CICLO: XXXIII

PHENOLOGY AND INTERDIPENDENT SOIL-PLANT DYNAMICS IN TWO DIFFERENT HIGH-ELEVATION ALPINE BIOMES

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Chapter 1: Introduction

General introduction

There is strong evidence that global warming is proceeding at a fast rate in the mountain areas; surface air temperature of such zones in America, Europe and Asia has clearly outpaced the average global trend (Williamson et al., 2020; Hock et al., 2019; Palomo, 2017). While there is still no unanimous confidence whether or not the warming trend globally varies at different rates in different altitudinal belts, some patterns have already been identified in various mountain regions of the world (Hock et al., 2019). In the European Alps, high-elevation biomes are currently witnessing a less dramatic impact of global warming when compared with their low-elevation correspondents (Du et al., 2019). Nonetheless, in the past few years, perceivable clues of this ongoing process have been already observed even at the higher belts of the alpine environment and it is already possible to identify major drivers of change.

The first recognizable trend is the steady rise of temperatures, which in the European Alps is more pronounced during the spring and summer seasons (Hock et al., 2019). The forecast of precipitation variations is subject to larger uncertainties. Nevertheless, while extreme precipitation events are likely to intensify, the accumulation of natural snow cover and the duration of the snowpack throughout the season are both going to dramatically decrease (Gobiet and Kotlarski, 2020; Marty et al., 2017a), while a slight decrease in summer liquid precipitations might cause unforeseen droughts in many alpine regions (Gobiet et al., 2014). Consequences of the climatic modification could highly impact numerous components of the alpine biome, which will be among the first to be threatened by such sudden modifications in temperatures and precipitations (Dirnböck et al., 2011). Since they are tightly interlaced with snow dynamics, high-alpine biomes are especially fragile and are deemed to undergo severe modifications (Rogora et al. 2018) either regarding soil microbiota (Adamczyk et al., 2019) and vegetation (Verrall and Pickering, 2020; Guisan and Theurillat, 2000).

Vegetation of the nival belt, albeit less rich and diverse when compared to lower elevation communities (Vittoz et al., 2010) is a composite blend of plants with specific strategies designed to survive the harsh conditions that can be found at high elevation. Depending on the microtopography, soil characteristics and patterns of snow distribution, a high species richness can be found in a relatively small space. Competitive grasses from the thermic slopes, dwarf shrubs of the snowbeds, pioneers of the rocky debris or cushion-like stress-tolerant on the windy ridges, many of these species might be susceptible to minor changes in their habitat. Plants with some specific adaptation to certain conditions might face disadvantage when compared with highly opportunistic species and the overall composition of alpine tundra vegetation could witness some major changes (Hamid et al., 2020; Schob et al., 2009). Snowbed specialists vegetate in areas with deep soils and long-lasting snow cover; such microbiomes, due to their peculiar characteristics of arctic tundra relicts in the Alps and their increased sensitivity to small climatic variations, are commonly considered as perfect “laboratories” to study the effects of global warming on the plant-soil system (Schöb et al., 2008; Björk and Molau, 2007). Alpine soils are strongly influenced by climatic variations, especially in the snow dynamics; such alterations might have a perceivable effect on the nutrients cycle and availability of carbon and nitrogen (Walker et al. 1999, Groffman et al. 2001). Regarding the vegetation, highly specialized plant species might undergo the risk of spring frosts if the protective layer of snow is to prematurely melt (Pardee et al., 2019). Other species might suffer from water shortage in the case of extremely reduced precipitation. On the other hand, there are species which could take advantage of some of the incoming variations in the alpine environment.

Silicic grasslands and snowbeds, representing the climax vegetation of the nival belt (Matthews, 1978), are not the only habitats which will witness drastic change in the near future. Among the most sadly spectacular consequences of global warming we can list the retreat and in some cases disappearance of numerous glaciers, which is already happening at a constant and alarming rate

(Sommer et al., 2020). There is evidence that, due to the imbalance with present-day climate conditions and the presence of mostly small glaciers, the Alps will lose up to 80% of their current ice mass by year 2100 (Zekollari et al., 2020; Hock et al., 2019). With the ongoing loss of glaciers in the Alps, more and more surface will go towards a vegetal colonization during the next decades. If on the one hand this dramatic disappearance might signify a chain effect resulting in an increased warming due to the loss of albedo (Di Mauro et al., 2020; Nageli et al., 2019) and carbon immobilized in ancient permafrost, on the other hand an increasingly large surface will be available for vegetation cover (Fickert and Grüninger, 2018). In the long run, with deglacialized soils being gradually colonized first by pioneer species and later by other plant communities, this may imply new possibility for carbon fixation (Varolo et al., 2016). The bare-soil colonization by plant communities might as well play a marginal role in controlling erosion and soil degradation which are already affecting alpine territories (Hudek et al., 2017).

The study of how mountain plant communities respond to the challenges of climate change still bears some uncertainties, mostly due to the increased time lag in the response of alpine plant communities and the multiple biotic and abiotic interactions involved (Malanson et al., 2019). Hence it is of the utmost importance to continue investigating how the alpine biome is responding to the ongoing alterations of its environment, in order to fully comprehend the ongoing processes which will shape the Alps in the decades to come.

General objectives

The alpine environment response to climate change has been the frequent object of studies in the past years. Nonetheless, there is still plenty to learn about the relations between plants, soil and climatic factors. In this thesis we aimed at exploring the complex ecology of high-elevation areas in the Western Alps, with a specific focus on shifting phenology, nutrients cycle in the soil and the vegetative colonization process relative to two habitats of great interest: snowbeds and proglacial chronosequences.

Phenology has historically been a useful tool to understand the basic biological dynamics of plants with commercial or agronomical purposes but has often been applied with an ecological insight. Phenology is still a valid tool to investigate the response of plants to a wide range of variations and could be useful to explore different types of behaviors or possibly the sensitivity of certain species to multiple environmental factors (Zhao et a., 2013). While there is strong evidence that the occurrence of phenological events for many alpine plant species is extremely correlated to seasonal factors such as thermal sum or the snowmelt (Kudo, 2020; Asam et al., 2018), literature often lacks thorough information about interannual variability or interspecific variability in the achievement of different phenophases. In this thesis we aim at clarifying the relation between plant phenology and both temporal and seasonal factors (photoperiod, snowmelt, thermal sum) by analyzing a range of different species in a snowbed habitat.

However, since vegetal communities are a complex system which could be intertwined with multiple factors, we addressed the need to explore further on the ecosystemic components which could be more influential on plants growing cycle. One of the most pivotal factors in plant development is the substrate, nevertheless the role of soil-plant interaction in relation to the timing of growing cycle is often overlooked, with exceptions such as Petraglia et al. (2013). Soil properties, moisture and nutrients content play a crucial role in biomass accumulation and plant development, but little research has been carried out on the influence that soil could have on plant phenology and or the consequences that different stages of plant development could have on soil chemistry and microbiota in the alpine belt (Adair and Burke, 2010; Nord and Lynch, 2009; Bilbrough et al., 2000; Mullen et al., 1998). Microbial communities are deemed to be extremely sensitive to even small-scale variations in temperature or moisture (Ding et al., 2019; Donhauser and Frey, 2018; Zhang et al., 2016), thus there is confidence their structure is going to be affected by the climatic outcome of global warming. However, there might be more derivative consequences to be taken into consideration, such as the possible shift in plant phenology. The uptake of nutrients by the vegetation is known to have a role in the seasonal fluctuation of soil nitrogen, carbon and micronutrients (Tian et al., 2017; Chen et al., 2019), but it is still unclear whether or not the different timing of certain events or the very extension of the whole growing season will impact the chemistry of alpine soils. Our purpose was to link carbon and nitrogen dynamics in the soil with plant phenological cycle throughout the growing season and the vegetation composition of snowbeds.

In addition, we further examined the topic of soil-plant interactions with a specific focus on newly deglacialized lands. In recent years, many studies aimed at investigating the interactions among ecosystem compartments to understand the role of biotic factors affecting proglacial chronosequences (Matthews and Vater, 2015; D’Amico et al., 2014). One of the key factors in understanding the succession dynamics is the evaluation of species patterns and traits, which can provide useful insight in the community ecology and interactions between biotic and abiotic factors (Těšitel et al., 2014; Caccianiga et al., 2006). The ameliorating impact of plants on either chemical and physical properties of bare soils has been addressed in several documents (Burga et al., 2010; Egli et al., 2006). Nevertheless, a thorough exploration of the effect of single herbaceous species on soil C:N:P stoichiometry is still missing. We focused on investigating the population structure of both a pioneer and a grassland species in a primary succession, and their role in soil formation and improvement by the tracking of carbon, nitrogen and phosphorus.

This manuscript is structured in 3 main chapters, of whom the first and third paper are respectively published in Journal of Mountain Science and Frontiers in Environmental Sciences. The second paper is currently submitted and undergoing the reviewing process. The first two papers are referred to the same habitat and location (snowbeds of the Cimalegna Plateau, VC), on permanent plots set in the framework of the LTER Italia monitoring network (https://deims.org/17210eba-d832-4759-89fa-9ff127cbdf6e). The last paper takes into consideration a different habitat, specifically a proglacial chronosequence in the Lauson valley (AO).

Specific objectives

Chapter 2: Alpine tundra species phenology is mostly driven by climate-related variables rather than by photoperiod

Quaglia E, Ravetto Enri S, Perotti E, Probo M, Lombardi G, Lonati M

The study of plant phenology has frequently been used to link phenological events to various factors, such as temperature or photoperiod. In the high-alpine environment, proper timing of the phenological cycle has always been crucial to overcome harsh conditions and potential extreme events (i.e. spring frosts) but little is still known about the response dynamics of the vegetation, which could shape the alpine landscape in a future of changing climate. In this chapter, we investigated the phenological behavior of a set of alpine tundra species present in eight different snowbeds but belonging to different functional pools (species typical of the rocky debris, grassland species and snowbed specialists). The aim was to understand which, among photoperiod, thermal sum and snowmelt date, are the major drivers for the timing of the phenological cycle and how it could vary through the array of species belonging to different functional pools. The data and methods to reach these objectives are analysed and discussed in Journal of Mountain Science (2020) 17:2081–2096. https://doi.org/10.1007/s11629-020-6079-2

Chapter 3: Snowbed community and soil C and N dynamics in high-elevation ecosystems (NW-Italian Alps)

Pintaldi E, Quaglia E, Viglietti D, Pittarello M, D’Amico ME, Lombardi G, Colombo N, Lonati M, Freppaz M

The snowbed is a peculiar habitat which not only hosts a well adapted pool of plant species, but which soil characterization is highly dependent on the specific ecological and climatic conditions of the biome. The chemistry of soil nutrients is directly influenced by precipitation dynamics, but factors such as the impact of the vegetation should be taken in consideration, especially in a global warming scenario where the plants’ growing cycle is likely to witness some major changes. In this chapter we aimed at linking seasonal fluctuations in soil pools of available carbon and nitrogen to the phenological cycle of the main species of the snowbed communities of the Cimalegna Plateau, the dwarf shrub *Salix herbacea* L. We also used different ecological indicators derived from four years of vegetation surveys to infere soil properties, arguing that the indirect use of plant communities might represent a valid tool to describe environmental conditions when compared with direct measurements of said soil properties. We further explore the data and methods to meet our aims in a manuscript submitted to Plant and Soil (2021).

