaphyllea* didn't show any influence of the SF deployment, while it was visible an enhanced mean height in SFtN plots, indicating the preferences of this species for cool and north exposed sites (**Tab. II.4.G.c.4.3**; Fig. S13).

The day of achievement of the maximal mean height (Hmean_DoY) and of maximum plant height (Hmax_DoY) were not affected by the snowmelt date and didn't showed any significant pattern among the treatments.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test.

				Hmean						Hmax		
year	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
2015-2017	SFtN	18	20	20	30	35	-	25	37	38	46	50
2015-2017	SF+	11	15	20	23	60		16	27	38	40	72
2015-2017	SFtS	12	15	20	25	50		23	31	35	38	100
2015-2017	SFc	10	12	20	25	30		15	24	32	40	54

Tab. II.4.G.c.4.3 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *A. pentaphyllea*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

Comparison of manipulation effects on plant height – A. pentaphyllea synthesis

During the study period the size of *A. pentaphyllea* was manily affected by warming and by it combination with drought, especially in the most water limited plant community (alpine grasslands), while the snowmelt date didn't have any appreciable influence.

Indeed the mean and maximum plant size (Hmean, Hmax) were both positive affected by warming (OTC), but with different responses in growth rate: faster in alpine grasslands and slower in snowbeds. The larger plant size and faster growth rate were maintained as long as did not occurs any drought events, especially in alpine grasslands, those otherwise were able to counteract the species responses under warming.

II.4.G.c.5. Reproductive investment and success – A. pentaphyllea

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the number of reproductive individuals (flowers_n) in alpine grasslands (CC) was on average 8 (median=4) while in snowbeds (SH) 58 (median=60), with a maximum value of 30 and 125 individuals, respectively, indicating a wide difference between plant communities (**Tab. II.4.G.c.5.1**).

Both at inter- and intra-annual levels, in OTC we found a lower flowers_n compared to control plots in alpine grasslands (CC) as well as in snowbeds (SH), except for year 2017 when in SH the number increased (**Fig. S14**). Between years, it must be noted the lower flowers_n reported in 2014 and 2016, characterized by cool and wet summers and therefore indicating the negative influence of these parameters on the number of flowers.

The reproductive success (rep_succ) in control plots was on average 29% (median=27) in alpine grasslands (CC) and 34% (median=32) in snowbeds (SH), but with maximum value up 100% of reproductive success in both plant communities (**Tab. II.4.G.c.5.1**).

Overall, in OTC the reproductive success decreased by -2% in median values but with a wide interannual and inter-communities variability. In CC rep_succ increased in OTC in years 2015 and 2017, whereas it decreased in 2014 and 2016, confirming how particularly cool and wet summers were able to reduce both flower number and their reproductive success. In SH the response of rep_succ was less affected by OTC and extreme years, showing an increase in 2014 and 2015 and a decrease in 2016 and 2017. Other factors would probably led this phenological stage (**Fig. S14**). However, none of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test.

				flowers_	n					rep_succ	;	
plant	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
all	OTC	0	2	4	40	100		0	0	25	75	100
all	OTCc	0	2	15	50	125		0	1	27	50	100
CC	OTC	0	0	2	4	70		0	0	25	80	100
CC	OTCc	0	2	4	15	30		0	0	27	40	100
SH	OTC	2	12	32	75	100		0	15	28	58	100
SH	OTCc	2	40	60	70	125		0	18	32	50	89

Tab. II.4.G.c.5.1 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) in warming (OTC) and control (OTCc) plots of *A. pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).



Fig. II.4.G.c.5.1 – Vegetative plant of *A. pentaphyllea* with seeds of *Salix herbaceae* between the leaves (27/08/2016; DoY 240)

Precipitation shields (PS)

During the four years of study (2014-2017), the number of reproductive individuals (flowers_n) in control plots was on average 16 (median=16) in alpine grasslands (CC) and 18 (median=20) in snowbeds (SH), with a maximum value of 62 and 36 individuals respectively (**Tab. II.4.G.c.5.2**). At the inter-annual level, this species decreased flowers_n in all the treatments in CC, with the decrements in PS (-63%; -10 n) and PSv1(-56%; -9 n) similar to each other, but without any consistent pattern between at the intra-annual level. Differently, in SH we found an increase in flowers_n in PSv1 (+35%; +7 n), reported also at the intra-annual level (**Fig. S15**), underlining the importance of soil moisture availability for the reproduction effort.

The reproductive success (rep_succ) in control plots was on average 40% (median=30) in alpine grasslands (CC) and 42% (median=36) in snowbeds (SH), but with maximum values up 100% in both plant communities.

In CC the rep_succ increased by +3% in PS at the inter-annual level, but it decreased at the intraannual level if compared to both PSv1 and control plots, indicating here the negative effect of warm and drought (PS) on the reproduction effort of *A. pentaphyllea* in this more water limited community (**Fig. S15**). To confirm the influence of soil water limitation on this point, was the higher rep_succ found in PSv1 of CC in all the years of study.

In SH it was not detected any clear of rep_succ pattern among treatments, underlining the lower sensitivity of this snowbeds species to any climatic forcing, especially in it typical plant community.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test.

				flowers_1	1				rep_succ	;	
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	1	2	9	27	120	0	0	29	57	100
all	PSv1	0	3	17	30	70	0	14	29	67	100
all	OTCc	0	4	17	25	62	0	20	32	53	100
CC	PS	1	2	6	25	120	0	0	33	57	67
CC	PSv1	0	2	7	20	70	0	15	27	100	100
CC	OTCc	0	2	16	24	62	0	13	30	76	100
SH	PS	1	8	11	38	120	0	3	16	89	100
SH	PSv1	2	15	27	45	70	0	0	33	67	78
SH	OTCc	1	5	20	30	36	0	24	36	53	100

Tab. II.4.G.c.5.2 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *A. pentaphyllea* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow Fence (SF)

During the three years of snow fence deployment (2015-2017), the number of reproductive individuals (flowers_n) in control plots was on average 11 (median=8), with a maximum value up to 30 (**Tab. II.4.G.c.5.3**). Despite the absence of the snow fence, in 2014 was visible the low number of flowers already reported for many species and in all the experiment types.

Compared to control plots, flowers_n increased of +63% (+5 n) in SFtN and of +13% (+1 n) in SF+, while decreased of -13% (-1 n) in SFtS (**Fig. S16**). At the intra-annual level the only well visible effect was the higher flowers_n in SFtN, whereas in SFtS and SF+ the patterns were not confirmed. However, an enhanced flowers_n (at least in its maximum values) compared to control plots was found in 2015 and 2016, indicating the ability of this species to increase the reproductive effort under a prolonged snow cover. Further years of study can clarify this point.

The reproductive success (rep_succ) in control plots was on average 36% (median=33), with a maximum of 100%. Compared to control plots, at the inter-annual level the rep_succ decreased of - 3% in SFtN, of -1% in SF+ and of -13% in SFtS. However, the intra-annual level led to a different response (**Fig. S16**). Indeed, SF+ increased the rep_succ in all years of study, while for instance in SFtN (natural delayed snowmelt, with north exposure) it decreased compared to the controls, at least in 2015 and 2017.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test.

			:	flowers_	n					rep_succ	•	
year	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
2015-2017	SFtN	10	10	13	25	36	-	8	19	30	33	67
2015-2017	SF+	0	4	9	18	31		0	20	33	78	100
2015-2017	SFtS	2	4	7	12	20		0	14	20	50	80
2015-2017	SFc	1	6	8	16	30		0	17	33	38	100

Tab. II.4.G.c.5.3 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *A. pentaphyllea*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

Comparison of manipulation effects on reproductive investment and success -A. pentaphyllea synthesis

Over the study period, in all the three experiment types we were able to find an inter-annual variability in both the number of reproductive individuals (flowers_n) and the reproductive success (rep_succ), with a particular lower flowers_n in 2014 and 2016 and a reduced rep_succ in 2016, independently by the plant community type.

The number of reproductive individuals (flowers_n) of *A. pentaphyllea* decreases in both plant communities under warming conditions (OTC), while the reproductive success (rep_succ) increased, despite the inter-annual variability indicated also a negative influence of extremely cool and wet summers on both flowers_n and rep_succ especially in alpine grasslands (CC).

The soil moisture did not affect the flowers number but affected (increased) their reproductive success in CC, while in snowbeds (SH) it led to an increase of flowers number but without any difference in rep_succ.

A prolonged snow cover (SF+) positively affected both the reproductive effort and success, indicating the ability of this typical snowbeds species to cope with a delayed snowmelt, despite more years of monitoring are needed to clarify this point.

II.4.G.d – Forbs – G. supinum

II.4.G.d.1. Snowmelt and greening – G. supinum

As the precipitation shields were set every year after the onset of SM, FS and NL, these stages are not shown in the snowmelt and greening part.

Open Top Chambers (OTCs)

At inter-annual level, SM in control plots ranged between DoY 155 and 183 (median = 173) (**Tab. II.4.G.d.1.1**), showing a wide inter-annual variability, with a general prolonged snow cover in 2014 and 2016 and a reduced one in 2015 and 2017, but without any effect of OTC deployment (**Fig. S17**).

The first shoot (FS) of *G. supinum* followed the snowmelt inter-annual variability, but with an advancement under the OTC deployment, particularly visible in alpine grasslands (CC) in all years of study and in snowbeds (SH) in 2017 only (**Fig. S17**). Differently from the previous species, here the new leaves (NL) advanced in OTC mainly in years with a reduced snow over (2015, 2017),

indicating the importance of a warmer temperature after an earlier snowmelt for the start of the greening stages.

However, none of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

				SM						FS						NL		
plant	treat	Min	25%	Med	75%	Max	N	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
all	OTC	155	159	171	175	183]	159	163	168	179	188		159	163	173	180	188
all	OTCc	155	160	173	175	183	1	159	163	173	179	188		159	165	175	180	188
CC	OTC	155	159	160	173	182	1	159	160	165	179	179		159	163	168	179	184
CC	OTCc	155	159	160	173	182]	159	163	165	179	184		159	165	168	179	184
SH	OTC	155	163	173	179	183	1	159	165	177	183	188		160	165	179	183	188
SH	OTCc	155	163	173	175	183]	160	167	175	180	188		160	171	178	182	188

Tab. II.4.G.d.1.1- Descriptive statistics of snow melt (SM), first shoot (FS) and new leaves (NL) computed in warming (OTC) and control (OTCc) plots of *Gnaphalium supinum* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

At inter-annual level, for the period 2015-2017 the snow melt (SM) in control plots (SFc) ranged between DoY 155 and 173 (median = 160), with a wide natural variability (earlier SM in 2015 and 2017; delayed in 2014 and 2016). (**Tab. II.4.G.d.1.2**).

In SF+ the SM was delayed of +7 days compared to control plots, as also visible at the inter-annual level. The other two treatments (SFtN, SFtS), characterized by natural delayed snowmelts, showed a lower delay, up to a none difference compared to control plots in 2016 (**Fig. S18**).

The first shoot (FS) and new leaves (NL) followed the snowmelt pattern and in particular were affected by the delay reported in SF+, even if in 2015 and 2017 we detected also an advance in SFtS, indicating also the influence of topography (south exposure) in years with a general advanced snowmelt (**Fig. S18**).

However, none of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

				SM					FS					NL		
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
2015-2017	SFtN	159	159	165	173	173	163	163	169	179	179	163	165	169	179	179
2015-2017	SF+	160	162	167	176	183	163	165	172	181	188	165	168	174	181	188
2015-2017	SFtS	155	160	163	173	173	160	163	165	179	179	160	163	165	179	179
2015-2017	SFc	155	157	160	173	173	159	163	168	179	179	160	164	168	179	179

Tab. II.4.G.d.1.2 - Descriptive statistics of snow melt (SM), first shoot (FS) and new leaves (NL) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *G. supinum*, at inter-annual level since the growing season 2015.

Comparison of the manipulation effects on snowmelt and greening -G. supinum synthesis

In agreement with the other species, snowmelt (SM) for *G. supinum* showed a wide inter-annual variability that followed the natural variability in snowfalls; in this frame, the OTCs treatment induced only a marginal or null effect while the snow fence induced an evident delay, especially in SF+.

The greening stages (FS, NL) followed the snowmelt patterns and, within its inter-annual variability, they advanced under the OTC deployment only in years with advanced snowmelt and, therefore, avoiding the damages on the greening stages due to early summer frost events.

The SF deployment confirmed the linkage of snowmelt and greening, but with interferences also from the surface topography, especially in years with advanced SM, when FS and NL advanced more strongly in the south exposed plots (SFtS).

In summary, warming was a key drivers on first shoot and new leaves mainly in years with advanced snowmelt.



Fig. II.4.G.d.1.1 – Example of new leaves (NL) of *G. supinum* (24/06/2015; DoY 176).

II.4.G.d.2. Flowering – G. supinum

Open Top Chambers (OTCs)

For G. *supinum* the OTC deployment induced an inter-annual advance of FB of -3 days in alpine grasslands (CC) and of -6 days in snowbeds (SH) (**Tab. II.4.G.d.2.1**), with differences well visible also at the intra-annual level (**Fig. S19**). 1. It is important to note the wide inter-annual variability, with FB advanced in warm and dry years (2015, 2017) compared to the other years, in both plant communities. Very important was also the lacking of any FB (and therefore of any further flowering and seeds stages) in year 2014, characterized by extremely delayed snowmelt and cool and wet summer; underlining the sensitivity of the species to these harsh conditions that the OTC deployment was not able to buffer.

Within the wide natural variability, the peak of flowering (MF) advanced in both plant communities under the OTC deployment (**Fig. S19**), both at inter-annual and intra-annual levels, with the missing year (2014) just mentioned for FB.

As tested by one-way ANOVA and Tukey post-hoc test, FB resulted statistically significant influenced by OTC treatment at inter-communities level (p < 0.05).

				FB					MF		
plant	treat	Min	25%	Med	75%	Max	 Min	25%	Med	75%	Max
all	OTC	173	183	187	198	203	191	195	198	208	216
all	OTCc	178	184	191	203	212	194	197	198	216	221
CC	OTC	173	181	184	193	198	191	195	197	201	216
CC	OTCc	178	184	187	203	208	195	197	198	216	221
SH	OTC	173	183	187	198	203	195	197	202	211	216
SH	OTCc	178	189	193	203	212	194	198	203	214	221

Tab. II.4.G.d.2.1- Descriptive statistics of flower bud (FB), begin of peak flowering (MF_beg), peak flowering (MF) computed in warming (OTC) and control (OTCc) plots of *G. supinum* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Precipitation Shields (PSs)

For what concerns flower bud (FB), at the inter-annual level *G. supinum* advanced in alpine grasslands (CC) of -4 days in PS and of -1 day in PSv1, whereas in snowbeds (SH) it advanced as well but with a reduced intensity (**Tab. II.4.G.d.2.2**).. At the intra-annual level, we were not able to detect any significant pattern between treatments but it must be noted (in agreement with OTC experiment) the lack of FB (and subsequent reproductive stages) in year 2014 (**Fig. S20**), characterized by early snowmelt and cold summer. This highlighted how such an extreme year was able to affect the biological answer much more than the experimental forcing and confirmed (as found in the OTCs) the species sensitivity to cold temperature.

The peak of the flowering (MF) showed similar answer of FB, with the most evident effect derived from the missing MF in the extreme year (2014) (**Tab. II.4.G.d.2.1; Fig. S20**).

None of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

				FB						MF		
plant	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
all	PS	187	187	190	196	208	-	194	195	200	212	221
all	PSv1	183	187	190	208	212		191	197	200	204	221
all	OTCc	178	187	191	203	208		191	197	200	212	216
CC	PS	187	187	187	191	194		195	197	198	201	201
CC	PSv1	183	187	190	191	212		197	197	198	202	221
CC	OTCc	178	190	191	203	208		191	198	204	208	212
SH	PS	187	187	190	203	208		194	195	204	216	221
SH	PSv1	183	187	190	208	208		191	198	202	210	221
SH	OTCc	178	187	191	203	208		195	197	198	216	216

Tab. II.4.G.d.2.2 - Descriptive statistics of flower bud (FB), begin of peak flowering (MF_beg), peak flowering (MF) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *G. supinum* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

Between the growing seasons 2015 and 2017, the day of first appearance of flower bud (FB) was delayed by +4 days in SF+ treatment, while in SFtN and SFtS it didn't show any variance compared with control plots, indicating the different ability of the species to cope with artificially and natural delayed snowmelts (**Tab. II.4.G.d.2.3**). At the intra-annual level, a delay was visible in SF+ in 2016

and 2017, but not in 2015 when otherwise the species showed the same value in all the plots (Fig. S21).