Chapter 4: Successional Herbaceous Species Affect Soil Processes in a High-Elevation Alpine Proglacial Chronosequence.

Mainetti A, D'Amico M, Probo M, Quaglia E, Ravetto Enri S, Celi L,
Lonati M

In the final chapter, the objective was to analyze plant-soil interactions in a different high-elevation habitat. We focused on the interdependent dynamics of vegetation structure and biogeochemical processes to evaluate the biotic patterns in ecosystem development of deglacialized soils along a proglacial chronosequence. Two vascular plant species with different life strategies were selected in order to compare properties and chemistry of the soil around their roots as well as the ecological structure of the community across six different stages of the chronosequence. We analyzed plant number and cover in order to highlight differences in the colonization process among the two species. Furthermore, we tested variation in the C:N:P stoichiometry of soils near the two species and bare, to verify whether or not the establishment of one vegetation type could contribute to an increase in nutrients supply and the development of soil functionalities. We face our aims with data and methods which are described and discussed in a paper published in Front. Environ. Sci. (2021)9 8:615499. https://doi.org/10.3389/fenvs.2020.615499

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Chapter 2: Alpine tundra species phenology is mostly driven by climate-related variables rather than by photoperiod

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Abstract

The study of plant phenology has frequently been used to link phenological events to various factors, such as temperature or photoperiod. In the high-alpine environment, proper timing of the phenological cycle has always been crucial to overcome harsh conditions and potential extreme events (i.e. spring frosts) but little is known about the response dynamics of the vegetation, which could shape the alpine landscape in a future of changing climate. Alpine tundra vegetation is composed by an array of species belonging to different phytosociological optima and with various survival strategies, and snowbed communities are a relevant expression of such an extreme-climate adapted flora. We set eight permanent plots with each one in a snowbed located on the Cimalegna Plateau in Northwestern Italy and then we selected 10 most recurring species among our plots, all typical of the alpine tundra environment and classified in 3 different pools: snowbed specialists, grassland species and rocky debris species. For 3 years we registered the phenophases of each species during the whole growing season using an adaptation of the BBCH scale. We later focused on the three most biologically relevant phenophases, i.e., flower buds visible, full flowering, and beginning of seed dispersion. Three important season-related variables were chosen to investigate their relationship with the phenological cycle of the studied species: (i) the Day Of Year (DOY), the progressive number of days starting from the 1st of January, used as a proxy of photoperiod, (ii) Days From Snow Melt (DFSM), selected to include the relevance of the snow dynamics, and (iii) Growing Degree Days (GDD), computed as a thermal sum. Our analysis highlighted that phenological development correlated better with DFSM and GDD than with DOY. Indeed, models showed that DOY was always a worse predictor since it failed to overcome interannual variations, while DFSM and marginally GDD were better suited to predict the phenological development of most of the species, despite differences in temperature and snowmelt date among the three years. Even if the response pattern to the three variables was mainly consistent for all the species, the timing of their phenological response was different. Indeed, species such as *Salix herbacea* and *Ranunculus glacialis* were always earlier in the achievement of the phenophases, while *Agrostis rupestris* and *Euphrasia minima* developed later and the remaining species showed an intermediate behavior. However, we did not detect significant differences among the three functional pools of species.

Keywords: Alpine plants; Climate change; Growing degree days; Italian Alps; *Salix herbacea*; Snowbed vegetation

Introduction

The most recent scenarios show that high-elevation and high-latitude biomes have been remarkably affected by Climate Change and the impacts will rapidly increase in the next future (Hock et al., 2019). Therefore, the European Alpine region will likely witness an increasingly dramatic warming and a severe alteration of snow dynamics (Rixen et al. 2008). In this biogeographic region, the so-called ‘snowbeds’ are landscape portions generally located at altitudes above 2500 m a.s.l. on small, flat surfaces, where snow cover lasts up to 10 months/year on relatively deep soils with considerable organic layers (Freppaz et al. 2010). During the snow cover period, thanks to the insulating properties of the thick snowpack, soil temperature seldom drops below 0°C and freeze-thaw cycles are rare (Carbognani et al. 2012; Petraglia et al. 2014). Although currently fragmented, snowbeds may help detecting which climatic alterations could potentially threaten the whole arctic and alpine biomes in the future (Björk and Molau 2007). According to the most recent reports, snow-dependent species of polar and mountain regions are already declining in abundance and their seasonal activities suffer from alterations due to changes in winter and spring climatic dynamics, causing an increase in their risk of extinction (IPCC 2019b). Even species that are not threatened in the short term, due to a higher ecological plasticity, are likely to suffer a decrease in their overall performances (Sedlacek et al. 2015).

The snowbed vegetation is composed by a specific array of species, characterized by a number of different survival strategies. Indeed, within this habitat, species with different phytosociological optima can coexist, and three main functional pools of species can be generally found, namely (a) snowbed species, (b) grassland species and (c) debris species. Also Ninot et al. (2013), studying functional traits of alpine tundra plants, grouped species into these three categories. More specifically, together with snowbed specialists, which are the most adapted to the long-lasting snow cover conditions, typical grassland species as well as ridge and pioneer species from surrounding grasslands and rocky outcrops can profit of small scale niche differentiation within the snowbed (Körner 2003). Unlike the ridge vegetation which is adapted to resist severe cold, strong winds and spring frost, snowbed vegetation benefits from the insulation provided by the thick, long-lasting snowpack. The persistence of this layer not only prevents possible late spring frost damages on plants reproductive structures, but it also maintains an ideal soil moisture content during the whole growing season (Isard 1986). Within this habitat, most of the species are perennials, for instance, the most distinctive snowbed species is the dwarf shrub *Salix herbacea* L., which despite the small size of the aerial parts can develop an impressive and extremely ramified root system and shows pronounced longevity (Wijk 1986; Beerling 1998). Some other species, such as *Poa alpina* L. subsp. *vivipara*, implement a strategy of agamic reproduction instead of the gamic one, which at this elevation requires a higher energetic effort and has a lower success rate.

Phenology has been frequently used to investigate the response of the life cycle of alpine plants to a wide range of environmental and climatic variables (Ernakovich et al. 2014; Khorsand et al. 2015; Ding et al. 2016). Additionally, phenology is an effective parameter to test whether or not species will be able to adapt with the progressive anticipation of snowmelt (Schmid et al. 2017), since the correct timing of sensitive phenophases is crucial for their survival. Indeed, in the alpine environment, rising temperatures and sudden alterations in the snow dynamics, such as reductions in volume and duration of the snow layer, are the most likely drivers of changes that could affect plant species survival (Huelber et al. 2006). The high-elevation environment is likely to maintain, in the short term, a certain degree of protection against spring frost, due to a less dramatic reduction of the amount and duration of the snow cover (Klein et al. 2018) when compared to low or medium-elevation environments, which are more at risk of frost-related damage (Vitasse et al. 2018). On the other hand, more competitive species of typical alpine grasslands could benefit from advantages such as the elongation of the growing season (Wang et al. 2016) or the increased nutrient input in the soil due to a higher bacterial activity (Freppaz et al. 2008) at the expense of the strongly adapted snowbed specialists. Most of the studies carried out up to now has mainly focused on the flowering phenological stages (Totland et al. 2002; Kawai and Kudo 2011; Carbognani et al. 2016; Richardson et al. 2017), while the analyses of other phenophases which could be relevant for the success of the species is far less common.

In our study, we assessed the linkages between plant phenology (expressed through phenophases) and three temporal and climate-related explanatory variables that are commonly used in literature to predict the progression of phenological events. The first one was the Day Of Year (DOY), the progressive number of days starting from the 1st of January, as it is widely considered to be an effective proxy of the photoperiod for alpine vegetation (Kimball et al. 2014) and has been frequently adopted to describe flowering phenophase, especially at low-elevation environments (for instance by Moriondo et al. 2001; Iannucci et al. 2008). A conspicuous number of studies have also explored its suitability to outline the response of alpine plant communities to photoperiod (Kimball et al. 2014; Petraglia et al. 2014). Snowbed vegetation is generally expected to be less responsive to this factor when compared to communities typical of early melting snow cover sites (Hülber et al. 2011) but, due to its composite pool of species and the interaction of multiple environmental factors, the correlation between snowbed species phenology and DOY is still far from being thoroughly inspected. The second variable we used was Days From Snow Melt (DFSM), which is the progressive number of days after snowmelt (used in Wang et al. 2015; Delnevo et al. 2018). It represents a simple yet effective explanatory variable to explore the relationship between plant phenology and the spring dynamics of the snowpack (Kimball et al. 2014; Petraglia et al. 2014) and to understand the relationships between the risk of late frost damage and an increasingly early snowmelt. In snow-dependent habitats this kind of variables are widely adopted to investigate the risk of frost damage correlated to an increasingly early snowmelt, but its use to describe the whole phenological cycle has been less frequent. The third explored variable was Growing Degree Days (GDD), which accounts for the sum of temperatures above which plants carry out their physiologic processes starting from a certain baseline temperature. GDD is largely adopted in agronomy and plant science and considered an effective predictor of plant phenological responses (Moriondo et al. 2001; Lonati et al. 2009; Sedlacek et al. 2015) . Nevertheless, it is still uncertain to what extent thermal requirements of alpine species are due to their plasticity or their genetic adaptation (Kawai and Kudo 2011; Domenech et al. 2016).

The aim of the present study was to investigate the occurrence of certain phenophases in a pool of alpine tundra species belonging to different functional groups (i.e. snowbed, grassland and debris species), taking into account interannual fluctuations. The objective of this research was to address the following questions:

1. Which temporal and climate-related variable is more related to the phenophases of different alpine tundra species?

2. Are there significant differences in the phenological development among these species?

3. Are there any differences among functional pools of species?

1. Materials and Methods

1.1 Study area

The experiment was carried out at the Cimalegna Plateau (Sesia Valley, North-western Italian Alps), an area close to Monte Rosa Massif (45°52'12.0"N, 7°53'3.0"E) and with an elevation ranging between 2600 and 2900 m a.s.l. The whole area is included in the international Long Term Ecological Research monitoring network (LTER 2020), within the site Mosso, Passo Salati-Col d'Olen (IT).

The bedrock was mainly micaschists, with some inclusions of ophiolites and calcicschists (Magnani et al. 2017) . Within the Plateau, the vegetation was composed by a mosaic of three main phytosociological communities: (i) *Androsacetum alpinae* (Br.Bl. in Br.Bl. et Jenny 1926) on the steeper slopes with medium or large silicic debris;

(ii) *Caricetum curvulae* (Rübel 1911) with acidic grasslands species on windy ridges and gentle slopes; and (iii) *Salicetum herbaceae* (Rübel 1911) on snowbeds with longer snow cover duration and deeper soils.

During the last decade the area was characterized by a mean annual air temperature of 7°C in the snow-free period, a cumulative average annual snowfall of 805 cm, and a mean annual liquid precipitation of 283 mm (Meteomont, Stazione Mosso). The snow cover duration lasted 263 days on average, with the snowpack developing by late October - early November, while snowmelt starts in late May to early June on the steeper and more sunny slopes and up to mid-July in some of the shadiest snowbeds.

1.2 Phenological monitoring

A set of eight permanent plots was established within eight snowbed sites (Figure 1), all including the *Salicetum herbaceae* association. Plots were located at an altitude ranging from 2686 to 2854 m a.s.l., on flat and homogeneous surfaces.

Each plot was a 4 m × 4 m square and was equipped at ground level with a HOBO Pro v2 U23-00x (Onset Corp., Pocasset, MA) thermal probe measuring hourly temperature since the beginning of the monitoring.

During the summer 2015, botanical surveys were carried out to describe the plot vegetation composition and identify the most frequent plant species. For the phenological monitoring we selected ten species which were widespread over the Plateau and common among the eight plots (Table 1). In this study, we grouped the species in three functional species pools that reflect the main habitats in the surroundings. In order to identify the different pools, which were characterized by similar ecological needs, we classified each species according to its phytosociological optimum at the class level, following Aeschimann et al. (2004). Moreover, species in phytosociological classes having physiognomic, ecological, and floristic similarity were pooled according to Aeschimann et al. (2004). Accordingly, we were able to identify three main functional species pools: snowbed specialist species (hereafter, snowbed), typical alpine grassland species (hereafter, grassland) and rocky debris species (hereafter, debris).

For the phenological monitoring each 4 m × 4 m plot was permanently divided in 16 subplots 1 m x 1 m used as pseudo-replicates, where we recorded the phenophase of the most advanced individual of each species. The phenophase was assigned with a visual observation using an adaptation of the BBCH scale (Hack et al. 1992) fitted for the snowbed plant communities as a reference (Annex 1). Such adapted scale consists in a series of progressive numbers ranging from 0 to 59, with the tens representing the major phenophases in chronological order (Shoots emergence, Leaves development, Flower buds, Flowering, Fruiting, Seed dispersal) and the units representing the advancement of said phenophases. The surveys were carried out every vegetative season along a three-year span (2016, 2017 and 2018) for seven up to nine times per year. Since the initial stages occurred very quickly right after snow melt, the monitoring dates were closer, thus the surveys were carried out every week at the beginning of the growing season (i.e. June-July). The frequency of the surveys was reduced to one monitoring date every two or three weeks towards its end (i.e. August-September), matching the deceleration of the growing cycle and the greater time lag occurring between recognizable phenophases. The average frequency at the beginning of the growing season was coherent with other studies in the Alpine area (Larl et al. 2006; Filippa et al. 2015; Carbognani et al. 2016). A photographic handbook of the phenophases was drawn during the first year of monitoring to avoid, as far as possible, interannual surveying bias. The subplots in which no individual for a given species reached at least the flowering stage at the end of the season were excluded from further analysis.

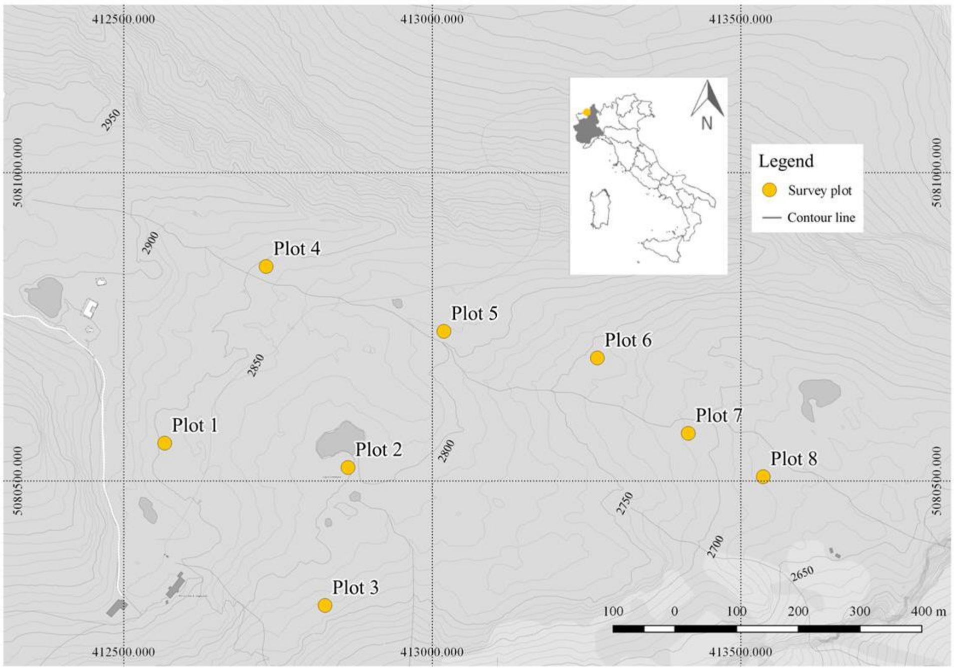

Figure 1 Distribution of the survey plots on the Cimalegna Plateau, in the Western Italian Alps. Coordinates are referred to the system WGS 84/UTM zone 32N.

Table 1 List of the target species selected for this study, with their growth form, optimum phytosociological class and the identification of functional pool.

Species	Growth form Landolt (2010)	Optimum class Aeschimann et al. (2004)	Functional species pool
Agrostis rupestris All.	Perennial graminoid	*Elyno-Seslerietea variae*	Grassland
Alchemilla pentaphyllea L.	Perennial forb	*Salicetea herbaceae*	Snowbed
Euphrasia minima Jacq. ex DC.	Annual forb	*Juncetea trifidi*	Grassland
Gnaphalium supinum L.	Perennial forb	*Salicetea herbaceae*	Snowbed
Leucanthemopsis alpina (L.) Heywood	Perennial forb	*Juncetea trifidi*	Grassland
Luzula alpinopilosa (Chaix) Breistr.	Perennial graminoid	*Salicetea herbaceae*	Snowbed
Poa alpina L.	Perennial graminoid	*Molinio-Arrenatheretea*	Grassland
Ranunculus glacialis L.	Perennial forb	*Thlaspietea rotundifolii*	Debris
Salix herbacea L.	Perennial shrub	*Salicetea herbaceae*	Snowbed
Veronica alpina L.	Perennial forb	*Thlaspietea rotundifolii*	Debris

1.3 Statistical analysis

We considered the raw observations as pseudo-replicates, therefore we did not use them as replicates in the further statistical analyses. For every monitoring date, the phenophase of the species occurring in each plot was calculated as the median value of the 16 subplots, in order to avoid outliers. We studied the relationships between plant phenophases and DOY, DFSM, and GDD to investigate the effects of these three temporal and climate-related variables as proxies of photoperiod, snow dynamics and temperature, respectively. The values of the three proxy variables were interpolated on three phenophases selected as the most relevant for their biological role: (i) Phenophase 20, corresponding to inflorescence or flower buds visible; (ii) Phenophase 35, corresponding to full flowering; and (iii) Phenophase 50, corresponding to the beginning of seed dispersal. Since plants are sensitive to climatic threats, such as late spring frost, these phenophases were selected as the most relevant, since they are critical for plant survival and reproduction. The GDD base 0°C was calculated for every date and plot starting from the 1st of January (Figure 2). This type of thermal sum was used instead of the more common GDD base 5°C, since alpine plants have lower thermal requirements when compared with lowland species (Legault and Cusa 2015; Carbognani et al. 2016; Mulder et al. 2017).

In order to explore the relationship between the phenological development and explanatory variables, a correlation analysis (Spearman's correlation) was carried out separately for each species. The median phenophases values, recorded during all the years and in all the plots, were plotted per each species against DOY, DFSM and GDD separately. Spearman’s coefficient was computed and used to compare the different correlations.

Then, in order to analyze the interannual differences we used Generalized Linear Mixed Models (GLMMs) for each species and for each phenophase. Separate models were run with DOY, DFSM and GDD set as response variables against year while the plot was used as random factor. We built models using both Gaussian and Gamma distribution, then we chose the best fitting one, i.e. that one showing the lowest Akaike Information Criterion (Zuur et al. 2009). When significant differences were found, Tukey’s post-hoc tests were used to assess variations in vegetation response amongst years.

Finally, to compare how the phenological development of different species could respond to different factors, GLMMs were again run separately on the values interpolated on the three phenophases. Models were built with DOY, DFSM and GDD as response variables, species as a fixed factor while plot and year were considered as random factors. When significant effects were found Tukey’s post-hoc test was used to assess differences amongst species. The same analysis was performed using functional pool of species as fixed factor, to compare difference between snowbed species, grassland species and species of the rocky debris. The DOY, DFSM and GDD were used as response variables against functional species pool, while species were set as fixed factors and plot and year random factor. We built models using both Gaussian and Gamma distribution, selecting the one with a lower AIC (Akaike Information Criterion) according to Zuur et al. (2009). All the statistical analyses were performed with R 3.5.2.

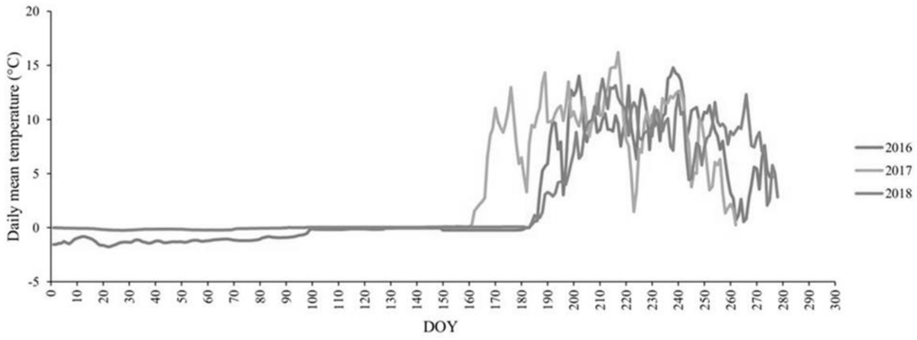

Figure 2 Daily mean temperatures (°C) of all the plots starting from the 1st of January, for the three studied years. Time is expressed as DOY, Day of Year.

2. Results

2.1 Interannual differences

The temperature trends of the three studied years within the eight monitored plots are reported in Figure 2. Over the course of the monitoring, average yearly temperatures of whole snow-free period had small differences (8.5°C in 2016, 8.6°C in 2017 and 7.9°C in 2018). On the other hand, both the length of the snow-free season and the date of snowmelt varied among years, highlighting how 2017 was different from years 2016 and 2018. The snowmelt date for all the eight plots was on average on DOY 187 during year 2016, on DOY 165 during 2017 and on DOY 194 during 2018, i.e. with a variation range of about a month. The average variation in snowmelt date among plots was 17 days throughout the whole monitoring period, with a difference of respectively 11 and 14 days between the earliest and latest melting plot during years 2016 and 2018, while a difference of 25 days was assessed during year 2017. The interannual trend of snowmelt date (represented by the first spike after the flat line in Figure 2) was consistent throughout all the plots.