Compared to controls, the peak of flowering (MF) delayed by +4 days in SF+ (**Tab. II.4.G.d.2.3**), confirming the influence of the snowmelt up to this phenological stage (well visible in 2015 and 2016 and to a lesser extent also in 2017). In SFtS and SFtN the shift with control plots was negligible (+1 days) or null and contrasting between the years of study (**Fig. S21**), indicating the presence of other major factors to control the MF of this species.

None of these effects are statistically significant compared to control (p>0.05), as tested by Tukey post-hoc test.

				FB						MF		
year	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max
2015-2017	SFtN	183	183	187	198	203	-	194	195	197	212	212
2015-2017	SF+	183	187	191	203	208		191	195	201	212	221
2015-2017	SFtS	178	187	187	187	198		195	197	198	201	208
2015-2017	SFc	183	185	187	201	208		191	194	197	208	216

Tab. II.4.G.d.2.3 - Descriptive statistics flower bud (FB), begin of peak flowering (MF_beg) and main flowering (MF) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *G. supinum*, at interannual level since the growing season 2015.

Comparison of manipulation effects on flowering – G. supinum synthesis

Over the study period we found a wide natural variability in the flowering stages of *G. supinum*, especially related to the occurrence of extreme year (2014), that indicated a strong relationship with the natural climatic variability, how reported also for *A. pentaphyllea*.

The prevalence of a warming control over the flower bud (FB) and the main flowering (MF) of G. *supinum* in both the plant communities derived from the OTC and PS experiments, as well as from



Fig. II.4.G.d.2.1 – Example of (a) flower bud (FB) and (b) main flowering (MF) of *G. supinum* (29/07/2016; DoY 211).

the lack of any reproductive stages in 2014 (irrespective of experiment, treatment and community types) that indicated a particular sensitivity to cold temperatures.

II.4.G.d.3 - Seed development and leaf senescence – G. supinum

Open Top Chambers (OTCs)

During the four years of study (2014-2017), at the inter-annual level in OTC we recorded an earlier seed development (SD) of -4 days in median values in alpine grasslands (CC) and a slight delay of +1 day in snowbeds (SH) (**Tab. II.4.G.d.3.1**). SD was not detected in year 2014 and a wide inter-annual variability was found between 2015 and 2017 (also for Sdisp).

At the intra-annual level, SD didn't show a clear pattern in response of OTC deployment, with only an agreement between CC and SH on the earlier SD in year 2017 (**Fig. S22**).

Unlike the SD, differences between treatments and communities were found in the seed dispersal (Sdisp). At the inter-annual level it was advanced of -5 days in alpine grasslands (CC) and of -6 days in snowbeds compared to control plots, considering only the difference in median values (**Tab. II.4.G.d.3.1; Fig. S22**). Except for 2014, when the sexual reproduction of *G. supinum* was stopped by the extreme cool and wet summers, all the years showed and advance in OTC compared to control plots, especially in SH, indicating therefore the higher sensitivity of the species within its typical plant community.

Differently to many of the previous phenological stages and in agreement with *L. helveticus*, leaf senescence (LS) delayed in both plant communities under the OTC deployment, but with a stronger delay of +5 days in SH and a weaker delay of +2 days in CC; this confirmed the differential response to warming of *G. supinum* LS between plant communities, also visible at the intra-annual level (**Tab. II.4.G.d.3.1; Fig. S22**). A weak advance was found only in 2016. This confirmed also the positive influence of warming on the growing season length of the species, especially in absence of water shortage (like in SH).

As tested by one-way ANOVA and Tukey post-hoc test, seed dispersal (Sdisp) was significantly influenced by OTC deployment at the inter-communities level, but not at intra-communities level.

				SD					Sdisp						LS		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	l	Min	25%	Med	75%	Max
all	OTC	201	204	211	221	230	211	214	219	240	249		191	204	211	230	255
all	OTCc	201	209	214	225	230	214	218	234	245	263		191	204	210	225	255
CC	OTC	201	202	206	210	230	211	214	214	223	240		194	204	210	230	255
CC	OTCc	201	204	210	225	230	214	214	219	240	240		194	202	208	225	255
SH	OTC	201	208	215	221	230	211	218	229	240	249		191	208	216	225	247
SH	OTCc	201	211	214	225	230	218	229	234	253	263		191	205	211	228	247

Tab. II.4.G.d.3.1 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaves senescence (LS) cmputed in warming (OTC) and control (OTCc) plots of G. supinum at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Precipitation Shields (PSs)

Overall we found a delay in seed development (SD) of +3 days in PS and an advance of -4 days in PSv1. Among plant communities, in alpine grasslands (CC) we found an earlier onset of SD in PSv1

(-1 day), while PS didn't change, and in snowbeds (SH) a delay of +5 days in PS and of +1 day in PSv1, as already found for *A. pentaphyllea* (**Tab. II.4.G.d.3.2**). In 2014 in all the treatments of both plant communities (except for PS in SH) it must be noted the lack of SD data, due to the extremely cold years that affected the reproduction of *G. supinum* since the flower bud onset. None pattern emerged from the intra-annual level, as we could see from the contrasting patterns between treatments in years with comparable climate (2015 and 2017) (**Fig. S23**).

At the inter-annual level, the seed dispersal (Sdisp) delayed by +4 days in PS and advanced by -1 day in PSv1 in alpine grasslands (CC) and delayed by +10 days and by +15 days in PS and PSv1 in snowbeds (SH), indicating at least for SH the prevalence of local conditions (vegetation type and related ecological niche) over the experiment forcing (**Tab. II.4.G.d.3.2**). At the intra-annual level the experiment didn't show any significative patterns between treatment (**Fig. S23**), except for the delay found in PSv1 in SH, that indicated a prolonged ripening time under an excessive soil moisture.

At the inter-annual level, leaf senescence (LS) of *G. supinum* delayed of +3 days and +2 days in PS and PSv1 in alpine grasslands (CC), while in snowbeds (SH) it delayed of +1 day and +7 days (**Tab. II.4.G.d.3.2**). Within the wide variability, also at the intra-annual level we found the same patterns, with a more intense delay in PSv1, especially in SH, indicating the positive influence of an enhanced soil moisture on LS (**Fig. S23**). However, the magnitude of the delay was much lower than in the OTC deployment, identifying the warming as the key drivers on this phenological stage.

As tested by one-way ANOVA and Tukey post-hoc test, none of these effects resulted statistically significant compared to control (p>0.05).

				SD					Sdisp					LS		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	197	204	217	225	230	211	214	224	245	272	198	208	215	225	247
all	PSv1	201	206	211	219	230	218	218	219	249	260	198	206	218	230	255
all	OTCc	204	206	214	225	230	214	218	219	245	249	190	201	211	230	255
CC	PS	204	204	210	225	225	214	214	223	245	249	198	202	219	225	247
CC	PSv1	204	208	209	214	225	218	218	219	219	249	198	204	211	230	255
CC	OTCc	206	208	210	221	230	214	219	219	245	249	190	198	208	230	255
SH	PS	197	211	219	225	230	211	214	229	245	272	202	210	212	221	247
SH	PSv1	201	205	215	222	230	218	218	234	249	260	202	210	218	230	247
SH	OTCc	204	206	214	225	225	214	218	219	240	249	198	202	211	230	247

Tab. II.4.G.d.3.2 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaves senescence (LS) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *G. supinum* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), at the inter-annual level *G. supinum* advanced the seed development of -4 days in SFtN and delayed it of +10 days in SF+, while in SFtS it didn't change (**Tab. II.4.G.d.3.3**). Also at the intra-annual level, the Sdisp was generally delayed in SF+ compared to the others treatments (SFtN, SFtS). This finding showed and confirmed the sensitivity of this snowbeds species to the snow melt date, more evident than in grasslands species (*L.helveticus, C. curvula*) (**Fig. S24**).

Seed dispersal (Sdisp) at the inter-annual level didn't show any important difference between treatments; compared to control plots, Sdisp delayed of +1 day in SFtN and SF+ and of +3 days in SFtS (**Tab. II.4.G.d.3.3; Fig. S24**).

Leaf senescence (LS) at the inter-annual level was delayed of +8 days in SFtN and of +3 days in SF+, while it was advanced of -3 days in SFtS, indicating the possible occurrence of a topographic effect on this stage, particularly visible in 2015 and 2016 (**Fig. S24**), despite the advance in the south exposed (and warmer) plots was in disagreement with the delays found in the OTC deployment.

				SD					Sdisp					LS		
year	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
2015-2017	SFtN	201	204	206	221	225	214	218	219	233	245	198	202	218	235	258
2015-2017	SF+	204	207	220	233	235	218	218	219	245	245	198	205	213	217	243
2015-2017	SFtS	201	204	210	214	225	214	219	221	240	267	191	206	208	216	225
2015-2017	SFc	197	206	210	221	230	214	218	218	235	249	191	201	211	230	258

Tab. II.4.G.d.3.3 - Descriptive statistics of seed development (SD), seed dispersal (Sdisp) and leaves senescence (LS) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *G. supinum*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

As tested by one-way ANOVA and Tukey post-hoc test, none of these effects resulted statistically significant compared to control (p>0.05).



Fig. II.4.G.d.3.1 – Examples of (a) senescent flower (28/07/2016; DoY 210) and of (b) leaf senescence (LS) of *G. supinum* (26/09/2016; DoY 270).

Comparison manipulation effects on seeds and leaves senescence -G. supinum synthesis

Over the study period, on seeds (SD, Sdisp) and leaf senescence (LS) of *G. supinum* we found influences due to experiments deployment and to climatic variability, but not due to terrain morphology.

The seed development (SD) of *G. supinum* didn't show clear responses to the OTC and PS deployments, while it still presented a delay in consequence of the prolonged snow cover of SF+, indicating the snowmelt as the major driver of this stage of the species.

Unlike to SD, seed dispersal (Sdisp) of *G. supinum* responded to warming with an advanced Sdisp, much pronounced in SH, indicating the higher sensitivity of the species in its typical plant community, characterized by the lower frequency of soil water shortage. At the same time, an excessive soil water availability could prolong the ripening time and could delay Sdisp, as found in PSv1 of SH. To better clarify the influence of soil moisture, a seed viability analysis under different soil moisture conditions would be really useful.

For what concerns the leaf senescence (LS), warming was the most important limiting factor that allowed to lengthen the growing season, with a magnitude of delays in OTC higher than the values reported in plots with increased soil moisture (PSv1). The SF deployment didn't affect the LS, with contrasting pattern of response between years of study.

II.4.G.d.4. Plant height – G. supinum

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the mean plants height (Hmean) of *G. supinum* increased by +3 mm (25%) in alpine grasslands (CC) and +1 mm (5%) in snowbeds (SH) (**Tab. II.4.G.d.4.1**). This was clearly visible in all the years of study and in particular in 2017 in CC (**Fig. S25**).

The comparison of Hmean and Hmean_DoY under warming condition indicated an enhanced size and a prolonged growing period, due to a slower growth rate of *G. supinum*.

The maximum plant height (Hmax) and the day of achievement of that size (Hmax_DoY) in OTC showed the same responses (increase), without any significative difference between plant communities (**Tab. II.4.G.d.4.1**; Fig. S26).

				Hmean						Hmax		
plant	treat	Min	25%	Med	75%	Max		Min	25%	Med	75%	Max
all	OTC	6	9	10	14	25	-	7	13	17	25	60
all	OTCc	4	8	9	10	15		4	10	12	15	35
CC	OTC	6	9	10	12	25		9	15	20	26	60
CC	OTCc	5	7	8	10	12		7	9	12	15	35
SH	OTC	6	10	11	14	18		7	13	17	24	45
SH	OTCc	4	8	10	10	15		4	10	12	17	27

Tab. II.4.G.d.4.1 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in warming (OTC) and control (OTCc) plots of *G. supinum* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

As tested by Tukey post-hoc test (p<0.05), Hmean and Hmax difference between OTC and controls resulted statistically significant at inter-community level and, for Hmean only, at intra-community level in alpine grasslands.

Precipitation Shields (PSs)

During the four years of study (2014-2017), the mean plants height (Hmean) of *G. supinum* increased of +1 mm (13%) in PS only in alpine grasslands (CC) and increased of +1 mm (13%) and +2 mm (23%) in PS and PSv1, in snowbeds (SH) (**Tab. II.4.G.d.4.2**). At the intra-annual level, in CC we didn't find any clear pattern, whereas in SH we found an increase in Hmean in both PS and PSv1 in

all years (except for 2015, when in PS it decreased). The more evident increase in PS (with reference to the one in PSv1) than in OTC, highlighted the absence of any water limitation on the Hmean, allowing to speculate the warming as the main driver for the mean plant size in its typical plant community (**Fig. S27**).

The day of achievement of the maximal mean height (Hmean_DoY) advanced of -11 days in PS in alpine grasslands (CC), while it advanced of -3 days in PS and delayed of +3 days in PSv1 in snowbeds (SH) (**Fig. S27**). At the intra-annual level, we didn't find any pattern in SH, while in CC was the inter-annual variability rather than the experiment deployment to regulate the growth rate, with an advance in warm and dry years (2015, 2017) and a delay in cool year (2014, 2016), in both PS and PSv1.

The maximum plant height (Hmax) in PS experiment showed the same response found in Hmean, with an higher sensitivity in SH than in CC, but with particular higher warming influence of PS (dry and warm) in years characterized by cool and wet summers (2014, 2016), indicating again the importance of warming on this biological variable (**Tab. II.4.G.d.4.1**; **Fig. S28**).

At the intra-annual level, none clear pattern of maximal growth rate (Hmax_DoY) was found, with contrasting responses also in climatically similar years (2014 and 2016; 2015 and 2017) (**Fig. S28**).

				Hmean					Hmax		
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	PS	5	7	9	10	13	7	10	13	16	43
all	PSv1	5	8	8	10	12	6	10	12	15	45
all	OTCc	5	7	8	10	15	7	10	12	15	30
CC	PS	5	7	9	12	12	9	10	13	16	32
CC	PSv1	5	7	8	9	12	7	10	10	15	20
CC	OTCc	5	6	8	10	15	7	8	10	12	30
SH	PS	5	6	9	10	13	7	10	12	18	43
SH	PSv1	6	8	10	10	11	6	10	12	22	45
SH	OTCc	6	7	8	10	10	10	11	12	23	26

Tab. II.4.G.d.4.2 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of *G. supinum* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

As tested by one-way ANOVA and Tukey post-hoc test, only the differences in Hmean_DoY reported for alpine grasslands resulted statistically significant (p<0.05).

Snow fence (SF)

During the three years of snow fence deployment (2015-2017), the snow fence (SF+) induced on G. *supinum* a decrease of -3 mm (-19%) in mean plant height (Hmean) and of -5 mm (-33%) in maximum plant height, confirmed also at the intra-annual level (**Tab. II.4.G.d.4.3**). Moreover, in SFtS we found higher Hmean and Hmax compared to control plots in all the years (**Fig. S29**), confirming the positive influence of the better exposed sites (south exposure) on the plant size and therefore confirming the influence of warming on these variables.

The day of achievement of the maximal mean height (Hmean_DoY) and of maximum plant height (Hmax_DoY) in SF+ advanced of -9 days and of -3 days, respectively, indicating therefore slower growth rate and a smaller size than in control plots (**Fig. S28**, **Fig. S29**). Differently, among the other

treatments, in SFtS we found a prolonged growth period combined with a bigger size, confirming the influence of surface topography and edaphic conditions on these variables).

None of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test.