All the correlations between plant species phenophases and DOY, DFSM and GDD were significant with high R values (Figures 3, 4 and 5). The value of the comparison of Spearman’s coefficients highlighted that correlation between plant phenophases and DOY was always lower than correlations between phenophases and DFSM and GDD, this trend being consistent throughout all the species and functional pools. Correlation coefficients of DFSM and GDD were considerably similar, with the first being slightly higher than the latter for eight out of ten species. The only exceptions were *P. alpina* and *A. rupestris* where the two values were equal. For all the species and phenophases, DOY values were the most unstable, with significant differences among years recurring in all the cases (Table 2). Moreover, while considering the DOY values of different species and phenophases, 80% of the cases showed exactly the same trend along the growing season. The DOY values for 2016 and 2018 were always grouped in the same post-hoc group and set apart from year 2017, which showed lower values. Not significant difference among years was detected in 56% of the cases for the DFSM models and 33% of the cases for GDD models. The results were not consistent throughout all the species and phenophases. For some species the response to the variables was mutually exclusive (*R. glacialis* and *A. rupestris* were responsive to DFSM only, not to GDD) while for *L. alpinopilosa* and *L. alpina* the responses to both variables were perfectly overlapped. *E. minima* was the only species insensitive to both DFSM and GDD and, to a lesser extent, *A. pentaphyllea* and *P. alpina*, which responded to DFSM only and in a single phenophase, were moderately insensitive. However, most of the species showed a lack of significant interannual variation. Seven species were responsive to DFSM or GDD in at least two phenophases, and for a total of four species (i.e. *G. supinum, S. herbacea, A. rupestris, V. alpina*) the DFSM alone or alternatively DFSM or GDD were fitting in the description of all the three selected phenophases.

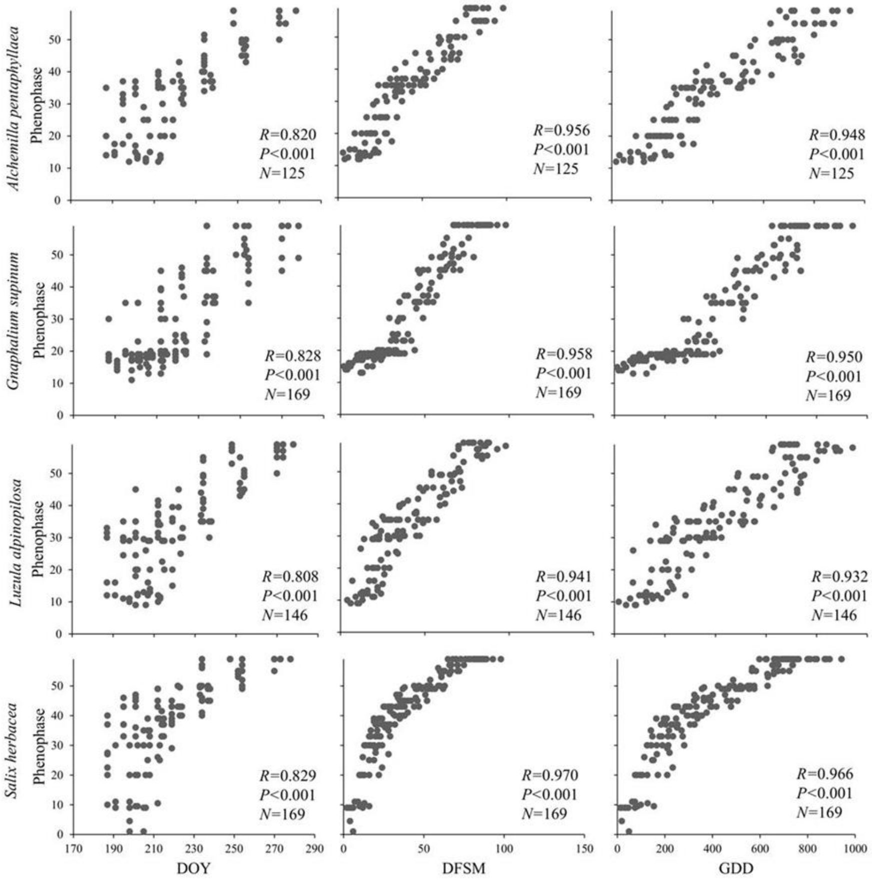

Figure 3 Correlations between phenophases of the snowbed species and Day Of Year (DOY), Days From Snow Melt (DFSM) and Growing Degree Days (GDD). Every point represents the median phenophase of the 16 subplots at a defined survey date in a single plot and year. Plant phenophases are expressed as a progressive number following the adaptation of BBCH scale (Hack et al. 1992). Spearman's *r* and significance of the correlation are indicated in every chart.

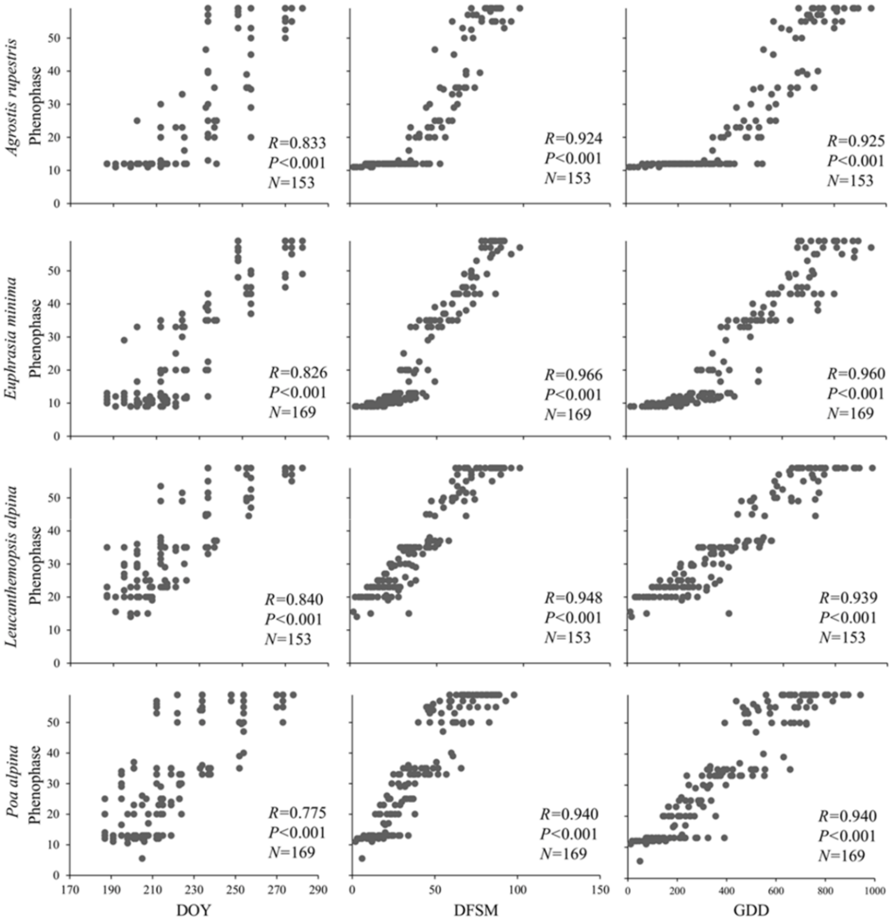

Figure 4 Correlations between phenophases of the grassland species and Day Of Year (DOY), Days From Snow Melt (DFSM) and Growing Degree Days (GDD). Every point represents the median phenophase of the 16 subplots at a defined survey date in a single plot and year. Plant phenophases are expressed as a progressive number following the adaptation of BBCH scale (Hack et al. 1992). Spearman's *r* and significance of the correlation are indicated in every chart.

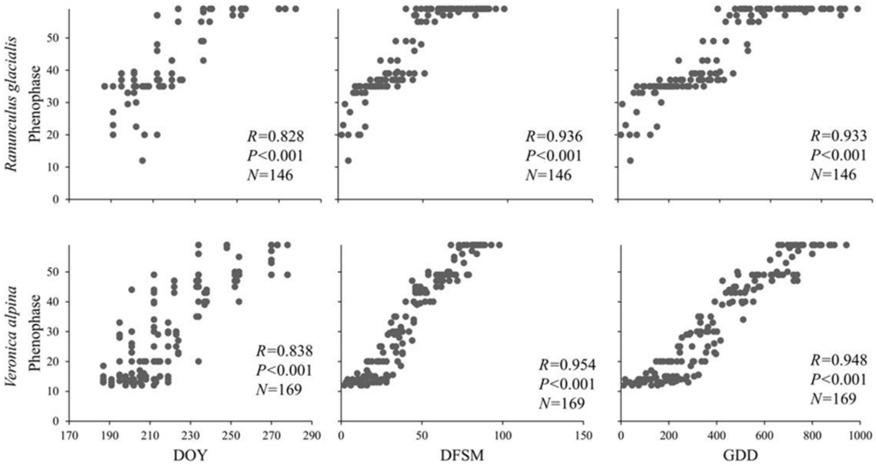

Figure 5 Correlations between phenophases of the debris species and Day Of Year (DOY), Days From Snow Melt (DFSM) and Growing Degree Days (GDD). Every point represents the median phenophase of the 16 subplots at a defined survey date in a single plot and year. Plant phenophases are expressed as a progressive number following the adaptation of BBCH scale (Hack et al. 1992). Spearman's *r* and significance of the correlation are indicated in every chart.

Table 2. Comparison of mean values of Day Of Year (DOY), Days From Snow Melt (DFSM) and Growing Degree Days (GDD). The mean value of DOY, DFSM and GDD for each phenophase and species is reported, alongside with the corresponding standard error (Phenophase 20=Flower buds visible, 35=Full flowering, 50=Beginning of seed dispersal). Letters are assigned with Tukey's post-hoc and listed only when there is a significant difference (*P* < 0.05). ns means the difference is non-significant, while the symbol - means there are no values available.