				Hmean			Hmax						
year	treat	Min	25%	Med	75%	Max	-	Min	25%	Med	75%	Max	
2015-2017	SFtN	5	7	9	10	12	-	6	10	11	25	40	
2015-2017	SF+	5	5	7	9	10		7	10	10	11	22	
2015-2017	SFtS	5	6	10	11	15		9	12	15	18	30	
2015-2017	SFc	5	7	8	10	20		7	12	15	29	36	

Tab. II.4.G.d.4.3 - Descriptive statistics of mean plant height (Hmean) and maximum plant height (Hmax) computed in plots with artificially delayed snow melt (SF+) and in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence for *G. supinum*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

Comparison of manipulation effects on plant height -G. supinum synthesis

During the study period the size of *G. supinum* was mainly controlled by warming that induced an increase in both mean and maximum plant height (Hmean, Hmax) and a prolonged growing period (delays in the days of achievement of the maximum sizes), without any clear influence of soil moisture, as reported in the precipitation shields experiment.

The importance of the warming arose also from the snow fence (SF) deployment, where in the south exposed plots (SFtS) we recorded the highest values of plant size and a lengthened growing period. Differently, the species was negatively affected by a prolonged snow cover, as emerged from the smaller sizes in SF+.





II.4.G.d.5. Reproductive investment and success – G. supinum

Open Top Chambers (OTCs)

During the four years of study (2014-2017), the number of reproductive individuals (flowers_n) of *G. supinum* in control plots was on average 4 (median=1) in alpine grasslands (CC) and snowbeds (SH) of 4 (median=2) as well, with a maximum value of 17 and 20 individuals, respectively (**Tab. II.4.G.d.5.1**). As already mentioned for the flowering and seeds stages, in year 2014 (characterized

by late snowmelt and by cool summer) it must be noted the extremely low number of flowers and how many of those were not able to reach the end of the reproductive stages, due to the extreme climatic conditions. Except for that year, in OTC we found an higher flowers_n of *G. supinum*, particularly visible in its typical plant community (snowbeds), indicating the positive influence of warming on its reproductive effort (**Fig. S30**).

The reproductive success (rep_succ) in control plots was on average 97% (median=100) in both alpine grasslands (CC) and snowbeds (SH), with maximum value up 100% (**Tab. II.4.G.d.5.1**). Overall, in OTC the reproductive success didn't show any clear response compared to control plots (**Fig. S30**).

However, none of these effects resulted statistically significant compared to control (p>0.05), as tested by one-way ANOVA and Tukey post-hoc test.

		rep_succ									
plant	treat	Min	25%	Med	75%	Max	Min	25%	Med	75%	Max
all	OTC	0	0	3	7	70	67	100	100	100	100
all	OTCc	0	0	2	6	20	67	100	100	100	100
CC	OTC	0	0	1	3	40	86	100	100	100	100
CC	OTCc	0	0	1	9	17	67	100	100	100	100
SH	OTC	0	3	5	10	70	67	95	100	100	100
SH	OTCc	0	1	2	5	20	67	100	100	100	100

Tab. II.4.G.d.5.1 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) in warming (OTC) and control (OTCc) plots of *G. supinum* at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Precipitation shields (PS)

During the four years of study (2014-2017), the number of reproductive individuals (flowers_n) in control plots was on average 3 (median=3) in alpine grasslands (CC) and 9 (median=7) in snowbeds (SH), with a maximum value of 15 and 33 individuals, respectively (**Tab. II.4.G.d.5.2**). Also in this experiment, in 2014 we recorded a low number of flowers and an even lower reproductive success, due to the harsh climatic conditions.

At the inter-annual level, this species decreased in all treatments compared to control plots: -50% (-2 n) in both PS and PSv1. Among plant communities, this response was more visible in SH: -71% (-5 n) in PS and -57% (-4 n) in PSv1. In CC was found an increase of +100% in median values of both PS and PSv1 (**Fig. S31**). However, at the intra-annual level was not visible any pattern among treatments in CC, while in SH we found an increase in flowers_n in PS and a decrease in PSv1 in 2015 and 2017, both years characterized by early snowmelt and warm summers. This confirmed how in the communities with general higher soil moisture and cooler temperature (snowbeds) it was soil warming rather than soil moisture that controlled the flowers_n of *G. supinum*, in agreement with the results of the OTC deployment.

The reproductive success (rep_succ) in control plots was on average 90% (median=100) in CC and 98% (median=100%) in SH (**Tab. II.4.G.d.5.1**).

Overall, compared to controls, we didn't find any difference in the reproductive success at the interand intra-annual level between treatments and plant communities (**Fig. S32**). In 2014, most of the flowering individuals didn't reach the seed dispersal, because of the harsh climatic conditions of that years that none of the treatment types were able to counteract.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by one-
way ANOVA and Tukey post-hoc test.

		flowers_n						rep_succ						
plant	treat	Min	25%	Med	75%	Max		Min	25%	Med	75%	Max		
all	PS	0	0	2	5	46		100	100	100	100	100		
all	PSv1	0	0	2	3	15		67	100	100	100	100		
all	OTCc	0	0	3	7	33		50	100	100	100	100		
CC	PS	0	0	1	3	8		100	100	100	100	100		
CC	PSv1	0	0	1	1	3		100	100	100	100	100		
CC	OTCc	0	0	0	4	15		50	100	100	100	100		
SH	PS	0	1	2	5	46		100	100	100	100	100		
SH	PSv1	0	2	3	6	15		67	93	100	100	100		
SH	OTCc	0	2	7	13	33		86	100	100	100	100		

Tab. II.4.G.d.5.1 - Descriptive statistics of number of reproductive individuals (flowers n), number of individuals in anthesis at peak of flowering (MF n) and reproductive success (rep succ) computed in dry+warm (PS), moist (PSv1) and control (OTCc) plots of G. supinum at inter-annual level, for both the plant communities (CC= alpine grasslands; SH=snowbeds).

Snow Fence (SF)

During the three years of snow fence deployment (2015-2017), in G. supinum the number of reproductive individuals (flowers n) in control plots was on average 3 (median=3), with a maximum value up to 15 (Tab. II.4.G.d.5.3). Despite the absence of the snow fence, in 2014 was visible the low number of flowers already reported for many species and in all the experiment types.

Compared to control plots, flowers n increased of +100% (+3 n) in SFtN and decreased of -67% (-2 n) in SF+ and SFtS. At the intra-annual level, a well visible effects, also reported for A. pentaphyllea, was the higher flowers n in SFtN and, only for G. supinum, also in SFtS, whereas in SF+ the flowers n was always lower compared to SFtN and SFtS (Fig. S32). This indicated the negative effect of a prolonged snow cover on the number of flowers of this species, but it indicated also the influence of the surface topography, appreciable in particular in year 2015, when in SFtN, despite the latest SM, the flowers n was still higher than in SF+.

The reproductive success (rep succ) in control plots was on average 97% (median=100) and at the inter-annual level it didn't show any difference with plots with artificial delayed snow melt (SF+) nor with natural delayed snow melt (SFtN, SFtS) (Tab. II.4.G.d.5.3). However, the intra-annual level highlighted a reduction of rep_succ in SFtN and, more pronounced, in SF+ (Fig. S32). Therefore, if these two treatments both showed a decrease in rep succ of G. supinum, only SF+ was not able to increase the flowers n, in disagreement with the results of A. pentaphyllea. At the end, the snow fence deployment (SF+) induced a decrease in both flowes n and rep succ.

None of these effects resulted statistically significant compared to control (p>0.05), as tested by oneway ANOVA and Tukey post-hoc test.

			-	flowers_	n		rep_succ					
year	treat	Min	25%	Med	75%	Max	_	Min	25%	Med	75%	Max
2015-2017	SFtN	0	3	6	11	19	_	71	80	100	100	100
2015-2017	SF+	0	0	1	5	8		0	0	100	100	100
2015-2017	SFtS	0	0	1	8	16		100	100	100	100	100
2015-2017	SFc	0	2	3	3	15		67	100	100	100	100

Tab. II.4.G.d.5.3 - Descriptive statistics of number of reproductive individuals (flowers_n), number of individuals in anthesis at peak of flowering (MF_n) and reproductive success (rep_succ) computed in plots with artificially delayed snow melt (SF+), in plots with naturally delayed snow melt induced by terrain morphology on the north side (SFtN) and south side (SFtS) of the fence and in control plots (SFc) for *G. supinum*, at inter-annual level since the growing season 2015 (the first influenced by the winter deployment of the snow fence).

Comparison of manipulation effects on reproductive investment and success – G. supinum synthesis

Over the study period, in all the three experiment types we were able to find an inter-annual variability in both number of reproductive individuals (flowers_n) and reproductive success (rep_succ), in particular in year 2014 when a delayed snowmelt and a cool and wet summers reduced the flowers_n and affected also their rep_succ.

The number of reproductive individuals (flowers_n) of *G. supinum* increased under warming (OTC), in particular in its typical plant community (snowbeds), whereas the reproductive success (rep_succ) was not affected, in disagreement with the results of *A. pentaphyllea* that decreased the flowers_n but increased the rep_succ.

The soil moisture did not affect these phenological stages, as the treatments of the precipitation shield experiment didn't have any difference, but confirmed how the increase in flowers_n was more effective in the species typical community, characterized by higher soil moisture and cooler soil temperature.

A prolonged snow cover (SF+) negatively affected the reproductive investment and effort, once again in disagreement with *A. pentaphyllea*, indicating a lower plasticity of this species on this quantitative variable. Moreover, the number of flowers was also affected by the topography, in particular by the concavity of the surface rather than its exposure. However, more years of monitoring will better clarify the influence of topography under different snow melt scenario.

II.5. DISCUSSION

After four years of monitoring, we assessed the influences of three different *in situ* manipulation experiments (Open Top Chambers, Precipitation Shields, Snow fence) on environmental data, vegetation composition and structure and plant phenology (in particular on the four most abundant species) of alpine grasslands and snowbeds, communities characterized by the highest regression risk due to climate change (Cannone et al., 2007; Cannone and Pignatti, 2014).

In the former part of this chapter we discuss the inter annual variability of meteorological parameter (II.5.A), of micro-climate and micro-topographical conditions (II.5.B) and of plant phenology (II.5.C), whereas in the latter we focus on the effect of manipulation deployment on environmental data (II.5.D), on vegetation composition (II.5.E) and on plant phenology (II.5.F).

Moreover, in the last part we highlight the points that sustain or reject the initial hypothesis of: (<u>*h1*</u>) a drought ability to buffer the biological answer to soil warming; (<u>*h2*</u>) a snowmelt influence on greening but not on flowering and reproductive phenological stages; (<u>*h3*</u>) a linkage between changes in species abundance and phenology; (<u>*h4*</u>) differential responses due to functional composition, species ecology, plant communities and terrain morphology; (<u>*h5*</u>) an higher sensitivity of alpine species to extreme years rather than to gradual and constant environmental forcing.

II.5.A. Inter-annual variability of meteorological parameters

Since the beginning of the experiment in 2014, we found inter-annual differences in meteorological and climatic parameters recorded at our study sites.

The growing season (GS) length showed an evident inter-annual variability with two years (2014, 2016) with a more delayed start of the growing season compared to the other two (2015, 2017). Moreover, one year (2014) resulted by far more cold then the further three, whereas one (2015) resulted by far more warm and dry than the others.

Despite this, meteorological parameters recorded at the two study sites were comparable, as tested by linear regression model.

II.5.B. Natural variability in micro-climate and micro-topographical conditions

Our study sites are completely comparable in terms of mean altitude (2612 vs 2638 m asl), mean slope (10 vs 11°), lithology (acidic glacial deposits) and plant community types (*Caricetum curvulae* and *Salicetum herbaceae alchemilletosum*).

However, the spatial variability of the ground surface in the alpine environment is very high, because morphology and grain size distribution produce a relief largely variable. Moreover, also the different plant communities and growth forms can influence strongly the ground surface temperature (e.g. **Guglielmin, 2004; Guglielmin et al., 2008; 2012; Gubler et al., 2011**). Because of this, the topographically induced mosaic of micro-climate conditions in an Alpine landscape are associated with local plant species distribution, and such thermal variability can exceed IPCC warming projections for the next 100 years (Scherrer and Korner, 2010; 2011).

In our study, because of the variability of meteorological parameters, we reported a large inter-annual variability in the ground surface temperature of the growing season (GST_GS). More in detail, we

found the occurrence of a significant difference in GST_GS in years (such as 2015, 2016 and 2017) without any significant changes in air temperature. This emphasized the importance of the snow melt (SM) and of the snow cover duration on regulating the GST of the growing season at inter-annual level and confirm their importance in the alpine ecosystems (e.g. **Zhang, 2005**).

Moreover, despite their comparability concerning the climatic, geological, vegetation and geomorphological condition, according to the inter-annual variability in GST_GS we found a wide and natural variability between the two study sites (Site 1, Site 2) that persisted for the whole period of study, confirming the findings of **Scherrer & Korner (2010; 2011)**.

Other than elevation, the spatial variability in GST could be influenced by many parameters, even within a small area: vegetation, thickness of the organic layer, snow depth and/or snowmelt, soil water content, micro-topography, geology and surface grain size as reported by several authors from polar to alpine environment (**Guglielmin et al., 2003; Cannone et al., 2006; Guglielmin et al., 2008; Cannone and Guglielmin, 2009; Gruber and Haeberli, 2009; Gubler et al., 2011; Guglielmin et al., 2012; Pogliotti et al., 2015**).

The absence of any effect due to vegetation coverage was also confirmed by the occurrence in each year of a similar differences of GST_GS within each plant communities and study sites, emphasizing the occurrence of other major factors.

The general aspect of the sites differed, with Site 1 and Site 2 having N-NW and S-SW exposures, respectively. Moreover, the terrain morphology of the snowbeds community in Site 2 was represented by a concavity that influenced the snow accumulation at the end of spring season, the snow melt time and therefore enhanced the GST. Here we found also the highest percentage of soil fine fraction (particles size < 2 mm) and water content (% of weight) of the four trenches excavated.

From the analyses reported above, we concluded that the difference in GST_GS was a structural difference, related to local aspect at the microscale level and to micro-topography features. Therefore the two study sites could be considered as true replicates within the experiment design. However, according to our data, despite the two sites were comparable and could be considered as true replicates, differences in biotic and abiotic elements were detected. For this reason, replication of each experiment types, communities and species were performed in order to better assess the experimental forcing drivers and the biological answers over the natural variability.

II.5.C. Natural variability in plant phenology

Plant phenology is among the most notable, widespread and sensitive indicator of climate change across all biomes (e.g. Walther et al., 2002; Parmesan and Yohe, 2003; IPCC, 2014; Prevey et al., 2017).

Several studies have documented the effect of global warming on the natural variability of plant phenological stages during recent decades in Europe (Menzel and Fabian 1999; Menzel et al. 2006) and North America (Schwartz and Reiter 2000). Some of these studies include data from a large latitudinal and longitudinal range (Chmielewski and Roetzer 2001; Menzel et al. 2006; Ernakovich et al., 2014; Prevey et al., 2017), while others have considered altitudinal differences over a short latitudinal range (Migliavacca et al. 2008; Vitasse et al. 2009; Ziello et al. 2009; Moser et al. 2010; Cornelius et al., 2012).

II.5.D. Effect of manipulation deployment on environmental data

II.5.D.a. Open Top Chambers (OTCs)

According to our data, the OTC deployment significantly increased the mean ground surface temperature (GST) of the growing season by $+0.5^{\circ}$ C, with intra-annual warming up to $+1.4^{\circ}$ C. With these results, we confirmed the suitability of the International Tundra Experiment (ITEX) hexagonal green house for passive warming experiments (Molau and Mølgaard, 1996; Marion et al., 1997) also for tundra sites of the Alps range.

This warming effect was comparable with the increasing air temperature reported at different spatial scale (IPCC, 2014; Cannone et al., 2007; Rebetez and Reinard, 2008) and was comparable with the warming expected in the next century (IPCC, 2014). Therefore, the biological answers that we reported in our manipulated plots are those that we could expect to see *in situ* in natural conditions under such a warming scenario.