Functional species pool	**Species**	**Pheno phase**	**Year**	**DOY**		**DFSM**		**GDD**	
Snowbed species	Alchemilla pentaphyllea	20	2016	208.5 ± 1.43	B	22.4 ± 2.05	ns	221 ± 18.21	B
			2017	190.2 ± 1.66	A	25.8 ± 2.2		251.6 ± 29.42	B
			2018	210.6 ± 4.08	B	17.4 ± 1.86		167.5 ± 18.52	A
		35	2016	227.9 ± 3.84	B	41.8 ± 4.11	B	413.3 ± 40.63	B
			2017	199.8 ± 2.81	A	37.8 ± 1.89	AB	371 ± 25.79	AB
			2018	225 ± 4.25	B	31.8 ± 2.19	A	316.6 ± 19.02	A
		50	2016	258.1 ± 1.78	B	71.9 ± 2.62	B	712.3 ± 20.72	B
			2017	237.7 ± 1.73	B	75.7 ± 1.43	A	760.8 ± 24.58	B
			2018	258.6 ± 2.62	A	65.4 ± 1.8	B	624.9 ± 26.66	A
	Gnaphalium supinum	20	2016	218.7 ± 2.2	B	31.4 ± 2.65	ns	300.3 ± 24.21	AB
			2017	198 ± 1.57	A	30.6 ± 1.39		320.4 ± 18.35	B
			2018	219.7 ± 2.75	B	26.2 ± 1.76		259.6 ± 18.52	A
		35	2016	234.5 ± 2.04	B	47.3 ± 2.08	ns	465.5 ± 22.86	ns
			2017	212.4 ± 3.59	A	47.1 ± 2.14		465.3 ± 24.98	
			2018	237.5 ± 3.65	B	44 ± 2.07		429.2 ± 15.82	
		50	2016	249.3 ± 1.11	C	62 ± 1.55	B	630.1 ± 17.19	ns
			2017	233.5 ± 3.38	B	68.3 ± 2.26	A	678.1 ± 29.44	
			2018	256.9 ± 3.43	A	64.2 ± 2.35	AB	604.2 ± 20.25	
	Luzula alpinopilosa	20	2016	207 ± 2.15	B	20.2 ± 1.99	ns	194.4 ± 25.43	ns
			2017	193.3 ± 1.98	A	27.2 ± 3.23		261.8 ± 26.85	
			2018	211 ± 3.89	B	16.7 ± 2.58		156.2 ± 26.3	
		35	2016	238.3 ± 0.78	C	51.5 ± 1.28	B	510 ± 13.74	C
			2017	204.9 ± 3.35	B	38.7 ± 3.64	A	407.1 ± 34.41	B
			2018	225.5 ± 3.6	A	31.3 ± 2.1	A	307 ± 20.35	A
		50	2016	259.7 ± 1.98	B	73 ± 2.01	ns	701.3 ± 20.95	ns
			2017	234.9 ± 2.12	A	68.7 ± 2.49		697.4 ± 30.07	
			2018	257 ± 2.28	B	62.7 ± 2.37		589.3 ± 29.38	

Functional species pool	**Species**	**Pheno phase**	**Year**	**DOY**				**DFSM**				**GDD**			
Snowbed species	Salix herbacea	20	2016	203.7	±	1.59	B	14.9	±	2.22	ns	141.4	±	22.75	B
			2017	188.5	±	1.5	A	13.5	±	1.5		118	±	19	AB
			2018	205.7	±	2.54	B	12.2	±	1.06		105.3	±	10.82	A
		35	2016	211.6	±	1.9	B	24.3	±	1.77	AB	233.8	±	19.62	ns
			2017	193.8	±	1.19	A	26.2	±	1.99	B	248.8	±	23.12	
			2018	214.1	±	2.48	B	20.6	±	1.39	A	198.2	±	15.85	
		50	2016	240.8	±	1.25	B	53.6	±	1.63	ns	535.1	±	20.51	ns
			2017	216.9	±	2.46	A	51.6	±	2.74		514.9	±	24.09	
			2018	245.5	±	3.18	B	52	±	1.97		500.2	±	19.33	
Grassland species	Agrostis rupestris	20	2016	232.9	±	2.32	B	46.2	±	2.46	ns	453.2	±	26.54	B
			2017	206.4	±	4.81	A	41.1	±	3.29		453.3	±	25.08	B
			2018	231.8	±	4.37	B	39.2	±	2.79		372.9	±	30.82	A
		35	2016	250	±	2.17	B	63.3	±	2.35	ns	642.5	±	27.93	B
			2017	222	±	4.99	A	56.8	±	5		604.3	±	21.47	B
			2018	246.2	±	4.8	B	53.6	±	2.89		516.9	±	20.37	A
		50	2016	266.9	±	0.44	B	80.2	±	1.38	ns	739.7	±	20.64	B
			2017	231.9	±	5.97	A	66.6	±	6.3		710	±	28.11	B
			2018	258.6	±	4.23	B	66	±	2.26		627	±	14.94	A
	Euphrasia minima	20	2016	224.1	±	1.73	B	36.9	±	2.25	C	354.9	±	23.07	B
			2017	206.4	±	3.97	A	41.1	±	2.53	B	425.9	±	27.99	A
			2018	226.5	±	3.33	B	33	±	1.54	A	328	±	13.28	B
		35	2016	237.4	±	0.18	B	50.1	±	1.36	B	496.2	±	16.71	B
			2017	222.5	±	3.31	A	57.3	±	2.56	A	571.9	±	30.49	A
			2018	240.5	±	3.76	B	47	±	1.92	B	454.8	±	13.02	B
		50	2016	262.1	±	0.66	B	74.9	±	1.43	AB	712.3	±	18.47	C
			2017	243.1	±	1.3	A	77.9	±	2.25	B	773	±	25.82	B
			2018	262.1	±	2.59	B	69.4	±	0.69	A	649.2	±	12.63	A
	Leucanthemopsis alpina	20	2016	204.1	±	1.67	B	16.6	±	2.92	B	157.8	±	29.6	B
			2017	189	±	2	A	20	±	8	AB	188.5	±	89.5	AB
			2018	200.2	±	3.34	AB	7.7	±	1.91	A	63.5	±	14.91	A
		35	2016	225.5	±	2.64	B	38.2	±	2.65	ns	370	±	27.51	ns
			2017	205.9	±	3.6	A	40.9	±	1.62		391.3	±	26.94	
			2018	227	±	3.44	B	34.5	±	1.45		349.7	±	15.8	

Functional species pool	Species	Pheno phase	Year	DOY				DFSM				GDD			
Grassland species	Leucanthemopsis alpina	50	2016	250.6	±	1.9	B	63.4	±	2.72	ns	638.1	±	28.69	ns
			2017	224.6	±	3.45	A	59.6	±	1.85		582.9	±	32.35	
			2018	249.7	±	2.86	B	57.2	±	1.17		547.4	±	17.65	
	Poa alpina	20	2016	211.1	±	2.42	B	23.8	±	2.61	ns	228.6	±	25.77	B
			2017	192.6	±	2.03	A	25.1	±	1.06		241.9	±	17.16	B
			2018	213.3	±	3.32	B	19.8	±	2.03		189.4	±	19.27	A
		35	2016	238.8	±	2.55	C	51.5	±	2.21	B	530	±	23.23	B
			2017	204	±	2.22	B	38.8	±	1.62	A	380.6	±	11.13	A
			2018	229.3	±	2.96	A	35.8	±	2.28	A	356.4	±	20.44	A
		50	2016	255.7	±	2.23	B	68.4	±	2.45	B	680.7	±	18.63	B
			2017	214.4	±	3.1	A	49.1	±	2.21	A	487.1	±	17.8	A
			2018	246.6	±	4.75	B	53.1	±	3.66	A	508.3	±	28.29	A
Debris species	Ranunculus glacialis	20	2016	209.3	±	-	-	23.3	±	-	-	222.2	±		-
			2017	191	±	-	-	41	±	-	-	339	±	-	-
			2018	203.8	±	4.48	-	8.3	±	2.59	-	65.1	±	24.4	-
		35	2016	206	±	1.85	B	19.3	±	2.43	ns	186	±	23.71	AB
			2017	188.6	±	1.07	A	24.7	±	3.72		227.9	±	32.88	B
			2018	210.3	±	2.49	B	17.7	±	1.15		153.1	±	19.02	A
		50	2016	232.5	±	0.29	B	45.8	±	1.62	ns	449.3	±	17.84	AB
			2017	207.7	±	3.88	A	43.9	±	3.37		459.7	±	20.39	B
			2018	232.6	±	2.49	B	40	±	1.94		397.6	±	21.11	A
	Veronica alpina	20	2016	213.6	±	2.31	B	26.3	±	2.77	ns	253.2	±	28.71	ns
			2017	195.6	±	1.95	A	30.4	±	1.68		289.5	±	19.71	
			2018	217.6	±	3.2	B	24.1	±	2.18		235.5	±	23.72	
		35	2016	231	±	0.76	B	43.7	±	1.68	B	427.4	±	18.02	ns
			2017	208.5	±	2.54	A	43.3	±	0.92	B	426.6	±	13.43	
			2018	230.9	±	3.14	B	37.4	±	2.03	A	372.2	±	19.63	
		50	2016	255.8	±	0.9	B	68.5	±	1.57	ns	681.3	±	17.31	B
			2017	230.5	±	2.88	A	65.3	±	1.97		650.5	±	26.41	AB
			2018	253.5	±	2.09	B	62	±	1.96		590.4	±	23.28	A

2.2 Interspecific differences

When analyzing interspecific differences (Figure 6) species had similar responses when considering DFSM and GDD, whereas with a slight difference concerning DOY in phenophase 20 (Flower buds visible) and 50 (Beginning of seed dispersal). The species were equally sorted also in phenophase 35 (Full flowering) for all the variables. The same species showed a recurring distribution throughout most of the phenophases: *R. glacialis* and *S. herbacea* always reached the three phenophases at the lowest values of DOY, DFSM and GDD, together with *L. alpina* during the flower buds phenophase. *E. minima* and *A. rupestris* always achieved the selected phenophases at the highest values of DOY, DFSM and GDD, as well as *G. supinum* during the flower buds and full flowering phenophases and with *A. pentaphyllea* during the dispersal phenophase. *L. alpina* had an early onset just for the first phenophase, while the remaining species have an intermediate phenological development.

The model run with species grouped in functional pools did not highlight any significant difference in the achievement of the three phenophases, regardless of the selected variables (Table 3).