To our knowledge, we reported and quantified for the first time the difference in GST between the edge and middle of the open top chambers that could be used for the evaluation at the small scale of the biological answer along a temperature gradient.

Indeed we confirmed that OTCs are a reliable, cheap, and easy to deploy and maintain method for simulating a warmer climate (**Aronson and McNulty, 2009**), particularly in this remote or un-staffed location without power supply. However, they might potentially cause confounding effects on microclimate variables that could influence the response of plant species to the warming treatment, such as modifications in wind patterns around the study plants (**Marion et al., 1997; De Boeck et al., 2012**), changes in soil moisture and vapor pressure deficit (**Wookey et al., 1993; Marion et al. 1997; Piiki et al. 2008**) and decrease of photosynthetically active radiation (**Debevec and MacLean, 1993** but see **Bokhorst et al., 2011**).

From field measurements in spot days, we reported the influence of OTCs on wind speed, that started to decrease just below the edge of the chambers and reached the lowest values close to the ground surface. This reduction in wind speed was one of the mechanisms by which GST in OTCs was warmer than outside (**De Boeck et al.,2011**). Moreover, during the summer season 2017, simultaneously with measurements of carbon ecosystem exchange, soil temperature and soil moisture measurements were conducted in both OTCs and control plots. Between chambers and controls, the warming effect was confirmed, while the values of moisture didn't show significant differences (personal communication N. Cannone). Therefore we could exclude any influence of the OTCs deployment on the soil moisture. To understand better this process, as future remarks, it will be of great interest and importance to perform long-term continuous monitoring of PAR, soil moisture and wind speed inside an outside of the chamber.

II.5.D.b. Precipitation Shields (PS)

Extreme drought events challenge ecosystem functioning and are predicted to increase in frequency and magnitude (**IPCC**, **2014**). Manipulation experiments in the field are powerful tools for the causal understanding of ecological processes in the face of climate change and provide complimentary tool that enable replication and control for confounding factors (**Beier et al.,2012**; **Smith et al., 2014**).

However, information on the impacts of changing water regimes through *in situ* rain-out shelter are only recently starting to be used worldwide (**Kreyling et al.,2017**), with a really low amount of data for tundra vegetation (**Beier et al.,2012**).

Our rain exclusion experiment (PS) could be classified as pulsed full exclusion (**Kreyling et al.,2017**): a complete precipitation withdrawal for defined periods of time (growing season), realized by fixed rain-out shelters.

In the two rain exclusion experiments equipped with sensors of volumetric water content (VWC; m^3/m^3), the occurrence of the expected pattern of soil moisture was not confirmed for all the years with available data (2015-2017). That is probably due to the co-occurrence of winds during the rain events, that allowed the rain to reach also the plots underneath the shields, and because of the influence of the precipitation shield in the redistribution of rain.

However, among the three plots (plus control) installed for each precipitation shields we were able to identify the two of those (PS, PSv1) where the soil moisture extremes were most frequent and to use those for the assessment of soil moisture impacts on the biological components.

Any experiment is inevitably associated with artefact. Rain-out shields are often criticized for unwanted side-effects on microclimatic conditions such as reduced wind speed, altered radiation and warming (Fay et al.,2000), but a detailed quantification of such microclimatological artefacts is still missing (Kreyling e al.,2017).

Our results allowed to fill the gap in knowledge and provided the evidences and the quantification of the warming effect underneath the precipitation shield that, on average, was of +0.8°C in GST, but with values up to 1.5°C in very hot and dry year (2015). The warming was comparable and even higher than the one reported for the OTCs. For this reason, we have further used the plots underneath the shields (PS) as indicative of warm and dry condition, instead of only dry condition.

The continuation of this experiment and the implementation of the monitoring sites of soil volumetric water content, will help in the future to better disentangle the influences of the precipitation shields on soil moisture and to address the "dilemmas" that still are unsolved on this type of field experiment (**Beier et al.,2012**).

II.5.D.c. Snow fence (SF)

There is still uncertainty on how climate change will influence snow cover in cold regions. Although snow amount is expected to increase in the Arctic and at high altitudes on mountains, snow cover duration will probably decrease in sub-arctic regions and at mid- to low altitudes (IPCC, 2014).

Studies that experimentally explored the effects of snow cover changes on arctic and alpine plant communities had often used snow fences, which accumulate snowdrifts in their leeward side and thus increase snow depths and extend snow cover duration (e.g. Cooper et al.,2011; Johansson et al., 2013; Rumpf et al., 2014; Legault and Cusa, 2015; Semenchuk et al.,2016).

Most of the researches in artic and alpine tundra used the delayed snow melt as treatment, obtained through a snow fence deployment (**Wipf and Rixen, 2010**). Other studies have investigated the advance or both the delay and advance in snow melt, using open top chambers (e.g. Petraglia et al.,2014) or through shoveling (e.g.: Gerdol et al.,2013; Bombonato and Gerdol, 2012). However,

only a limited number of studies used snow fence in alpine terrain (e.g. Seastedt and Vaccaro, 2001; Wipf and Rixen, 2010) and no one on the Alpine range, to our knowledge.

Our results confirmed the suitability of the snow fence for a snow manipulation experiments in an alpine tundra ecosystem. The effect exerted by the fence was an artificial delay of the SM from 5 up to 10 days compared to control plots, with a longer permanence of the snow cover on the summit of the moraine ridge.

Thanks to the complexity of alpine terrain, beside the artificially delayed snow melt (SF+) we had part of the monitoring plots those showed a natural delayed snow melt (SFtN, SFtS) comparable in magnitude to those of SF+, because of their position in topographically low areas. Therefore, compared to most of the researches those involved artificially delayed or advanced snow melt *versus* control plots, in our study we were able to compare artificially induced snowmelt with natural snowmelt and to assess their different influences on the biological component.

Moreover, conversely to most of the studies with a snow fence deployment (**Wipf and Rixen, 2010**), our experiment is the first one that combined the GST and digital images from cameras to assess the snow redistribution and to identify the snowmelt day. The combined analyses of these two source of data would be useful to validate the application of GST as a proxy for the snowmelt date in our study sites (e.g. Schmidt et al., 2009; 2012; Danby and Hick, 2007; Gadek and Leszkiewicz, 2010; Mutter and Phillips, 2012; Jean and Payette, 2014).

Future remarks should take into account the installation of a new line of snow stakes, that run parallel to the already installed three lines but outside of the influence of the fence, in order to give for each growing season the natural variability of snowmelt and snow depth along the entire moraine profile. This will be of big importance to validate the assignation of the treatments (SF+, SFtN, SFtS) to each of the plots of this experiment.

In order to increment the replication and enhance the data quality, it will be useful the installation of at least two more snow fence. However, on this latter part we are conscious of the difficulties of such an implementation in a roughness terrain, like here in the Alps. Moreover, the study area is located within the Stelvio National Park and in order to avoid disturbance to local wild life and flora, the installation of snow fence experiment was limited in space.

II.5.E. Effect of the manipulation experiments on vegetation composition

Changes in species composition in alpine and arctic plant communities have already been recorded (Capers and Stone, 2011; Erschbamer et al., 2011; Callaghan et al., 2013). Many long-term studies have shown that vegetation abundance and dominance have changed in response to climate change (Sturm et al., 2001; Cannone et al.,2007; Myers-Smith et al., 2011; Cannone and Pignatti, 2014; Hobbie et al., 2017), despite the extreme longevity of many alpine species is able to counterbalance the impact of environmental forcing on species composition and abundance (e.g. Windmaißer and Reisch, 2013).

In the following paragraph I discuss the changes in the main vegetation layers and growth forms, as well as in the species selected for the phenological analyses, in order to detect the effects of the manipulation experiment after four years of deployment (3 year for the snow fence) and to give a supporting information for the interpretation of the phenological monitoring data.

II.5.E.a. Open Top Chambers (OTCs)

The past few decades have experienced dramatic shifts in vegetation composition, biomass and diversity in several polar and alpine tundra sites and ecosystems (e.g. Grabherr et al. 1994; Sturm et al.,2001; Tape et al. 2006; Cannone et al.,2007; Hudson and Henry 2009; Cannone and Pignatti, 2014; Cannone et al.,2016) as a results of recent climate warming.

Because of this particular sensitivity to climate change of the tundra biome, recently there has been an increasing numbers of warming experiments that aimed to assess changes in vegetation composition and structure (e.g **Elmendorf et al., 2012a, 2012b**). This experimental warming studies have offered different results, with vegetation changing, not changing or changing in unexpected directions.

Evidences of species loss have been reported in several warming experiments (Wahren et al., 2005; Walker et al., 2006; Hudson and Henry 2010).

After 4 year of passive warming through OTCs our results showed the occurrence of a moderate changes in the total species richness, with the ingression of 2 new species (*Agrostis schraderana*; *Anthoxanthum odoratum*) compared to control plots. This finding confirm the responsiveness of the alpine ecosystem to an increased temperature, much more pronounced than in the lower elevational belt (e.g. **Anadon-Rosell et al., 2017**).

In agreement with large scale studies (e.g Elmendorf et al., 2012), among the two plant communities we found a general decrease of cryptogamic layer (mosses and lichens) under warming condition, confirming the sensitivity of this vegetation layer to soil warming (e.g. Walker et al., 2006; Gerdol and Brancaleoni, 2015), especially with the co-occurence of vascular plants species (Alatalo et al., 2017). A decrease in cryptogams coverage is able to modify soil properties, decreasing the litter decomposition, because of the lower decomposition rate of tissues compared to those of vascular plants (Hobbie et al., 2000; Faquiang et al., 2010).

Alpine grasslands resulted less sensitive to soil warming compared to snowbeds communities. Indeed if in the alpine grasslands the total coverage as well as the coverage of the main growth forms didn't change, in the snowbeds we reported an increase in total coverage, mainly due to an increment of forbs, in agreement with large-scale studies (e.g. **Elmendorf et al.,2012**). Differently to most of the previous results and evidences (i.e. **Sturm et al.,2001; Cannone et al.,2007**), shrub cover decreased. However it must be noted how here the shrubs layer was composed only by the prostrate dwarf shrub *Salix herbacea*, species well known to be strictly negative affected by climate change (Wheeler et al., 2016) and its indirect consequences (Little et al., 2016).

The warming forcing on the vasculars layer changed among species and plant communities. Other than the already mentioned *A. pentaphyllea* and *S. herbacea* and for *C. curvula* and *G. supinum*, soil warming had a positive effect on *L. helveticus* that increased its coverage mainly in grasslands but with an enhanced abundance in snowbeds as well.

II.5.E.b. Precipitation Shields (PS)

Despite in the results we reported the changes in species richness and abundance of all the plots within the rain exclusion experiment (PS, PSv1, PSv2, PSc), concerning the abiotic gradients obtained with this experiment, in particular the soil moisture difference among plots, here we decided to discuss

only the changes in species richness and abundance found in warm and dry (PS) and moist (PSv1) plots, as we already did in the results concerning the impact on the phenological stages.

Soil moisture is an important determinant of community composition, and generally mesic sites have the greatest species diversity and are most responsive to environmental change (e.g. **Grime, 2001; Korner, 2003; Walker etal.,2006**).

In many places, soil moisture determines the local distribution and productivity of alpine plants (Jonas et al., 2008, Engler et al., 2011) and can determine how responsive or resistant a community will be to increased temperatures (Walker et al., 2006, Pauli et al., 2012).

After 4 year of PS experiment our results showed the occurrence of a moderate changes in the total species richness, with the ingression of 2 new species and the disappearance of 1 species in the warm and dry treatment (PS).

In the frame of substantial unchanged total coverage, among the two plant communities we found a general decrease of cryptogamic layer (mosses and lichens) under warm and dry conditions, confirming for these component a strong sensitivity to moisture availability.

Moreover the results showed a general increase in vascular coverage (up +20% of relative cover), independently by treatment and plant communities. Among growth forms, the results confirmed the variation of graminoids coverage depending on the soil moisture status (Elmendorf et al., 2012), with a more evident increase in graminoids coverage in PSv1 (moist) compared to PS (warm and dry). Forbs were considered to be less responsive to temperature and moisture (Elmendorf et al., 2012): conversely, our results demonstrated a consistent decreasing abundance in warm and dry conditions, in both plant communities, in agreement with De Boeck et al. (2011, 2016), mainly associated to a decrease of *A. pentaphyllea*. Shrubs, in this experiment only composed by *S. herbacea*, seems not responsive by the treatment, but showed a slightly enhanced coverage in moist condition. This is in agreement with wide long-term experiments and monitoring, in which total shrub expansion due to warming was mainly located in regions with moist or wet soils (Elmendorf et al., 2012), as well as with landscape level research (i.e. Pouliot et al., 2009).

The influence of a warm and dry condition (PS treatment) on our growth form is not clear and consistent within growth forms. Differently, in situation of increased soil moisture (PSv1) graminoids seems able to outcompete forbs (confirming **Elmendorf et al.,2012**), especially in grasslands, but its interaction with shrubs need to be better assessed.

This finding confirms the responsiveness of the alpine ecosystem to an increased temperature, much more pronounced than in the lower elevational belt (e.g. **Anadon-Rosell et al., 2017**).

Consistent with other alpine sites (e.g. Winkler et al., 2016), our experiment's results suggest that the effects of future, warmer temperatures on alpine productivity are largely contingent upon available growing season soil moisture and underlined how the magnitude of drought effect is species-specific and plant species combination-dependent, as shown for other water limited environments (i.e. Rodriguez-Ramirez et al., 2017). However, patterns in responses can vary also depending on the interaction between drought sensitivity and rainfall event size and timing (i.e. Cherwin and Knapp, 2012).

II.5.E.c. Snow fence (SF)

In the longest running snow manipulation experiment to date (also in the Arctic alpine), **Wahren et al. (2005)** found that after 8 years of additional snow, overall plant diversity had decreased significantly, with the most substantial losses among the bryophytes and lichens.

The length of the growing season is an important limiting factor for total vascular coverage, that increased in early-snow condition (e.g. **Carbognani et al.,2012**), in terms of coverage as well as of height (Johansson et al., 2013). A review of papers on snow manipulation experiments in Arctic and alpine tundra ecosystems by **Wipf and Rixen (2010)** concluded that increased snow accumulation resulted in a decrease in graminoid productivity and abundance, while in the subalpine belts an advanced snowmelt enhance shrub growth, but only in years low frequency of frost events (**Gerdol et al.,2013**).

From our results, compared to the natural delayed snow melt, a future shortening of the growing season would led to a decreasing vascular coverage, due to a reduction in shrubs firstly and graminoids secondly. However, here changes in absolute coverage are less important than in OTC or PS experiments, and none of those resulted statistically significant (perhaps due to the relatively short temporal period of the experiment), indicating a slower response of alpine vegetation to an altered snow melt pattern.

Within the frame of low changes, snowbeds species (*A. pentaphyllea* and *G. supinum*) resulted the only two species that increased in coverage, confirming their ability to respond and benefit from a prolonged snow cover (e.g. **Carbognani et al., 2012**).

II.5.F. Effect of the manipulation experiments on plant phenology

Shifts in phenological events due to climate change have been documented in numerous global-scale studies (**Parmesan and Yohe, 2003; Menzel et al., 2006; Cleland et al., 2007**).

Phenological plasticity may play a crucial role in the adjustment to the novel conditions and can promote long-term adaptive evolution by buffering against rapid change (**Price et al., 2003; Richter et al., 2012; Gugger et al., 2015**).

Many studies have used *in situ* warming manipulation experiments (OTCs) to assess and quantity the phenological changes under a warming scenario (i.e. Ernakovich et al.,2014; Prevey et al.,2015), from the alpine and mountain regions (i.e. Erschbamer, 2007; Carbognani et al.,2016; Dorij et al.,2013; Meng et al.,2016;) to the arctic tundra (i.e. Klady et al.,2011; Bjorkman et al.,2015; Rosa et al.,2015).

Unlike the OTCs, to date there has been only one formal meta-analysis published on the results of precipitation manipulations through rain out shelters (**Wu et al., 2011**) and few other through water table manipulation (e.g. **Cornelius et al., 2014**). Moreover, only a really limited amount of researches were conducted on tundra vegetations (**Beier et al., 2012**). Our research must be seen in this frame of data gaps on the answers of alpine species to a changing water availability.