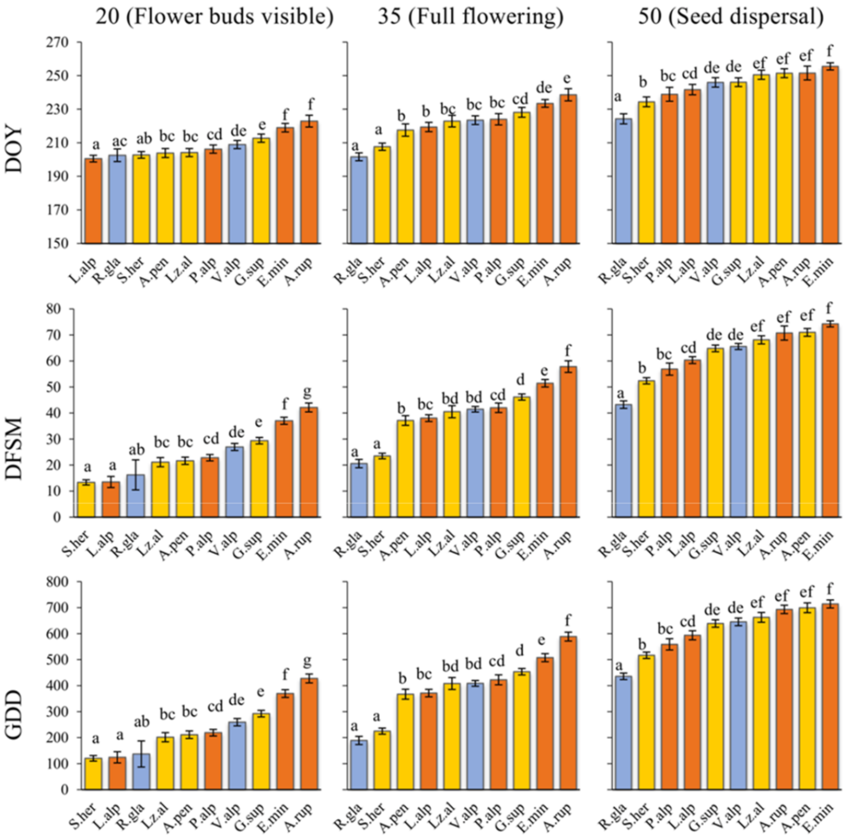

Figure 6 Interspecific differences through three selected phenophases and Day Of Year (DOY), Days From Snow Melt (DFSM) and Growing Degree Days (GDD). The chart is referred to all the monitoring years and sites. The height of the column represents the

mean value of DOY, DFSM or GDD at which the given phenophase was reached, together with its corresponding standard error. Different letters highlight significant differences among species (letters assigned with Tukey's post-hoc tests for $P < 0.05$). The colors identify the three functional species pools: blue: debris species; yellow: snowbed species; red: grassland species. A.rup=*Agrostis rupestris* A.pen=*Alchemilla pentaphyllea*; E.min=*Euphrasia minima*; G.sup=*Gnaphalium supinum*; L.alp=*Leucanthemopsis alpina*; Lz.al=*Luzula alpinopilosa*; P.alp=*Poa alpina*; R.gla=*Ranunculus glacialis*; S.her=*Salix herbacea*; V.alp=*Veronica alpina*.

Table 3 Differences through the selected phenophases and variables in terms of functional species pools. Mean value of Day Of Year (DOY), Days From Snow Melt (DFSM) and Growing Degree Days (GDD) for each pool is expressed alongside with the corresponding standard error.

Phenophase		**20**				**35**				**50**			
Variable	**Group**	**Mean**		**S.E.**		**Mean**		**S.E.**		**Mean**		**S.E.**	
DOY	Debris	203	±	8.6	ns	213	±	9.3	ns	236	±	9.4	ns
	Snowbed	204	±	7.4		219	±	8.2		246	±	8.4	
	Grassland	211	±	7.4		229	±	8.2		247	±	8.4	
DFSM	Debris	22	±	6.3	ns	31	±	6.6	ns	54	±	6.2	ns
	Snowbed	22	±	4.7		37	±	5.0		64	±	4.6	
	Grassland	29	±	4.7		47	±	5.0		66	±	4.6	
GDD	Debris	199	±	67.4	ns	298	±	69.5	ns	538	±	60.0	ns
	Snowbed	209	±	51.0		360	±	52.4		625	±	46.0	
	Grassland	288	±	51.0		473	±	52.4		640	±	45.9	

3. Discussion

3.1 Interannual differences

Although Climate Change models foresee a significant snowfall reduction in the Alps, higher elevation areas located in the alpine belt (i.e. above the treeline), such as the Cimalegna Plateau, are currently facing a less dramatic change in snow dynamics than lower elevation areas located in the mountain and subalpine belts (IPCC 2019a). Moreover, the timespan of this study was not sufficient to witness evidence of Climate Change. Nevertheless, we detected a pronounced interannual variability especially in the date of snowmelt, which ranged up to one month over the three years of monitoring. Although the effect of microtopography determined some consistent variations in temperature and snowmelt across the plots, the weight of interannual differences was overwhelming on the general trend which set apart year 2017 from 2016 and 2018, enabling us to explore different scenarios. The snowmelt shift was mainly caused by various conditions of the snowpack, which can last longer according to spring temperature and snowfall dynamics during winter. For instance, the consistent amount of precipitation during winter 2017-2018 caused a thicker snowpack, resulting in a later snowmelt, while the relatively dry winter 2016-2017, with snowfall accumulation centered in December-January led to an early snowmelt. The change in the snowmelt date resulted in a broad variation of the beginning of the vegetative season since, in snowbed communities, the onset of the first phenophases is believed to be strongly dependent on snowmelt (Körner 2003; Lessard-Therrien et al. 2014; Wang et al. 2015). Indeed, findings from the correlations underlined how climatic differences among 2016, 2017 and 2018 heavily affected the whole temporal distribution of the phenological cycle. Despite the high correlation values between plant phenophases and DOY, DFSM or GDD, the first variable had always a lower Spearman's r than the other two. The phenophases of most of the species were strongly correlated to DFSM and slightly less correlated to GDD with two exceptions, having nearly identical values. Photoperiod, which is proxied by DOY, is frequently referred to as an important cue in the timing of phenological events since it can help controlling extremely early or late development (Hülber et al. 2010; Steltzer and Post 2009). Our results though addressed us towards the hypothesis of the relatively poor relevance of photoperiod as a single factor in the prediction of the phenological cycle of alpine plants when faced with the chance of interannual variability.

The interactions between photoperiodism, temperature, and alpine plant phenology has been investigated with an experiment by Keller and Körner (2003), who highlighted a strong dependence of flowering on photoperiod for most of the species (seven of them are in common with the present study). However, whereas at longer day length flowering dynamics were mostly influenced by photoperiodism, at shorter day length species were more sensitive to temperature regimes. Moreover, some species were found to be insensitive both to light and temperature (*R. glacialis*), thus possibly influenced by different ecological factors such as snow disappearance. Further links between flowering phenology and photoperiodism were studied by Larl et al. (2006). This study focused on the pioneer species *Saxifraga oppositifolia*, proving how the initiation of the flower primordia was controlled solely by photoperiod, while the time of flowering, seed development, and shoot growth depended on the date of snowmelt. Regarding arctic, sub-arctic and nival biomes, the date of snowmelt is widely recognized to have an overwhelming effect on plant species onset (Totland and Alatalo 2002; Wipf 2009).

While the effect of DOY always expressed a high variability throughout the years, with a consistent trend that identified 2017 as fairly different from 2016 and 2018, the effects of DFSM and GDD were less variable among years. Indeed, while considering DFSM and GDD, we observed for most of the species the lack of significant differences amongst the three monitoring years. This result led us to recognize these two variables better predictors of the phenological development even across very dissimilar vegetative seasons. When the three years of the experiment were modelled together, we found most of the species appearing more strongly controlled by DFSM and GDD than by DOY, even if the trend was not consistent throughout all the species and phenophases. If such a finding highlights the presence of some fairly good predictors of the phenological cycle of the overall plant community, on the other hand it reflects some profound differences in the behavior of different alpine tundra species. Indeed, the study of plant phenology should address each species individually since either an overall approach and grouping in functional species pool would not grant reliable results. For some species (*A. rupestris, R. glacialis* and to some extent *P. alpina* and *A. pentaphyllea*) we were able to identify DFSM as a major driver of the phenological development. For most of the species, phenology seems to be related to both DFSM and GDD depending on the selected phenophase. For *E. minima*, the only studied annual species, we were not able to identify which variable best suited the description of the phenological cycle, even though results from the correlation analyses stressed how DFSM and GDD are always slightly superior to DOY. Likely, widespread annual species will persist in the next changing climate due to the ability to quickly adapt their phenology in order to track ideal seasonal conditions (Hereford et al. 2017). From our study, *E. minima* phenophases seemed not to rely on a specific temporal and climate-related variable. However, since a single species is not sufficient to assess the response path of the whole annual species group, we suggest as a forthcoming step to possibly analyze a larger number of plant annual species since the population dynamics between opportunistic widespread species and specialists will be crucial in shaping the future of such environments.

3.2 Interspecific differences

Literature is mostly concordant on behalf of the potential fragmentation and disappearance of snowbeds in the current Climate Change scenario due to alteration in snow dynamics (Hülber et al. 2011; Legault et al. 2015; Sedlacek et al. 2015) and input of competitive, non-specialist vegetation from the nearby habitats (Heegard and Vandvik 2004; Komac et al. 2015). Species with phytosociological optimum in different associations tend to show a wide range of strategies; snowbed specialists for instance are generally poor competitors (Kudo and Suzuki 1999; Onipchenko et al. 2004). They are more likely to struggle in shifting their phenological cycle when compared with opportunistic grassland species or pioneer species of the rocky outcrops (Gugger et al. 2015; Carbognani et al. 2014). Nevertheless, no significant difference emerged between snowbed, grassland and debris species, displaying how the phenological development is not affected by functional species pool and should be rather explored at a species level. When we tested in detail interspecific differences, the variability was significant. Such variability was expressed in a consistent tendency of each species to reach phenophases with a certain advance or delay, while confronted with the others. Due to the high number of species it was not possible to identify a consistent threshold value in order to separate them in categories. Even though such an objective value could not be found, observation of the charts suggested a well expressed pattern that led us to the identification of three phenological behaviors. We defined “early species” as the ones which were able to reach all the selected phenophases quite in advance, as opposed to “late species” which always achieved phenophases with a certain delay. Species with an in-between behavior and the tendency to sway in the middle of the first two groups were defined “intermediate”. This classification could be applied to all of our three variables with some minor variations. Overall, DFSM and GDD show more similarities since the behavior did not change depending on the selected variable nor on the phenophase. DOY exhibited a solid consistency with the other variables only when examined on the phenophase of full flowering. The variations illustrated in the remaining phenophases are mostly referred to the category of intermediate species, namely the ones not showing a strongly polarized behavior. Species with a pronounced early or late behavior in the achievement of the phenophases (*S. herbacea, R. glacialis, E. minima, A. rupestris*) were not affected by said variations, but they maintained respectively their early or late conduct. The occurrence of different behaviors represents the practice of different strategies, each of which bearing benefits and disadvantages. In case of an early disappearance of the snow layer, an extremely fast onset could determine exposure to structural damages, especially for flower buds, linked to spring frosts (Inouye 2008; Klein et al. 2018; Vitasse et al. 2018). On the other hand, an exaggerated delay in the occurrence of late phenophases could lead to the failure to complete the reproductive cycle. Our findings highlighted not only how relevant the interspecific differences are when analyzing plant phenology, but also how the selection of a specific season-related variable could have a major role in describing the plant phenological cycle. Further research, based on similar comprehensive methods for the monitoring of alpine plant phenology, appears advisable to take in account additional environmental factors (for instance chilling, soil nutrients or precipitation) to contribute to a more accurate modelling of the phenological behavior.