If **Sherry et al. (2007)** and **Cornelius et al. (2014)** report that changes in water table level had almost no effect on plant phenology, our results gave important evidences of the different answers of species and plant communities to an altered soil moisture availability and indeed underlined their different sensibility. Moreover, the founding of a warming effect underneath the shields, allowed us to use this treatments (PS) as representative of a dry and warm future scenario and compare the reported biological answer with those from OTC, representative of a warming alone. Changes in snow cover characteristics can considerably influence plant growth and performance. For this reason another topic largely studied by experiments is the influence of snow cover depth and snowmelt on the vegetative and reproductive phenology of alpine and arctic species ((i.e. Cooper et al., 2011; Johansson et al., 2013; Rumpf et al., 2014; Legault and Cusa, 2015; Gillespie et al., 2016; Semenchuk et al., 2016).

In the following paragraphs we will discuss the influence of our manipulation experiments (OTCs, PSs, SF) on plant phenology, to assess the potential phenological responses of alpine species to different environmental drivers in a future climate change scenario.

II.5.F.a. Snowmelt and greening

The timing of snowmelt is an important determinant of the beginning of above-ground plant activity on the tundra (**Molau et al. 2005**). In a cold environment, an advanced snowmelt could be beneficial for plant growth and reproduction, but only if coupled with favorable climatic conditions (e.g. **Gerdol et al.,2013**).

Since the precipitation shields were positioned after the snowmelt, when the greening was already started and/or completed, no information can be provided on these stages by the PS deployment and the differences that we reported are included in local natural variability of these phenophases.

Despite the inter-annual variability in snowmelt date, according to our data the OTC deployment did not influence the snow melt pattern, even if in the first snow fall events in Autumns we personally found a potentially snow trap effect, as reported in polar environments (e.g. **Bokhorst et al.,2013**), although the duration of this effect was very limited in time.

However, using GST as a proxy for snow occurrence (e.g.: Schmidt et al., 2009; 2012; Danby and Hick, 2007; Gadek and Leszkiewicz, 2010; Mutter and Phillips, 2012; Jean and Payette, 2014), our data allowed to exclude any effect of the OTC on snowmelt and snow coverage onset. Because the vegetative stages are consistently associated with the snowmelt, as emerged from both monitoring (i.e. Gerdol et al., 2013; Julitta et al., 2014; Wheeler et al., 2016) and manipulative experiments (i.e. Cooper et al., 2011; Legault and Cusa, 2015), we could exclude any combined effect of longer snow cover and warming on the begin of the growing season in the OTCs and therefore avoided any external influence on the mono-factorial experiment design.

Unlike the OTC, the snow fence deployment strongly affected the snowmelt onset in all the species analysed, with a delay comparable to those found in other study sites (i.e. **Rumpf et al.,2014**; **Legault and Cusa, 2015**).

In the last few decades the earlier snow melt was reported by several authors at different spatial scales, from satellite data (e.g. Jeong et al., 2011; Garonna et al., 2016; Mao et al., 2016) and from climatological analyses (e.g. Kunkel et al., 2016; Klein et al., 2016). The onset of plant growth and the greening stages are strongly determined by the patterns of snow melt in Arctic and alpine regions (Arft et al., 1999; Steltzer et al., 2009; Wipf and Rixen, 2010; Julitta et al., 2014; Chen et al., 2015; Filippa et al., 2015; Livensperger et al., 2016; Vitasse et al., 2016). However, plants that start early the growing season because of an advancing snow melt may be susceptible to frost damage if temperatures remain cold or freezing events occur (Inouye, 2008; Wipf et al., 2009). This is because the temperature soon after the melting is of crucial importance.

Our results confirmed these findings, showing a close linkage between snowmelt and first shoot (FS) appearance in all the analyzed species, except for *A. pentaphyllea* that under warming conditions was already able to advance the onset date.

Similarly to FS, new leaves (NL) generally follows the snowmelt variability, but it advances also under warming, except in this case for the other snowbeds species *G. supinum*, that advanced only in years with early snowmelt, confirming therefore the importance of the air and soil temperature soon after the snow melt date and the differential ability to cope with the snowmelt between species.

II.5.F.b. Flowering

Changes in temperature associated with climate warming are expected to affect tundra plant sexual reproduction (e.g. Ernakovich et al.,2014; Prevey et al.,2015).

Our results confirmed the occurrence of a strong advances in flower bud (FB) and peak of flowering (MF) in all the selected species, both in alpine grasslands and snowbed communities, confirming the prevalence of the warming effect on other possible factors (i.e. **Petraglia et al.,2014; Carbognani et al., 2016**), such as photoperiod (e.g. **Keller and Korner, 2003**), vegetation type (e.g. **Kopp and Cleland, 2015**) or local environmental conditions.

However, a different sensitivity to drought was detected between species, with alpine grasslands species (*L. helveticus*, *C. curvula*) more affected than the snowbeds ones (*A. pentaphyllea*, *G. supinum*). Indeed, in the firsts two while the warming alone induced an advance in FB, the combination of heat and drought induced a delay, supporting two of our hypotheses (<u>*h1*</u>, <u>*h4*</u>).

The lacking of any reproductive individuals of *G. supinum* in any of the experiments (OTCs, PSs, SF) in year 2014, characterized by extremely short and cold summer, confirmed the warming limitation on these stages and underlined the particular sensitivity of the species to such extreme condition. Despite effects depended on species, the type and magnitude of the event and the period of the growing season (i.e. **Orsenigo et al.,2014**), this finding support our fifth hypothesis (<u>*h*5</u>) and the conclusion that frost, combined with an earlier snowmelt, is likely to be an important factor affecting the flowering (**Inouye, 2008**), with important demographic consequences.

Our results indicated also a snowmelt control on flowering, visible for *C. curvula* and, even more clear, for *A. penthaphyllea*, those delayed the onset dates in response of a prolonged snow cover, The results confirmed how, without any other external factor (such as warming or topography), the date of snowmelt was significantly and strongly related to the flowering time (e.g. **Bijorkman et al., 2015**), despite it is known to not likely cause any changes in flowering synchrony (e.g. **Bienau et al., 2015**).

However, other species, such the two Asteraceae (*L. helveticus*, *G. supinum*), were able to recover quickly to a later snowmelt, possibly thanks to a flower bud pre-formation, as documented for many arctic and alpine species (i.e. **Diggle, 1997**; **Meloche and Diggle, 2001**; **Larl and Wagner, 2006**; **Ladinig and Wagner, 2009**; **Semenchuk et al., 2013**).

II.5.F.c. Seed development and leaf senescence

For a proper seed formation there must be enough time, thermal energy and resources (Körner, 2003). Species-specific differences were found in the final part of the reproductive process.
For *L. helveticus* and *C. curvula* the advancement of the reproductive stages under warming was maintained up to the seed dispersal (Sdisp), indicating an energy threshold that regulates begin and end of the reproductive stages (**Hulber et al. 2010**; **Petraglia et al.,2014**). In support of our first hypothesis (<u>*h1*</u>), drought had a further influences on the specific response to warming, indicating a higher drought sensitivity in their typical plant community (alpine grasslands), already characterized by a warmer soil with lower water content, than in their sub-optimal community (snowbeds). Indeed, both *L. helveticus* and *C. curvula* delayed the seed dispersal under drought and warming,

conditions too extreme for this phenolgical stages that otherwise require higher amount of water and nutrient accumulation for a successful future seeds viability (e.g. Bernareggi et al.,2016).

Differently from the previous two, from our results the seed development and dispersal of snowbeds species (*A. pentaphyllea*, *G. supinum*) were mainly related to snowmelt and warming limitation, together with an increasing influence also of the surface topography, thus supporting the hypothesis of a snowmelt effect on the late phenological stages (<u>*h2*</u>) and the differential responses between species and plant communities (<u>*h4*</u>).

For what concerns the leaf senescence (LS), in contrast with the effect on many of the other qualitative phenological data, under warming condition (OTC) we observed a general delay of LS, and thus a lengthening of the growing season (e.g. **Menzel et al., 2006**) in all of the species, meaning that alpine plants are able to profit of an extended growing season due to Autumn warming. *C. curvula* was the most affected species, in both CC and SH emphasizing the plasticity of the graminoids growth form (**Cannone and Pignatti, 2014; Elmendorf et al., 2012**).

Moreover, from the results of the precipitation shields, as well as from the comparison with the warming experiment, we reported a drought limitation to leaf senescence (LS), that confirmed our first hypothesis (<u>*h1*</u>) and highlighted how under the double forcing of warming and drought, alpine species would reduce the growing season length. This is in agreement with the responses of deciduous species from lower elevational belts (e.g. **Schuster et al.,2014; Estiarte and Peñuelas, 2015**), but is in contrast with other alpine study that reported an unaffected plant senescence under warming conditions (**Shi et al.,2015**).

As we have just reported, later phenophases are generally controlled by temperature rather than snowmelt (**Wipf, 2010**), despite some findings indicated a longer lasting green leaves in relation to the increased snow depth (**Johansson et al., 2013**).

Our results indicated how the influence of the snowmelt went lost for the leaf senescence and indicated a more intense relation with the local topography (Scherrer and Körner, 2010; 2011). However, in some cases it was reported a lengthening of the growing season as consequences of an altered snow melt (Menzel et al., 2006), as we found in the snowfence experiment for A. *pentaphyllea*.

II.5.F.d. Plant height

Other vegetative factors could influence winners and loosers under climate change. For instance, flowering can be affected indirectly by climate through its influence on plant size, since reproduction is typically size-dependent, and plant size is known to be affected by temperature and water availability (**Bloor et al., 2010; Kardol et al., 2010; Meineri et al., 2014**). Moreover, once a minimum size for flowering has been reached, flowering probability and flower production often increase with plant size (**Obeso, 2002**).

Plant height is one important plant functional traits (PFTs) that could influence inter-specific interaction (e.g. **Cornelissen et al., 2003**), as it is correlated with competitive ability in light capture (e.g. **Westoby et al., 2002; Hudson et al., 2011; Anadon-Rosell et al., 2014**).

The responses in plant height in a warming condition could differ among species and growth forms (**Hudson et al.,2011**). Our analysis confirmed a general increase in size, with specific differences between the selected species. The sizes of *A. pentaphyllea* and *C. curvula* increased, in percentage, more than those of *G. supinum* and *L. helveticus* and indeed the first two species resulted as possible winners under climate warming scenario.

However, if for *A.pentaphyllea* the increment in size was supported by an increased abundance in its typical communities, for *C. curvula* at the moment we didn't find agreement between plant height and cover changes between year 2013 and 2017, that decreased in both grasslands and snowbeds.

Starting from our four year of warming experiment, we can forecast that in a long term scenario these two species would keep increasing their sizes within each typical community, with consequences on resources availability, competitive interactions and then on species composition (e.g. **Westboy et al.,2002**).

Similarly of few of the previous phenological stages, warming is the most important limiting factor of plant size, but drought is able to limit or counterbalance it, especially in term o maximum size, supporting again our first hypothesis (<u>h1</u>). Moreover, it must be also remembered the negative influence of an excess of soil moisture on plant size of *L. helveticus*.

The influence of a delayed snow melt could have different responses to the vegetation size, but most of the results reported a general increase size (Walker et al.,,2006; Johansson et al.,2016) or above ground biomass (Carbognani et al.,2012).

In our study, the positive influence of a delayed snow melt was overcame by local terrain morphology (Scherrer & Korner, 2010; 2011). Indeed, on the top of the moraine where the artificially delayed snow melt occurred, all the selected species grew less (or at least didn't grow, compared to control) in mean size, possibly because of the high exposition to the wind effect.

The maximum plant size confirmed the lower increase (or higher decrease) on the top of the moraine, especially for *C. curvula* and *L. helveticus*, that are the two species those reach the maximum plant height and, therefore, more influenced by the exposure at the top of the moraine.

Moreover, in the natural delayed snow melt areas, different exposure allowed to species with different ecological requirements to grow more than in control plots. For instances, the chionophilous *G. supinum* grew less in SF+ treatment and more in SFtN and SFtS; differently, *L. helveticus* grew less in SFtN and SFtS and more in SF+.

However, to disentangle or to better understand the relation with snowmelt and terrain morphology and their influences on plant growth, it would be useful a new line of control plots outside the influence of the snow fence but that crosses all the topographical gradient, in order to disentangle the topographical effect from the manipulative effect.

II.5.F.e. Reproductive investment and success

Other important information on the possible responses of alpine plant to climate are observed in the quantitative data at the species level. Indeed, the onset days of each phenological stages are good indicators of the answers to environmental drivers, but the number of reproductive individuals (flowers_n) and the reproductive success (rep_succ) provide a more accurate assessment of the reproductive effort and success and, therefore, of the possible future persistence of a species in the

environment under future climate warming. Indeed, reproduction by seed is a key event for plant population persistence and species range dynamics.

Warming could significantly decrease flower density, as reported in few studies in the arctic tundra (i.e. **Ellebjerg et al.,2008; Høye et al., 2013**). We observed different responses among the 4 species representative of the two plant communities. We identified *A. pentaphyllea* as the most negatively affected species and identifying this species as possible loser in a climate warming scenario. However, its coverage increased between 2013 and 2017. This apparent anomaly, can be explained by the ability of this species to perform lateral growth, that allowed to increase in coverage even if its reproductive success is declining. The changes in ratio between sexual and clonal reproduction must be considered in the evaluation of the climate change sensibility both at species and community levels (e.g. **de Witte and Stöckli, 2015**). Moreover, even if *A. pentaphyllea* decreased its flowers number under warming, the reproductive success was enhanced compared to control plots.

Unlike to the previous species and in agreement with other studies in the Arctic (i.e. **Kopp and Cleland, 2015**), *L. helveticus* and *C. curvula* resulted the species with most enhanced number of reproductive individuals and reproductive success under warming conditions, in both snowbeds and grassland communities, underling the ability of this grasslands species to invade and persist in new communities, in agreement with the reported climate change impacts in the Stelvio pass area. Indeed, our study site is located within the altitudinal range where alpine grasslands are invading snowbeds (**Cannone et al.,2007**), thus confirming the climatic signal in the enhanced reproductive investment of *C. curvula*, and in agreement also by the promoted seed production and size in warmth and longer growing season found in other alpine sites (i.e. **Wagner and Reichegger, 1997**).

The small *G. supinum* is a species that was not influenced by warming in terms of reproductive success, that remained high in OTC and control plots as well. However, warming increased its number of reproductive individuals confirming this species as a possible winner in a climate warming scenario (e.g. Hulber et al.,2010; Petraglia et al.,2014). Its low coverage and its low number of reproductive individuals (as absolute number) in alpine grasslands, allow to suppose a different performance in the future between the optimal (snowbeds) and sub-optimal (grasslands) conditions. Moreover, the increasing coverage of other species, such as *A. pentaphyllea*, allows to assume a future increase competition for space and nutrient uptake.

Also soil moisture, along with temperature, is a key element for the reproductive investment and reproductive success (**Körner**, 2003; **Bernareggi et al.**, 2016). From our results we confirmed the influence of moisture on the reproductive investment and success, but with difference among species and their typical ecological requirements.

If for the grasslands species we didn't find any drought influence on the reproductive effort, the snowbeds species showed an increased sensibility to moisture, especially in their typical plant community. Indeed, *A. pentaphyllea* increased flowers_n in the moist treatment (PSv1) of the precipitation shield experiment, whereas in *G. supinum* it increased more in snowbeds than in alpine grasslands under the OTC deployment, indicating a preference for the cooler soil temperature and higher water availability.

Reproductive responses to snowmelt manipulation are quite variable, ranging from increased fecundity (**Wipf, 2010**) to decreased floral abundance (**Ellebjerg et al.,2008**), up to a complete insensitivity (**Cooper et al. 2011**).

Our results showed how during the years of study a shorter growing season due to both natural and artificial snowmelt lead to a general decrease flowers number (<u>h2</u>) but with responses species-specific, supporting the forth hypothesis (<u>h4</u>).