4. Conclusions

The snowmelt date and, to a certain extent, the thermal sum, resulted effective predictors for the phenophases of most of the species under consideration. The photoperiod, on the other hand, proved to be a less valuable predictor due to the failure to offset interannual variability. The synchronization of the phenological cycle with the disappearance of the snowpack and the temperatures is an evidence of the remarkable adaptation of these species to the peculiar conditions of the snowbed environment. Forthcoming changes in temperature and snow dynamics will have an impact on the vegetation communities in the long term. However, we highlighted the relevance of considering each species individually, since we reported major differences in the phenological behavior of a relatively homogenous plant community. The consistent achievement of all the phenophases at lower or higher values of DFSM and GDD determined the classification in three distinct groups. Species were defined early, late or intermediate regardless of their functional pools, and each of these strategies could bear benefits or disadvantages. While early species could profit from elongation of the season, they could also incur in the risk of frost damages to the reproductive structures. Late species will avoid such risks but could fail in concluding the phenological cycle.

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Annexes

Annex 1. Adaptation of the BBCH scale used during the phenological surveys. To meet the needs of describing the phenology of alpine tundra species, stages are described with a numeric code ranging from 0 to 59 and they are divided into “Principal growth stages”. For the sake of clarity the description of stages is given separately for Annual, Perennial and Graminoid species (A, P, G)

Code		Description
	Principal growth stage 0: Germination, sprouting, bud development	
00	A	Hypocotyl with cotyledons growing towards soil surface
	P	Shoot growing towards soil surface
	G	Coleoptile emerged from caryopsis
09	A	Emergence: Cotyledons break through soil surface
	P	Bud shows green tips
	G	Emergence: Coleoptile breaks through soil surface
	Principal growth stage 1: Leaf development	
10	A	Cotyledons completely unfolded
	P	First leaves separated
	G	First true leaf emerged from coleoptile
11	A,P,G	First true leaf, leaf pair or whorl unfolded
12	A,P,G	2 true leaves, leaf pairs or whorls unfolded
13	A,P,G	3 true leaves, leaf pairs or whorls unfolded
1 .	A,P,G	Stages continuous till . . .
19	A,P,G	9 or more true leaves, leaf pairs or whorls unfolded
	Principal growth stage 2: Inflorescence emergence/heading	

20	A,P	Inflorescence or flower buds visible (soil level)
	G	Beginning of heading
23	A,P	Inflorescence or flower buds visible (beginning of elongation)
	G	Less than an half of inflorescence emerged
25	A,P	First individual flowers/flower styles visible (still closed)
	G	Half of inflorescence emerged (middle of heading)
29	A,P	First flower petals/flower visible (in petalled forms)
	G	Inflorescence fully emerged (end of heading)
	Principal growth stage 3: Flowering (gamic/agamic reproduction for G)	
30	A,P	First flowers open (sporadically)
	G	First stamens/bulbils visible (sporadically), sheaf still closed
33	A,P	< 50% of flowers open
	G	< 50% of stamens/bulbils visible, sheaf begins to open
35	A,P	> 50% of flowers open, first petals may be fallen
	G	> 50% of stamens/bulbils visible, opened sheaf
37	A,P	Majority of petals dry or fallen
	G	Majority of stamens fallen/stem may begin to bend
39	A,P	End of flowering: fruit set visible
	G	End of flowering: fruit set visible/bent stem
	Principal growth stage 4: development of fruit	
40	A,P	Fruit visible (unripe)
	G	Caryopsis watery ripe
43	A,P	<50% have reached final size
	G	Early milk
45	A,P	>50% have reached final size
	G	Milky ripe, medium milk

47	G	Late milk
49	A,P	Nearly all fruits have reached final size
	G	Nearly all caryopsis have reached final size
	Principal growth stage 5: Dissemination of fruit and seed (caryopsis/bulbils for G)	
50	A,P	Beginning of fruit abscission
	G	Beginning of dissemination (first glumes may be dry and empty)
53	A,P	<50% of seed dissemination
	G	<50% of caryopsis/bulbils disseminated
55	A,P	>50% of seed dissemination
	G	>50% of caryopsis/bulbils disseminated
59	A,P	Full dissemination
	G	Full dissemination (glumes totally dry and empty)

Chapter 3: Snowbed community and soil C and N dynamics in high-elevation ecosystems (NW-Italian Alps)

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Abstract

Aims. This work aims to investigate the effect of climatic, pedoclimatic and vegetation variables on soil C and N dynamics in snowbed communities.

Introduction. Snowbed communities develop in areas that accumulate high amounts of snow, resulting especially vulnerable to the predicted decrease in snowfall and change in snowmelt date combined with increased temperature.
Methods. The work was carried out at seven permanent sites belonging to *Salicetum herbaceae* plant community, located at the LTER Site Angelo Mosso Scientific Institute (NW-Italian Alps). During a four-year monitoring, we studied the floristic composition, phenology and soil C and N dynamics, evaluating, among climatic, pedoclimatic and vegetation variables, the main drivers acting on soil C and N forms in these high-elevation ecosystems.

Results. The results indicated that the soil water content, the melt-out day and the duration of soil freezing were the main abiotic factors driving soil C and N dynamics. A greater soil water content and a delayed melt-out day increased N-NH4^{+}, N-NO^{3-}, DOC, TDN, Nmicr, Cmicr and C:Nmicr ratio, while the duration of soil freezing increased DON and TDN. Considering the vegetation variables, the phenology of *Salix herbacea* played a fundamental role, reducing the N-NH^{4+} and increasing DOC concentrations in soil, revealing a strong plant-soil relationship.

Conclusion. Our results suggested that the predicted changes in snow dynamics might alter the biogeochemical cycles and phenology of snowbed communities. Especially, variations in the amount of snow precipitation, its timimg as well as an anticipation of melt-out day and an increased air temperature, due to interannual variability and climate change, are likely to affect high-elevation ecosystems and the plant-soil relations.
Keywords: LTER; *Salix herbacea*; topsoil; phenology; carbon and nitrogen; alpine tundra

Introduction

The seasonally snow-covered regions, such as the Alps, are particularly sensitive to climate change (Cebon et al., 1998; Beniston, 2006), because small changes in temperature or precipitation may result in large modifications in the amount and timing of snow cover (Brooks et al., 2011). In particular, global warming might introduce a number of different effects on the seasonal snow cover: increasing temperatures result in a higher percentage of liquid precipitation falling instead of snow, causing a thinner snow layer which is also more prone to earlier melting, a phenomenon already visible in many mountain ranges (e.g. Laternser and Schneebeli, 2003; Viglietti et al., 2014). Since snow dynamics control organic matter decomposition in the soil and nitrogen (N) dynamics (Walker et al. 1999, Groffman et al. 2001), variations in the thickness and duration of snow cover (e.g. Notarnicola, 2020) result not only in remarkable differences in pedoclimatic conditions and nutrient cycling, but also in the composition (Walker et al. 1993; Fisk et al. 1998) and phenology of plant communities (Khorsand Rosa et al., 2015). As also reported by Hock et al., (2019), the combined effect of higher spring and summer temperatures and reduced snowfall leads to a thinner snow cover, receding melt-out dates, and longer growing seasons for vegetation (Dye, 2002). In particular, the reduction in snow cover and its variation in density and thickness (Rixen et al., 2008) likely contributes in decreasing soil thermal insulation, with the counterintuitive result of colder soil temperatures, an increase in soil freezing periods and a rise in freeze/thaw episodes (Edwards and Cresser; 1992; Groffman et al., 1999). Such variations are already deeply influencing soil carbon (C) and N dynamics (e.g. Freppaz et al., 2008, 2018). Thus, the presence of a substantial and long-lasting snow cover holds a prominent relevance for the ecology of a large part of the Earth’s biomes, especially at high latitude and in mountain regions (Jones et al., 1994). Indeed, the seasonal snowpack controls the onset and duration of the growing season in cold regions (e.g. Cooper et al., 2011; Freppaz et al., 2018), protects against extreme cold environmental conditions (Billings and Bliss, 1959, Körner, 2003), affects soil temperature and microbial activities (e.g. Edwards and Cresser, 1992, Mikan et al., 2002, Panikov et al., 2006, Freppaz et al. 2018), soil nutrient cycling (e.g. Bilbrough et al., 2000, Hiltbrunner et al., 2005, Filippa et al., 2010), soil gas fluxes (e.g. Filippa et al., 2009;Brooks et al., 2011), pedogenesis (Schaetzl, 1990; Holtmeier and Broll, 1992) and soil properties (e.g. Hiller et al., 2005; Pintaldi et al., 2016; Freppaz et al., 2018).

Snowbed habitats form in small areas that tend to accumulate winter snow, mainly due to topographical factors and wind redistribution (e.g. Gjaerevoll, 1956; Billings and Bliss, 1959; Johnson and Billings, 1962), representing a relevant component of the alpine tundra biome (Björk and Molau, 2007). These habitats, located above the treeline, are characterized by specific environmental properties, such as a limited growing season, extended periods with air temperatures below freezing, and prolonged snow-covered soils (Edwards et al. 2007, Knowles et al. 2015). In fact, snow is considered as a key factor due to its capability to control distribution and abundance of plant species and shape the spatial patterns of alpine vegetation (Braun-Blanquet, 1932; Gjaerevoll, 1956; Bliss, 1963; Scott and Billings, 1964). Within the alpine life zone, snowbed communities (e.g. *Salicetum herbaceae* Braun-Blanquet and Jenny 1926) are especially vulnerable to the forecasted decrease in snowfall and change in snow cover duration coupled with increased temperature (Carbognani et al., 2012), as the date of snowmelt and the related beginning of the snow-free period sets the start and the duration of the whole growing season, conditioning plant survival, distribution and growth(Walker et al., 1993; Stanton et al., 1994; Huelber et al., 2006).

Phenological processes are among the most sensitive plant responses to climate change and have crucial effects at all levels within the ecosystem (Khors and Rosa et al. 2015). While investigating the drivers of plant phenology is rather common in literature, it is fairly rare for alpine ecology experiments to address the issue of whether or not phenology itself could have an effect on soil nutrient dynamics throughout the growing season. The uptake of elements depends both on the phenology of plants and the availability of the resource, therefore it is not simple to discriminate between the contribution of the factors affecting the availability of soil nutrients (e.g pedoclimatic conditions) and the plants acquisition of nutrients. Nitrogen supplies often show a pronounced seasonality, which is run by the spur of mineralization by seasonal variations of temperature or moisture levels while its uptake is related to different phenological phases of plants. Given that snow beds are more altered by climate change than other subalpine and alpine plant communities (Matteodo et al., 2016), they are often considered as a “natural laboratory” where the effects of global warming on alpine vegetation can be thoroughly explored(Björk and Molau, 2007; Schöb et al., 2008).