Indeed, as expected, the snowbeds species resulted more adapted to a prolonged snow cover (**Carbognani et al.,2012**; **Petraglia et al.,2014**), both in natural and artificial delay conditions; otherwise, the alpine grasslands species showed a lower attitude and plasticity.

As previously mentioned for the flower density, also the final reproductive success of a species could have variable responses to a delayed snowmelt, depending on the site and on the duration of the experiment (e.g. **Cooper et al.,2011; Legault and Cusa, 2015**).

Our results reported how the reproductive success of the snowbeds species were affected by snowmelt (**Carbognani et al.,2012**; **Petraglia et al.,2014**), but with a differential direction; if in *A. phentaphyllea* it increased, in *G. supinum* it decreased. Differently, in both the alpine grasslands species, the reproductive success were more affected by the topographical position and by their ecological niches.

An extremely advanced snowmelt, in extreme years, usually increases the risk of frost events (**Inouye 2008**), therefore frost damages could negatively affect the development of reproductive structures of Arctic and alpine plants occurring earlier than usual (**Ladinig et al. 2013**).

Here we found that in particularly cold year (2014), with delayed snowmelt but with cold summers, all the species decreased the flower density and in some cases, such for *G. supinum*, the reproductive structure completely lacked, confirming how extreme weather events are able to alter the flowering phenology of alpine species (i.e. **Inouye, 2008**; **Jentsch et al., 2009**; **Orsenigo et al., 2014**), in support of our fifth hypothesis (<u>h5</u>).

As species shift their ranges in response to climate change (i.e. Walther et al., 2002; Root et al., 2003; Lenoir et al., 2008), novel species interactions (such as shading by newly arriving overstory species) could arise with the potential to alter phenological and performance responses to climate change (i.e. Elzinga et al., 2007; Kopp and Cleland, 2015).

Indeed, interspecific competition for resources could also influence phenology; for instance shading by overstory species can delay flowering and reduce reproductive success in understory species (i.e. **Galloway and Etterson, 2009**).

The alteration of temperature, water supply and growing season length due to climate change could preclude, delay or enhance regeneration from seeds (**Walck et al., 2011**) and factors that affect the ability of seeds to develop into adult plants are likely to affect the fate of plant populations and communities (i.e. **Mondoni et al., 2012**).

Vegetative variables (Hmean, Hmax) and quantitative reproductive variables (flowers_n, rep_succ) are indeed indicators of the ability of the species to respond to climate and, together with the soil seeds bank and seeds viability (i.e. Marcante et al., 2009; Mondoni et al., 2012; Bernareggi et al., 2016), can be used in our experiment as proxy to predict the future species abundance under different environmental change (i.e. Johansson et al., 2013).

After four year of experiments deployment, our results indicated an agreement between phenology and abundance responses for some, but not all, the four selected species and with differences between plant communities, supporting in part our third hypothesis ($\underline{h3}$).

For instance, *L. helveticus* increased the coverage in both the plant communities under warmer but not water shortage scenario, in agreement with its increase in size, flower number and reproductive success. Also *C. curvula* showed the same relation but only in snowbeds, whereas in its typical community we found an agreement between phenology and coverage only under a delayed snowmelt, that reduced the number of flowers as well as the species abundance. However, changes could also

be affected by clonal growth, well known for this alpine species (i.e. **de Witte and Stöcklin, 2010; de Witte et al., 2012**).

Among the snowbeds species, the increase in size and reproductive investment of *G. supinum* under warming was not combined with an increase species coverage, indicating a reduced seed viability (i.e. **Høyle et al.,2013**) or an negative influence on seed germination (i.e. **Shevtsova et al., 2009**). Drought resulted a limiting factor only in its sub-optimal community where, however, we didn't find any influence on phenology.

For *A. pentaphyllea* the response differs between plant communities. The increasing coverage in snowbeds under the OTC deployment together with the reduction of flowers number and the increase in plant size indicate the preference of the species to increase the abundance by vegetative growth, as reported also by other *Alchemilla* species in wooden meadows (e.g. **Tamm et al., 2002**), probably with a phalanx spreading strategy through its compact rhizome (e.g. **Herben et al., 1993**).

However, unexpected discordances in the answers between species phenology and abundance could also be attributed to the extreme longevity and persistence of many alpine species (**de Witte and Stöcklin, 2010**), those don't change theirs coverage despite the occurrence of environmental forcing (e.g. **Erschbamer and Winkler, 2005**; **Windmaißer and Reisch, 2013**).

The systematic continuation of plot observations under the different experiments over the next years is highly desirable to answer questions about the impact of environmental drivers over the population development and over the linkage between species phenology and abundance.

III. NORTHER MARITIME ANTARCTICA

III.1. STUDY AREA

III.1.A. Signy Island, South Orkney Islands, northern maritime Antarctica

The foreign activity for this PhD was carried out at Signy Island (South Orkneys Islands, northern maritime Antarctica) with the founding of the Italian Antarctic Program (PNRA) and the logistic support of the British Antarctic Survey (BAS), that led to the enrolment in the XXX and XXXI Italian Antarctic Campaign (austral summers 2014/2015 and 2015/2016) and to the permanence at the Signy BAS Research Station.

The activities fall in the frame of the project "Interaction of permafrost with climate and its significance for biodiversity along a latitudinal gradient in maritime Antarctic", with Prof. PhD. Cannone Nicoletta (University of Insubria, Department of Science and High Technology) as principal investigator and Prof. Peter Convey (British Antarctic Survey, Natural Environment Research Council) as BAS line manager.

The general aim was to understand the relationships between vegetation (e.g. biodiversity, spatial distribution) and soil characteristics (e.g. periglacial landforms, active layer depth) in order to evaluate impacts and potential feedbacks of climate change on Antarctic terrestrial ecosystems. The aim of the project was reached through the performance of the vegetation, geomophological and soil maps of the accessible terrestrial area of Signy Island, as well as through the installation of long-term *in situ* manipulation experiments similar to those already presented in chapter for the Alps.

III.1.B. Geography and climate

Signy Island ($60^{\circ}43'S$, $45^{\circ}38'W$) is a small island (10 km^2) located in the South Orkney Islands (Maritime Antarctic) (**Fig. III.1.B.1**). It is characterised by a cold oceanic climate, with mean annual air temperature of -3.5 °C, mean monthly air temperatures above 0 °C for at least one (but up to three) months each summer (**Guglielmin et al.,2012**), annual precipitation of 400 mm, primarily as summer rain (**Smith 1990; Royles et al. 2012**) and average cloud cover of 6–7 oktas year-round (**Guglielmin et al.,2012**).

The island lies south of the much larger and higher Coronation Island, which generates regular Föhn winds, which bring moist misty air over Signy Island. The longest climatic records indicate a progressive warming of air temperatures of 2 °C \pm 1 over the past 50 years in the closest long-term station at Orcadas, Laurie Island, South Orkney Islands (**Turner et al., 2005**) and a warming of +0.21 °C per decade since 1904 (**Turner et al., 2014**).

III.1.C. Geology and geomorphology

The bedrock is mainly quartz–mica-schist, although in some parts of the island there are small limestone, marble and amphibolites outcrops (**Smith, 2007**). Signy soils are mainly Gelisols, with a prevalence of Psammoturbels, Haplorthels, Haploturbels, and Psammorthels. Histoturbels, Histoturbels, and Fibristels may occur in lowlands and on the western coast (**Guglielmin et al.,2012**).



Fig. III.1.B.1 – Geographical location of Signy Island (b). Detailed description (b): red stars = study sites (1a, 1b: Backslope and CALM grid: 2a, 2b: Express Cove). Black squares = Signy BAS Research Station and huts on the island. Light blue area = glacier extent (year 2016); contour lines: 10 m asl. Overview of Signy BAS Research station (c). © PNRA

An ice cap covers about half of the island's area (**Smith**, **2007**), although due to recent climate change it is currently shrinking rapidly with a glacier retreat of more than 1 m/y in the last 20 years (**Favero-Longo et al. 2012**).

Despite the debate on the reconstructions of the ice cover on Signy Island over the past 7000 years based on the radiocarbon ages of re-exposed moss peat (Fenton, 1982; Smith, 1990), palaeolimnological (Jones et al.,2000) and geomorphological (Guglielmin et al.,2008) studies, it is generally accepted the occurrence of several glacial fluctuations over the last 1500 years. The most important glacial expansion (LIA) took place between 1720 and 1750 AD according to the palaeolimnological profiles reported by Jones et al. (2000).

Studies carried out from the 1960s and 1970s on Signy Island (Chambers, 1966a, 1966b, 1967, 1970; Holdgate et al., 1967; Collins et al., 1975) reported the occurrence of discontinuous permafrost with an active layer ranging between 40 cm and 2 m. More recently, a permafrost model (PERMDEM, Guglielmin et al., 2008b) based on the digital elevation model and the air lapse temperature shows that permafrost conditions in 2006 (Mean Annual Ground Surface Temperature, MAGST=0 °C) occurred over the entire island from the sea shoreline upward.

The geomorphology of the island is characterized mainly by periglacial landforms, which were described in detail and monitored by **Chambers (1966a, 1966b, 1967, 1970)**. Low-centered sorted circles, sorted stripes and stone-banked lobes are the most widespread landforms.

Low-centered sorted circles occur preferentially in flat and depressed areas, at elevations lower than 80 m, and exhibit large variability in size, with diameters ranging between 2 and 5 m. At higher elevations high-centered sorted circles (ranging between 10 and 150 cm) occur, generally on flat summits. Unsorted circles are common, generally of similar size to the high-centered sorted circles, but these do not show a clear distribution pattern. Frost boils also occur in some depressed and peaty areas, with diameters ranging from 0.2 to 0.7 m. No ice wedge polygons or sand wedge polygons were found, although some poorly defined frost fissure polygons occur on the flat and highest ice-free areas of the island. Soil stripes are mainly sorted with a width ranging from 10 to 210 cm at the same site. Soil stripes are developed in till and on colluvial deposits where the slope is greater than 4° – 6° . Transitional features from low-centered circles to sorted stripes are also present. Usually the coarse bands are raised with respect to the finer ones; although sometimes the opposite can occur, with unsorted stripes located close to the sorted ones (**Guglielmin et al., 2012**).

Gelifluction lobes are the most common periglacial feature: often occurring on the same slope with a high variability of shape and size from stone-banked terracettes, to lobes and stone streams (sensu Chambers). These gelifluction features are sometimes aggregated to form sheets. The gelifluction features are almost all stone-banked and develop on a wide range of slopes (always greater than 4° – 5°), with frontal ramps between 0.5 and 3 m in height. On the western side of the island moss banked lobes, which can be over 3 m high at the frontal ramp, are widespread (**Cannone et al.,2017**). Only one active rock glacier has been observed on the island, located just northward of Sombre Lake. The rock glacier shows a convex profile along the flow direction and a very steep frontal scarp, indicating the presence of ice within it (**Guglielmin et al., 2008b**).

III.1.D. Flora and vegetation

Vegetation on Signy Island is composed of both the Antarctic herb tundra formation, where the two native vascular plants *Deschampsia antarctica* Desv. and *Colobanthus quitensis* Bartl. occur, as well as the more common Antarctic non-vascular cryptogamic tundra formation (Gimingham & Smith, 1970; Smith, 1972; 1984); Fig. III.1.D.1)

Most of the island is covered by cryptogamic vegetation and, among the most widespread communities, are the *Usnea–Andreaea* association, distributed in the drier and more exposed sites while more barren ground is colonized by crustose and foliose lichens. Moss turf (e.g. *Polytrichum strictum-Chorisodontium acyphyllum* association) and cushion sub-formations (e.g. associations with *Sanionia uncinata* (Hedw.) Loeske) occur mainly in the wetter areas (**Gimingham & Smith, 1970; Smith, 1972**).

Communities of crustose lichens characterize littoral and supralittoral rocks, coastal rocks influenced by seabirds and inland dry rocks and soils at higher elevations (Smith, 1972; 1997; 2007).

Changes in cover of the dominant moss species (c. 15% of the island's bryophyte vegetation), have been monitored in adjacent fenced and unfenced areas, and correlated with both physical disturbance and changes in soil chemistry due to seawater and sweat washed out of the fur seals (Mg and Na increases) and to urine and excrement deposition (nitrate and ammonium increases) (Smith 1997). While more anecdotal observations on the general disappearance of macrolichens in seal-damaged areas has also been reported (Smith, 1988; 1997), changes in lichen variability and abundance have been only recently quantified (Favero-Longo et al., 2011; Favero-Longo et al., 2012).

Marine vertebrates play an important role both in the transfer of nutrients from marine to coastal terrestrial ecosystems in Antarctica, as well as by having direct impacts through trampling in the vicinity of breeding colonies and other large concentrations of animals. Lack of feeding competition



Fig. III.1.D.1 - Vegetation on Signy Island (a) is composed of both the Antarctic non-vascular cryptogamic tundra formation (b, c) and the Antarctic herb tundra formation, where the two native vascular plants *Deschampsia antarctica* Desv. and *Colobanthus quitensis* Bartl. (d) occur. © PNRA.

(due to anthropogenic reductions in great whale populations), coupled with recent changes in krillbased food webs in the Southern Ocean (due to changes in the extent of winter sea ice), has resulted over the last 60 years in a rapid recovery and a distributional expansion of Antarctic fur seal (*Arctocephalus gazella* Peters 1875) populations, previously harvested almost to extinction at their main population centers on South Georgia and the South Shetland Islands (**Waluda et al., 2010**). Non-breeding fur seals were first seen on Signy Island in the late 1970s, with numbers then increasing rapidly to 10–20000 individuals being present during the 1990s, and this number remaining relatively stable to the present day (**Smith, 1988; 1997; Waluda et al., 2010**). Fur seal activity has drastically impacted vegetation on the island, both through trampling/crushing (**Smith 1988**), and polluting levels of nitrogen release in faeces/urine (**Favero-Longo et al. 2011**).

The recent increase in summer populations of Antarctic fur seals is already known to have had major impacts on elements of the island's vegetation. Due to excessive trampling and increased nutrient input, these highly mobile and gregarious marine mammals have caused the eutrophication of previously oligotrophic lake systems (**Butler, 1999; Quayle and Convey, 2006**) and the severe or complete destruction of c. 15% of the island's bryophyte on terrestrial ecosystems (**Smith, 2007**). Cover of the dominant moss species has been reduced over large areas, and totally lost in others, and there has been a large increase in abundance of the nitrophilous alga *Prasiola crispa* (Lightfoot) Kutzing (**Smith, 1990**).

The interaction of climatic factors and animal disturbance, between 1960s and 2009 the two native Antarctic vascular plant species have exhibited significant increases in number of occupied sites and

percent cover. *Deschampsia antarctica* increased in coverage by 191 % and in number of sites by 104 %, *Colobanthus quitensis* increased in coverage by 208 % and number of sites by 35 %, with an heterogeneity of responses across an elevation gradient (**Cannone et al.,2016**). Below 20 m asl fur seal activity exerted negative impacts, whereas between 20 and 60 m asl, both plant species underwent considerable increases in the number of sites and percent cover, likely influenced by both climate warming and nutrient input from seals. Above an elevation 60 m asl the sites occupied decreased for both species, perhaps as a consequence of physical disturbance at higher elevations due to the permafrost conditions and/or the snow cover thickness and persistence.

The effect of wildlife on native vegetation is confirmed in other sub-antarctic island, where seal affected sites had a significantly higher number and cover of exotic plants (*Poa annua* and *Sagina procumbens*), whereas some of the native species become significantly less abundant, as a result of both physical trampling and nutrient enrichment of the soils (**Haussmann et al., 2013**).

Other wildlife trend are reported in the last decades in the population size of breeding pygoscelid penguins, nesting giant petrels (*Macronectes giganteus*) and skuas (*Stercorarius* spp.) at Signy Island (**Dunn et al.,2016a; 2016b; Carneiro et al.,2016**).

Signy Island, which is one of the most important hot-spots of terrestrial (and marine) biodiversity in the whole of the Antarctic biome (**Barnes et al. 2009**) and one of the best paradigmatic example of polar cryptogam-dominated terrestrial ecosystems (**Smith, 1990**), thus provides a case study for the consequences related to the climate and anthropogenic driven increase of the Antarctic fur seal population on terrestrial and freshwater ecosystems (**Smith 1988, 1990, 1997, Butler 1999**).

III.2. MATERIALS AND METHODS: FIELD WORK AND DATA COLLECTION

III.2.A. Manipulation experiments

III.2.A.a. Experiments design and aims

Similarly to what already done at the Stelvio pass area since 2014, during the austral summers 2014/2015 and 2015/2016 at Signy Island were set up some *in situ* manipulation experiments, in order to simulate and detect the effects of climate change on vegetation, active layer thickness and surface disturbance over Antarctic terrestrial ecosystems and over sensitive periglacial landforms.

The research activity followed the same protocol already adopted in Victoria Land since 2015 in five different sites across a latitudinal gradient from Apostrophe Island (73°S) to Finger Point (77°S), within a long-term monitoring network (Cannone, 2006; Dalle Fratte, PhD thesis; unpublished data).

We replicated the experiments in 2 sites located at the opposite side of Signy Island, at the same elevation but with differences in the general aspect (**Fig. III.1.B.1**). Each site was further divided in 2 sub-sites one below (Sites 1a, 2a) and one above (Sites 1b, 2b) 60 m asl, the elevation threshold above which the fur seal damage disappears and the physical disturbances due to the permafrost conditions and/or the snow cover thickness and persistence take importance (**Cannone et al.,2016**).

Site 1 – Backslope and CALM grid (60.71016 N, 45.59210 W; WGS84: dd.ddd°). It is located on the East coast of the island, on the North facing 'back slope' (unofficial name) area, near to the BAS Signy Research Station and up to the flat summit at 80 m asl that connects Factory Bluffs (121 m asl) to Observation Bluffs (108 m asl), close to the CALM grid site equipped with a data logger system to monitor the active layer thermal regime since December 2005 (Guglielmin et al.,2012). At Site 1a (40 m asl) the vegetation is composed by *Polytrichum alpestre-Chorisodontium aciphyllum* sociation, frequently colonized by lichen species (Smith, 1972). Where mosses are present, the community is dominated by *Polytrichum strictum* Brid. and *Chorisodontium aciphyllum* (Hook. f. & Wils.) Broth in Engl., whereas the lichen community is dominated by *Usnea antarctica* Du Rietz. (Bokhorst et al., 2007). At Site 1b (90 m asl), and on the plateau west of Observation Bluff, occurs a mosaic of different vegetation/ecosystem communities, including bare ground, *Andrea-Himantormia lugubris* and *Usnea-Himantormia lugubris* sociations (Smith, 1972; Cannone et al., 2006; Guglielmin et al., 2012).

Site 2 – Express Cove (60.69010 S, 45.63421 W).
It is located on the West coast of the island, on the west facing slope that from the Express Cove shore line moves upward to Spindrift Col (104 m asl). At Site 2a (27 m asl) and between Express Cove and North Point, the vegetation is mainly composed of *Brachythecium cf. antarcticum* and *Sanionia uncinata* sociations, whereas at Site 2b (106 m asl) and along the ridge between Jane Peak and Express Cove, *Andreaea* and *Andrea-Himantormia lugubris* sociations are more frequent (Smith, 1972).

The manipulation effects were performed through different structures that influenced the local characteristics (plot scale) in different ways, with a mono-factorial design: 1) air and soil warming; 2) water availability and soil moisture; 3) snow-depth and snow-melt time.

The experiments were replicated on moss dominated (M) and lichen dominated communities (L). In sub-sites above 60 m asl (1b, 2b) a replicate of each treatment was installed for each vegetation type

both with and without the occurrence of small patterned grounds (p). Therefore, except for the SF, in total we had the following ecosystem types for each experiment: moss dominated, no patterned ground (-M); moss dominated, with patterned ground (-Mp); lichen dominated, no patterned ground (-L); lichen dominated, with patterned ground (-Lp) (**Fig. III.2.A.a.1**):

1) Open Top Chamber (OTC) - air and soil warming

To passively warm small vegetation plots, modified ITEX polycarbonate hexagonal greenhouses open at the summit were installed. The chamber is constructed of 3-mm thick polycarbonate panels with an inclination of 60°. It is 17 cm tall and the distance between parallel sides is 68 cm at the base and 36 cm at the top. The size reduction adopted here (respect to the normal size of the ITEX OTC) follows both logistical requirements (winds are very strong in Antarctica and a smaller size allows a more efficient fixing at the ground) as well as the small minimum area requirements of the vegetation, allowing to perform the monitoring on 50 x 50 cm plots (**Cannone, 2004, 2006**). The OTCs were placed during the 2014/2015 summer season, between 24 and 28 February 2015 and were kept in their position year round. In the near vicinity of each OTC, a control plot was established. Hence, two treatments were applied: Open Top Chambers (OTC-M, -Mp, -L, -Lp) and control plots (OTC-Mc, -Mpc, -Lc, Lpc) (**Fig. III.2.A.a.2**).

2) Precipitation Shield (PS): water availability and soil moisture

A 2x1 m polycarbonate sheets, 3-mm thick, installed at 30 cm above the ground, subparallel to the soil surface and open on the borders was used to recreate drought and moist scenarios. The PSs were placed during the 2014/2015 and 2015/2016 summer seasons, between 2 and 3 March 2015 (Site 1) and on 7 March 2016 (Site 2), further kept in their position year round. For each precipitation shield, four treatments were applied: drought (under the precipitation shield; PS); great water increase and erosion (next to the PS on the down slope side, it gets the precipitation that are intercepted by the shield; PSv1); moderate water increase (about 1 m down slope from PS; PSv2) and control plots (located up slope than the shield; PSm) (**Fig. III.2.A.a.2**). This manipulation has been performed only on moss dominated vegetation as it could potentially provide larger responses in a shorter time than lichen dominated vegetation.

3) Snow Fence (SF): snow-depth and snow-melt time

Plastic barrier, 0.5 m tall and 5 m long, aligned perpendicular to the main western winds, was used to manipulate the snow depth and the snow-melt time due to the wind effects (snow erosion, transport, accumulation). On each side of the SF, was

• /	• •	3.5.()	T ()		ID()
site	experiment	M (n)	L (n)	MP (n)	LP (n)
1a	OTC	1	1	-	-
	PS	2	-	-	-
1b	OTC	1	1	1	1
	PS	1	-	-	-
	SF (x10)	1	-	-	-
2a	OTC	2	-	2	-
	PS	1	-	1	-
2b	OTC	1	1	1	1
	PS	1	-	1	-

Fig. III.2.A.a.1 - List of the manipulation experiments installed at Signy Island, in each of the study sites. OTC = Open Top Chambers; PS = Precipitation shield; SF = Snow fence. M = mosses dominated community; L = lichens dominated community; MP = mosses dominated with patterned ground; LP = lichens dominated, with patterned ground.



located a 5 m-long line, composed of 5 plots, with 0.5 m distance from each other. We hypothesized two main treatment: plots on the leeward side (SFv) that undergone to snowdrifts events and delayed snowmelt and plots on the windward side (SFm) that undergone to snow erosion and advanced snowmelt (**Fig. III.2.A.a.2**).

The SF was placed during the 2015/2016 summer season (6 March 2016) and further kept in their position year round.

Based on previous analysis of the minimum area of the continental Antarctica vegetation communities (**Cannone, 2004**), for each of these experiments and relative control conditions we installed a 50 x 50 cm permanent plot.

All the delimited plots received a code, results of the combination of the sub-site, the treatment type, the plant community and the occurrence of patterned ground. The detailed list of the investigated plots within each study site is reported in **Tab. S4**.

The configuration of the structure for each site is as follow. Site 1: 6 OTCs (moss: n = 3; lichen: n = 3), 3 PS (moss: n = 3), 1 SF (moss: n = 10); Site 2: 8 OTCs (moss: n = 6; lichen: n = 2), 4 PS (moss: n = 4).

Overall, the investigated plots were 66 (Site 1a: 12; Site 1b: 22; Site 2a: 16; Site 2b: 16), of which 48 are on moss dominated community (Site 1a: 10; Site 1b: 18; Site 2a: 16: Site 2b: 12) and 10 on lichen dominated community (Site 1a: 2; Site 1b: 4; Site 2a: 0; Site 2b: 4).



Fig. III.2.A.a.2 - Example of a) Open Top Chamber (OTC), b) precipitation shield (PS) and c) snowfence (SF) installed at Signy Island, CALM Grid site (site 1b).

III.2.A.b. Environmental data collection

In order to know the effect of the structures installed and their manipulation effect on soil temperature, soil moisture and snowmelt time different devices were installed (see **Tab. S4**).

Ground surface temperature (GST; °C) data at 2 cm-depth were derived from Hobo Pro V2 U23-003 thermistor by Onset (accuracy ± 0.2 °C, resolution ± 0.02 °C). Temperature loggers were installed at 2 cm depth (according to **Guglielmin, 2006**) in correspondence of 16 moss-dominated plots (n. 6 OTCs, n. 6 control plots, n. 2 SF leeward side, n. 2 SF windward side) and programmed to record and store data every hour. Dataloggers sensor were distributed in the middle of the selected plots (e.g. **Cornelius et al.,2013**). Dataloggers in the OTCs were installed on 25 February (Site 1) and on 8 March 2015 (Site 2), whereas those in the SF on 8 March 2016.

Soil volumetric water content (VWC; m^3/m^3) and total precipitation (TP; mm) were derived from a five channel EM50 datalogger by Decagon Devices. Four channels were equipped with 5TM soil moisture sensors (accuracy: $\pm 0.03 \text{ m}3/\text{m}3 \text{ or } \pm 3\%$ VWC; resolution: 0.0008 m3/m3 or 0.08% VWC) at 2 cm-depth and one was equipped with an ECRN100 high resolution rain gauge sensor at 160 cm above the ground (resolution 0.2 mm; funnel size 17 x 14.2 cm). Data were recorded every 10 mins. During the 2014/2015 summer season, the soil VWC sensors and rain gauge were installed in one moss-dominated Precipitation Shield (PS) treatment and relative control plots (PSv1, PSv2, PSc) in Site 1 for few days only (from 1 to 12 March 2015). During the 2015/2016 summer season, sensors and rain gauge were installed (Site 1: 5 March; Site 2: 7 March 2016) and left in place in one moss-dominated PS treatment for each site year round.

III.2.A.c. Species composition assessment

Moreover, as a baseline for future monitoring, a phytosociological survey (PS 50x50 cm and PS 5x5 cm) and a point intercept (PI 5x5cm) for each of the installed plot (n = 66) were run in 2014/2015 and 2015/2016 summer season, before the manipulation had started, following methodology already used in continental and maritime Antarctica (**Cannone, 2004; 2006**). Species nomenclature follows **Øvstedal & Smith (2001)** for lichens, **Ochyra et al. (2008)** for Bryophytes.

GPS coordinates [dd.ddddd°S; dd.ddddd°E], elevation [m asl], slope [degree], aspect [°N] and surface texture [% of each grain size] were recorded for each plots as well.

III.3. RESULTS

III.3.A. Effect of manipulation deployment on environmental data

In the following chapter, will be provided a preliminary assessment of manipulation experiment on the abiotic component of the studies Antarctic ecosystems.

Open Top Chambers (OTC) deployment on ground surface temperature (GST) was assessed after one year of manipulation experiment (26/02/2015 - 24/03/2016) in all the manipulated and control plots, with soil temperature sensors since the 2014/2015 summer season at Signy Island.

Precipitation shield (PS) deployment on GST and volumetric water content (VWC) was assessed only for few days (05/03/2016 - 24/03/2016) in a moss dominated community ad Site 1, because of the really short time span between the installation and the last download of soil moisture sensors and rain gauge data during the 2015/2016 summer season. For logistical constrain we were not able to re-visit and download data from site 2.

Snow fence (SF) deployment on GST was assessed only for few days as well (04/03/2016 - 20/03/2016), for the same above mentioned reason of summer season 2015/2016.

For these reasons, we could provide really preliminary results only for part of the manipulation experiment deployment, whereas others require longer time period to become evident.

III.3.A.a. Open Top Chambers (OTC)

Ground surface temperature data were collected from 26/02/2015 to 24/03/2016. Within this period of experiment, we observed a large variability in the OTC deployment over the MAGST, in both the moss dominated communities studied (**Tab. III.3.A.1.1**).

site	community	treatment	plot	Ν	Mean	SD	Min	25%	Med	75%	Max	Diff
1b	М	OTC	OTCca-M	2465	-1.0	3.1	-19.2	-0.2	0.0	0.1	6.8	1 7*
1b	М	OTCc	OTCca-Mc	9416	-2.7	4.8	-19.4	-5.7	-0.7	0.1	12.1	1./"
1b	MP	OTC	OTCca-MP	9416	-2.5	4.5	-15.6	-5.5	-0.7	0.3	12.4	0.0*
1b	MP	OTCc	OTCca-MPc	9416	-2.5	5.0	-18.7	-5.5	-0.5	0.3	15.8	0.0*
2a	М	OTC	OTCeb-M	8750	-3.4	4.5	-16.4	-6.5	-1.9	-0.1	10.1	0.2*
2a	Μ	OTCc	OTCeb-Mc	8750	-3.6	5.2	-20.3	-6.6	-1.6	-0.1	11.0	0.2"
2a	MP	OTC	OTCeb-MP	8800	-1.9	4.8	-13.7	-5.1	-1.1	0.0	28.7	0.1
2a	MP	OTCc	OTCeb-MPc	8800	-2.0	5.2	-18.1	-5.2	-0.8	0.1	32.4	0.1
2b	Μ	OTC	OTCea-M	8800	-1.9	4.0	-14.3	-4.7	-0.8	0.3	13.7	0.2
2b	Μ	OTCc	OTCea-Mc	8800	-2.0	4.5	-19.3	-5.0	-0.9	0.1	17.4	0.2
2b	MP	OTC	OTCea-MP	8751	-2.9	4.3	-12.9	-6.3	-2.1	0.0	16.8	0.0
2b	MP	OTCc	OTCea-MPc	8751	-3.0	4.1	-13.7	-6.3	-2.1	0.0	14.6	0.0
all	М	OTC	all	20015	-2.4	4.2	-19.2	-5.4	-0.7	0.0	13.7	0.4*
all	Μ	OTCc	all	26966	-2.8	4.9	-20.3	-5.7	-1.0	0.1	17.4	0.4"
all	MP	OTC	all	26967	-2.4	4.5	-15.6	-5.8	-1.2	0.0	28.7	0.0
all	MP	OTCc	all	26967	-2.5	4.8	-18.7	-5.8	-1.0	0.1	32.4	0.0

Tab. III.3.A.1.1 – Ground surface temperature (2 cm depth) of the period 26/02/2015-24/03/2016 in the manipulated (OTC) and control (OTCc) plots, within mosses dominare communities with patterned ground (-MP) and without patterned ground (-M). N = number of hourly recorded data. Diff = difference in means between OTC and OTCc; * = p < 0.05, as tested by one-way ANOVA).



Fig. III.3.A.1.1 - Evidence of increase snow cover permanence in OTC (a) compared to control plot (b) in March 2016.

The largest increase of mean temperature was +1.7°C (despite the influence of data gaps during the winter season in OTCca-M compared to OTCca-Mc), whereas in some plots we didn't record any increase in ground surface temperature over the study period compared to relative control plots. Therefore we did not observe such a clear effect of OTC on ground surface temperature. More year of monitoring are needed to implement and better evaluate the effect of OTC deployment on ground surface thermal regime.

Moreover, for future evaluation other influence of the OTC deployment on the abiotic variables needs to be taken into account when analyzing the GST in warming and control plots. Indeed as already evidenced by previous authors (see **Bokhorst et al., 2011; 2013**), OTCs are able to influence the persistence of snow cover inside the chamber, as recorded in several OTCs in March 2016 (**Fig. III.3.A.1.1**).

III.3.A.b. Precipitation shields (PS)

Over the very short investigated period (05/03/2016-24/03/2016), the total precipitation recorded at Site 1 was 121.4 mm.

	site	community	treatment	plot	Ν	Mean	SD	Min	25%	Med	75%	Max	Diff
GST	1b	М	PS	PSca-M	920	1.4	1.7	-1.4	0.5	0.7	2.1	11.4	0.151
	1b	М	PSv1	PSca-Mv1	920	1.4	2.1	-1.5	0.3	0.7	2.0	14.7	0.151
	1b	М	PSv2	PSca-Mv2	920	1.1	1.9	-2.1	0.1	0.5	1.9	11.2	-0.095
	1b	М	PSc	PSca-Mc	920	1.2	1.7	-1.7	0.3	0.6	1.9	9.4	
VWC	1b	М	PS	PSca-M	920	0.117	0.033	0.031	0.105	0.116	0.133	0.236	-0.021*
	1b	М	PSv1	PSca-Mv1	920	0.127	0.077	0.029	0.093	0.123	0.140	0.549	-0.010*
	1b	М	PSv2	PSca-Mv2	920	0.116	0.062	0.039	0.093	0.115	0.123	0.470	-0.021*
	1b	М	PSc	PSca-Mc	920	0.137	0.071	0.040	0.112	0.137	0.151	0.632	

Significant differences in GST and VWC between manipulated and control plot were assessed though post-hoc Tuckey test (p < 0.05).

Tab. III.3.A.2.1– Ground surface temperature (GST; °C) and volumetric water content (VWC; m^3/m^3) of the period 05/03/2016 – 24/03/2016 in a rain exclusion experiment at Site 1b, on moss dominated community. Total precipitation for the period investigated was 121.4 mm. N = number of hourly recorded data. Diff = difference in means between manipulated (PS, PSv1, PSv2) and control (PSc) plots. * = p < 0.05, as tested by posy-hoc Tuckey test)



Fig. III.3.A.2.1 – Pattern of volumetric water content (m^3/m^3) in manipulated and control plots of a rain exclusion (PS) experiment on mosses dominated community in Signy Island.

GST didn't show any significant difference, with a mean value between $+1.1^{\circ}$ C and $+1.4^{\circ}$ C. Otherwise, VWC show significant differences, with a mean values between 0.116 m³/m³ and 0.137 m³/m³ (**Tab. III.3.A.2.1**).

The precipitation shield effect on rain exclusion on the underneath plots was more evident after intense precipitation events, such as that occurred on 18/03/2016 (Fig. III.3.A.2.1).

III.3.A.c. Snow fence (SF)

We were not able to quantify the effect of SF on snowmelt and snow distribution, since the snow cover was not already appeared at the end of the summer season of the second year of experiment (austral summer 2015/2016), the same when SF was installed for the first time.

Moreover, because of the logistical constrain we were not able to re-survey the experiment during the austral summer 2016/2017 and, therefore at the moment we don't have any information on the SF deployment on snow cover thickness.

site	community	treatment	Ν	Mean	SD	Min	25%	Med	75%	Max	Diff
1b	М	SFv3	383	0.2	1.1	-2.4	0.0	0.1	0.2	4.5	а
1b	М	SFv2	383	0.2	1.2	-3.4	0.0	0.0	0.2	5.1	а
1b	М	SFm2	383	0.2	1.0	-2.9	-0.1	0.0	0.1	4.5	b
1b	М	SFm3	383	0.1	1.1	-3.0	-0.1	-0.1	0.1	4.7	b

Tab. III.3.A.3.1 - Ground surface temperature (2 cm depth: °C) of the period 04/03/2016-20/03/2016 in the leeward (SFv3, SFv2) and windward (SFm2, SFm3) side of the snow fence (SF), within mosses dominated (M) communities. N = number of hourly recorded data. Diff = different letters represent statistic significant differences in mean GST, as tested by post-hoc Tuckey test (p<0.05).

However for what concerns the GST, from the short time period with available data we found significant difference between leeward (SFv2, SFv3) and windward (SFm2, SFm3) side of the SF, where GST has been recording since 04/03/2016 (last download: 20/03/2016) (**Tab. III.3.A.3.1**).

Leeward side plots resulted significantly warmer than windward side (leeward: +0.24°C; windward: 0.11°C), as tested by post-hoc Tukey test (p<0.05).

IV. SOUTHERN MARITIME ANTARCTICA

IV.1. Study area

IV.1.A. Marguerite Bay, southern maritime Antarctica, Western Antarctic Peninsula (WAP)

Additional activities during the PhD period dealt with the elaboration of data recorded during previous Antarctic research campaigns, funded by the Italian Antarctic Program (PNRA) and with the logistic support of the British Antarctic Survey (BAS) that leaded to the enrolment in the XXIX Italian Antarctic Campaign (austral summer 2013/2014) and to the permanence at the Rothera BAS Research Station. During the further PhD period, the data were elaborated in order to assess and evaluate on a small scale basis the plant species richness and biodiversity and the soil characteristics of the Antarctic terrestrial ecosystems, as well as their sensitivity to environmental change.

The results were showed in three oral presentation at the XXXIV Scientific Committee on Antarctic Research biennial meeting and Open Science Conference in Kuala Lumpur on 22-26 August 2016, within the following sessions: S27. Impacts of environmental changes on Antarctic ecosystems and biota; S11. Antarctic permafrost, periglacial processes and soil development.

Moreover, analyses on samples of mosses and underlying organic sediments collected along a glacier retreat front at Rothera Point were performed, in order to asses past climate change and glacier dynamics and to evaluate the possible consequences on moss vegetation after re-exposure due to glacier retreat (see **chapters IV.3.A** and **IV.3.B**).

IV.1.B. Geography and climate

Rothera Point is a rocky promontory with an ice-free area of c. 1000×250 m at the southern extremity of the Wormald Ice Piedmont (WIP), on the east side of Adelaide Island, south-west Antarctic Peninsula (**Fig. IV.1.B.1**). Since year 1985, it includes a Site of Special Scientific Interest (SSI), reclassified as Antarctic Specially Protected Area (ASPA) n. 129 in 1998. ASPAs are the highest level of environmental protection for a site within the Antarctic Treaty area (**Hughes et al., 2013**). The ASPA n. 129 itself has little intrinsic nature conservation value. However, it has scientific value as a control area, against which the effects of human impact associated with the adjacent BAS Rothera Research Station could be monitored in an Antarctic fellfield ecosystem (**ATCM XXXV Final Report**).

Climate change is particularly evident along the WAP over the last 60 years, with a mean annual air temperature (MAAT) increase of +0.54°C per decade and a more evident warming of + 1.01°C during the winter in the period 1951-2011 (**Turner et al., 2014**). This findings are in agreement with the trend recorded at Rothera weather station, with a warming in MAAT over the last 35 years of +0.5°C per decade, more pronounced during winter with an increase of + 0.8°C per decade (**Guglielmin et al., 2014**). Snowfall trends vary from region to region, for instance increasing on the western side of the Antarctic Peninsula since 1957 (**Convey et al., 2009; Turner et al., 2014**). Between 2009 and 2011, maximum snow depth recorded at Rothera Point ranged between 1 and 142 cm, with a large spatial (intra-annual) and temporal (inter-annual) variability depending on micro topographical characteristics, and snow-free days spanning from 13 to 301 (**Guglielmin et al., 2014**).



Fig. IV.1.B.1 - Geographical location of Rothera Point (a, b). Red star: Rothera BAS Research Station. Overview of Rohera BAS Research station (c).

IV.1.C. Geology and geomorphology

The bedrocks of the Rothera Point is predominantly heterogeneous intrusions of diorite and granodiorite of mid-Cretaceous to early Tertiary age (**Dewar**, **1970**). On Anchorage Island (close to Rothera Point) the soils show different physical and chemical characteristics (e.g. TOC, %C, C/N ratio, and grain size) in relation to the vegetation type (**Cannone et al., 2012**) but are all characterized by a low pH value and an high nitrogen content linked to the abundance of birds (and of their guano) on the island.

The deglaciation age of the Marguerite Bay area is still not well known. Radiocarbon dates on organic remains from an abandoned penguin colony near Rothera Point estimated that deglaciation occurred about 6000 yr BP (**Emslie, 2000**), whereas though new and previously published radiocarbon dates on penguin remains and shells and on two isolation basins dating back to 6500 yr BP, other authors estimated about 9000 yr BP (**Bentley et al.,2005**). A more recent study combined Schmidt Hammer R-values and ¹⁴C ages and it showed that local deglaciation of the Marguerite Bay area could have taken place about 12000 year BP, with a possible delay in Rothera than Anchorage Island due to its less exposed position and its proximity to grounded glaciers (**Guglielmin et al.,2012**).

The distribution and properties of permafrost and active-layer dynamics are still not completely known in the Antarctic Peninsula region, despite a great progress in the last 10 years in research on periglacial geomorphology and permafrost (Vieira et al., 2010; Guglielmin, 2012). Several methods has been employed to estimate the distribution of permafrost, including: isotherms of mean annual

air temperature; distribution of periglacial features indicative of permafrost; shallow permafrost excavations and boreholes along the Antarctic Peninsula; geophysical techniques; models for predicting the occurrence of permafrost especially in relation to variable conditions of snow distribution (**Bockheim et al.,2013** and related references). The map based on these approaches, suggests that permafrost likely is continuous in ice-free areas of the South Orkney Islands, along the eastern Antarctic Peninsula (EAP) and ca. 69° to 74°S along the WAP.

In Marguerite Bay area, permafrost is probably continuous in nature, although detailed small scale spatial and temporal data are still lacking. However, between 2009 and 2011 in a 30 m depth borehole at Rothera Point (Memorial Hill, 31 m asl), the active layer thickness (ALT) ranged between 0.76 and 1.40 m and the permafrost thickness was calculated to range between 112 and 157 m (**Guglielmin et al., 2014**).

Except for raised beaches around Rothera Point and Anchorage Islands and for a small number of frost-sorted circles (**Convey and Smith, 1997**), any other relevant periglacial landforms couldn't be detected.

IV.1.D. Flora and vegetation

The vegetation of the maritime Antarctic includes both the Antarctic herb tundra formation, with the occurrence of the vascular plants *Deschampsia antarctica* Desv. and *Colobanthus quitensis* Bartl, and the Antarctic non-vascular cryptogam tundra formation, with different sub-formation depending on several morphological and environmental variables. (Gimingham and Smith, 1970; Smith, 1972, 1990).



Fig. IV.1.D.1 – Vegetation at Rothera Point is very scattered and mainly composed by epilithic lichens: a) *Acarospora macrocyclos* and *Buellia latemarginata*; b) *Caloplaca hookeri* c) *Umbilicaria decussata*). Bryophyte development is fragmentary and mainly associated with sorted circles, melt water seepage, or damp crevices: d) *Pohlia nutans*.

Due to the prevailing outcropping bedrock and the coarse texture of the loose sediments, at Rothera Point the vegetation is very scattered and mainly composed by epilithic lichens (dominated by *Usnea sphacelata* and *Umbilicaria decussata*), with 42 species present (Antarctic Plant Database – BAS Herbarium; **Peat, 1998**). Other than two small patches of the moss *Sanionia uncinata* (Hedw.) Loeske, bryophyte development is fragmentary, with about 21 species present in very small quantities and mainly associated with sorted circles, melt water seepage, or damp crevices (**Convey and Smith, 1997**).

IV.2. MATERIALS AND METHODS

IV.2 A. Paleo-climate investigation

This work was performed at Rothera Point (67°34'S; 68°07'W), in Marguerite Bay, at the southern limit of the Wormland Ice Piedmont (WIP), locally known as "ice ramp" (**Fig. IV.2.1**). It has been monitored since 1989 and it shows a rapid retreat in recent decades (**Smith et al., 1998**).

For these study area, we presented for the first time evidences consistent with a) Medieval Warm Period (MWP) glacial retreat, b) subsequent Little Ice Age advance and c) glacial retreat associated with the rapid regional warming in recent decades. Moreover, past rates of glacial retreat were compared with contemporary rates in order to assess whether the impacts of recent climate warming on local glacial dynamics are comparable with those over the past millennium.

More detailed information about methods concerning this section are provided in the paper **Guglielmin et al. (2015),** see chapter IV.3.A.



Fig. IV.2.1– Location of the study area. The red dashed line represents the direction of glacial variations and the transect along with samples of mosses and organic sediment were collected. In the background, Rohera BAS Research station. \bigcirc PNRA.

IV.2.B. Moss regeneration and cryptobiosis

Cryptobyosis is a reversible ametabolic state of life that has been considered as third state between life and death, documented in several invertebrate groups (Clegg, 2001; Neuman, 2012) and particularly suited for permafrost environment. (Yashina et al., 2012).

In the same study area and along the same transect at Rothera Point mentioned above (**Fig. IV.2.1**), moss survival (cryptobiosis) after six centuries of glacier burial was assessed through growth experiments and, for the first time, through vitality measurements.

Using samples of two moss species (*Bryum pseudotriquetrum* (Hedw.) P. Gaertn. and *Sanionia uncinata* (Headw.) Loeske) exposed by recent glacier retreat, were presented evidence of: a) *in situ* moss survival for over than 600 yrs in the natural habitats through cryptobiosis and their subsequent

spontaneous recovery to active metabolism after glacier retreat; b) persistence of active metabolism and growth in surviving exhumed mosses; c) regeneration with development of green apices from moribund stems of exhumed mosses.

More detailed information about methods concerning this section are provided in the paper **Cannone** et al. (2017), see chapter IV.3.B.

IV.3. RESULTS

IV.3.A. Paleo-climate investigation

Several recent global scale studies have emphasised the absence of evidence for globally synchronous multi-decadal warm or cold intervals for the last millennia (e.g. Masson-Delmotte et al., 2013; Mann et al., 2009).

Synchronicity in the timing of temperature anomalies, such as Medieval Warm Period (MWP) and Little Ice Age (LIA) is not always apparent between the two hemispheres (**Neukom et al., 2014**). As well as at global scale, in the Antarctic Peninsula (AP), studies dealing with MWP and LIA reconstruction show contradictory data. More in detail, LIA is also not well defined or synchronous, especially on both sides of the AP (**Bentley et al., 2009; Domack et al., 1995; Liu et al., 2005; Heroy et al., 2008; Milliken et al., 2009**).

At a global scale, there is good correspondence in timing between the MWP and a phase of glacier retreat, as well as between the LIA cold phase and a period of glacier advance that represented the Holocene glacier advance peak (e.g. **Humlum et al., 2005; Nesje et al., 2011**). In the AP region, there are few terrestrial records of glacial advance and/or retreat spanning the last millennium.

In the first paper listed below in this chapter (**Guglielmin et al., 2015**) we try to fill this gap and we present a record of terrestrial organic material re-exposed by recent glacier retreat at a location in the WAP region that provides evidences consistent with (1) MWP glacial retreat, (2) subsequent LIA advance, and (3) glacial retreat associated with the rapid regional warming in recent decades. Furthermore, we compare past rates of glacial retreat with contemporary rates in order to assess whether the impacts of recent climate warming on local glacial dynamics are comparable with those over the past millennium.

IV.3.B. Moss regeneration and cryptobiosis

Cryptobyosis is a reversible ametabolic state of life that has been considered as third stat between life and death, documented in several invertebrate groups (**Clegg**, 2001; **Neuman**, 2012) and particularly suited for permafrost environment. (**Yashina et al.**, 2012).

Mosses are major components of plant communities in terms of diversity and biomass in Antarctica, where macroscopic vegetation is dominated by cryptogams (Longton, 1988; Ochyra et al.,2008). Mosses are poikilohydric organisms with high degree of phenotypic plasticity and among the key traits allowing mosses to persist in harsh environments, one of the most important is tolerance to desiccation and drought, also contributing to their ability to resist freezing (as during the Antarctic winter) (Turetsky et al., 2012; Schlensog et al., 2004).

In the second paper presented in this chapter (**Cannone et al., 2017**), we show evidence of: a) *in situ* moss survival for over more than 600 y in the natural habitat through cryptobiosis, and their subsequent spontaneous recovery to active metabolism after glacier retreat, b) persistence of active metabolism and growth in surviving exhumed mosses, c) regeneration with development of green stem apices from moribund stems of exhumed mosses.