In these environments, the locally microtopography-influenced distribution of the snowpack produces a spatial gradient in soil water availability, timing of soil dry-down following snowmelt (Taylor and Seastedt, 1994) and soil temperatures, which in turn affect plant communities distribution (Fisk et al., 1998), species growth (Walker et al., 1994) and build-up of soil organic matter (Burns and Tonkin, 1982). Thus, the C and N biogeochemical cycles of these soils have a wide variation range throughout the season, and are influenced by multiple factors (Schmidt and Lipson, 2004). Snowbeds generally have deeper and more developed soils than other high-elevation habitats, and their most relevant plant species (e.g. *Salix herbacea* L., *Poa alpina* L., *Luzula alpinopilosa* (Chaix) Breistr.) happen to bear complex and structured root systems (Wijk, 1986; Pohl et al., 2011). The effect of plant uptake on the availability of nutrients should be taken into consideration (Tian et al., 2017). To match their biological and developmental needs, plants tend toward different uptake intensities throughout the growing season (Onipchenko et al., 2009). Since the early stages such as foliation and flowering are the most demanding for the majority of alpine plants, during this phenological phase the nutrients uptake is higher (Mullen et al., 1998). In some cases, uptake of certain nutrients such as N could be activated even while the plant is still under the snow cover (Bilbrough et al., 2000). In Nord and Lynch (2009), uptake of mobile resources is proportional to the plant transpiration. Soil N and C pools increase toward the end of the growing cycle, in accordance with a diminishing in the uptake (Adair and Burke, 2010). Furthermore, nutrient dynamics in the complex alpine environment are tightly interlaced with microtopography (Moser et al., 2009), but sampling soil in a small patch could fail in a comprehensive description of the plot characteristics, diminishing or accentuating certain properties due to positioning bias of the samplings. It is possible to identify such a topography mosaic with the tool of vegetation surveys on a larger area, since the floristic composition is highly sensitive to micro variations in soil characteristics (Sterling et al., 1984; Sakai and Ohsawa, 1993). Through vegetation-derived indexes, such as Ecological Indicator Values (EIVs), it is possible to ecologically characterize an area from plant species and cover. The accuracy of such EIVs lies in the knowledge and extensive field experience of botanists and ecologists, which enable the characterization of the ecological condition of an area, e.g. soil moisture, nitrogen content and reaction (Ellenberg et al., 1992; Pignatti et al., 2005; Landolt, 2010). Moreover, EIVs are a valid tool for habitat estimation and could be used to indirectly describe the corresponding pedoclimatic properties (Schaffers and Sykora, 2000).

Alpine ecology literature lacks papers with a specific insight in the interdependent dynamics of soil biogeochemical cycles and plant phenology, and few studies are found on the relation between seasonal variations in soil C and N cycles and vegetation composition. Despite the reliability of such a tool, few studies utilize EIVs and few of them in the snowbed habitat. Therefore, to add knowledge about the dynamics of the snowbeds, in the framework of long-term ecological research, we studied the floristic composition, phenology and soil C and N forms of 7 homogeneous permanent plots (all belonging to *Salicetum herbaceae* community), during 4 years of monitoring. In particular, our work aims at evaluating: i) the interannual variability (2016-2019) of climatic and pedoclimatic conditions and soil C and N forms and plant phenology dynamics; ii) the effect of climatic and pedoclimatic variables as well as vegetation variables on soil C and N dynamics.

1. Materials and methods

1.1 Study area

The study was carried out at the LTER Site Angelo Mosso Scientific Institute (https://deims.org/17210eba-d832-4759-89fa-9ff127cbdf6e), located in the Western Italian Alps at the foot of the Monte Rosa Massif (4634 m a.s.l.), on the watershed between Piemonte and Valle d’Aosta regions (Fig. 1). The research was performed at seven permanent study sites (named site 1, 2, 6, 7, 8, 9, 10, according to the LTER site numerations), located between 2686 (site 10) and 2854 m a.s.l. (site 6). Since 2007, meteorological parameters of the study area were continuously recorded by an Automatic Weather Station (AWS) located at 2901 m and belonging to the Italian Army (Comando Truppe Alpine – Servizio Meteomont). From 2007 to 2019, the area was characterized by a mean annual air temperature of –2.2 °C and a cumulative annual snowfall of

805 cm, while the mean annual liquid precipitation was of ca. 370 mm (Table 1). Generally the snowpack developed by late October–early November, while snowmelt started once the snowpack became isothermal, typically in late May to early June. The mean snow cover duration was 263 days, ranging between 236 (year 2011) and 312 (2014). Mean soil temperature during the snow-covered season was 0° C, while it was 7° C during the snow free season. Generally, the mean duration of soil freezing lasted for 52 days, ranging between 0 and 162, with a mean temperature of –1 C°. From 2016 to 2019, the mean snow density before snowmelt onset was equal to 341 kg m^{-3}, while the mean snow water equivalent was around 1113 mm; the N-NH_4^+ and N-NO_3^- stocks in the snowpack ranged between 0.34–0.97 and 0.53–0.88 kg ha^{-1}, with median values 0.82 and 0.55 kg ha^{-1}, respectively while dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) stock ranged between 7.4-10.9 and 0.85-2.34 kg ha^{-1}, with median values 8.70 and 0.91 kg ha^{-1} respectively. The mean wind speed of the area was about 3.4 m s^{-1}.

Each study site consisted of paired plots for soil and vegetation survey. The bedrock was primarily micaschists, with some inclusions of amphibolites and calcschists. Soils belonged mainly to Regosol (sites 1, 8), Leptosol (site 2), Cambisol (sites 6, 7), and Umbrisol (sites 9, 10) great groups (IUSS Working Group WRB, 2015) (Table 1). Soil total organic carbon (TOC) and nitrogen (TN) ranged from 0.1 to 18.4 % and from 0.02 to 1.23 %, respectively; soil pH ranged from 4.2 to 6.1. Detailed soil classification, physical and chemical parameters are summarized in Table 1.

The vegetation of the sites was included in the ‘Siliceous alpine and boreal grasslands’ (habitat 6150, according to the EU Habitat Directive). Small differences in plant species composition was observed among the sites, according to contrasting extremes of exposure and snow cover duration, nevertheless all of the sites fall in the association *Salicetum herbaceae*. The vast majority of the species could be described, based on vegetative optimum, either as a snowbed specialist, a debris or a grassland species, according to Aeschimann et al. (2004).

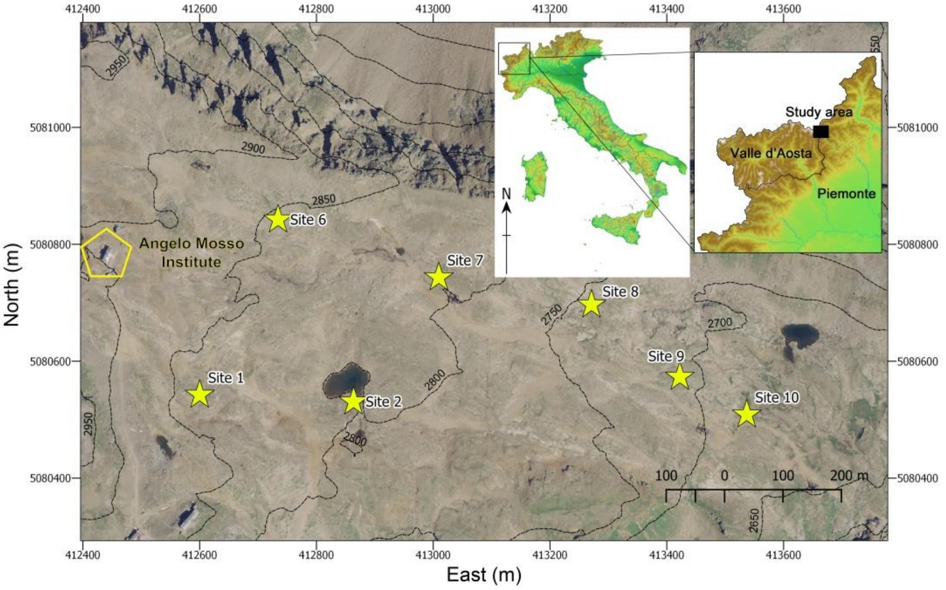

Figure 1. Location of the study research area in Italy and in the NW Italian Alps (www.pcn.minambiente.it), aerial overview of the study area (orthoimage year 2006) (coordinate system WGS84/UTM 32N) and localization of the 7 permanents study sites at the LTER Site Angelo Mosso Scientific Institute.

Table 1. Classification of physical and chemical parameters of soils in the 7 sites. TOC=Total organic carbon, TN=Total nitrogen, C/N=Carbon nitrogen ratio

Site	Soil (WRB)	Elevation (m a.s.l.)	Slope and aspect	Horizons	Depth (cm)	pH	CEC (meq 100 g^{-1})	TOC (%)	TN (%)	C/N	Polsen (mg kg^{-1})	Clay (%)	Silt (%)	Sand (%)
1	Leptic Regosol Dystric	2840	0° -	A AC C	0-7 7-20 20-40	4.6 5.4 7.2	7.8 4.8 8.8	1.84 0.65 1.49	0.14 0.05 0.08	13 14 18	N/A N/A N/A	1 1 1	13 17 17	86 82 82
2	Lithic Leptosol Dystric	2800	0° -	A C	0-3 3-10	4.4 4.7	13.1 14.3	3.79 2.62	0.30 0.18	13 14	N/A N/A	1 2	22 31	77 67
6	Dystric Skeletic Cambisol (Humic)	2854	15° SE	A AB BW	0-3 3-12 12-50+	4.5 4.7 4.9	39.0 11.4 13.1	6.2 1.6 1.1	0.44 0.16 0.11	14 10 10	67.22 13.10 13.63	4 1 2	8 13 13	88 86 85
7	Dystric Skeletic Cambisol	2813	5° S	A AB BW	0-5 5-10 10-33+	4.2 4.8 5.4	18.5 6.2 8.8	5.4 0.8 0.7	0.36 0.07 0.06	15 12 11	86.15 10.16 9.32	2 1 0	18 14 32	80 85 68
8	Eutric Endoskeletic Regosol (Arenic, Turbic)	2749	0° -	A1 A2 A3 AC1 AC2 CA	0-3 3-8 8-13 13-25 25-33 33+	5.3 5.4 5.7 6.1 6.1 6.1	14.9 7.1 5.1 6.5 6.9 11.2	4.2 0.9 0.6 0.8 0.8 0.8	0.34 0.08 0.06 0.06 0.06 0.07	12 11 11 13 12 11	28.98 7.53 5.85 9.42 8.16 11.53	1 1 1 2 2 0	15 16 15 11 22 22	84 83 84 87 76 78

9	Skeletic Umbrisol (Endoeutric, Arenic, Turbic)	2720	5° SE	A1 A2 2BC1 3BC2	0-3/5 3/5-36 36-40 40-50+	4.6 4.7 5.2 5.3	38.9 6.7 4.7 5.4	14.5 0.9 0.1 0.5	0.92 0.08 0.02 0.09	16 11 5 6	63.00 10.10 2.10 5.64	2 2 1 1	31 8 10 12	67 90 89 87
10	Skeletic Umbrisol (Arenic)	2686	2° S	A1/OH A2 BC	3-5 5-28/35 28/35-60+	4.6 4.8 5.1	89.0 10.4 7.9	18.4 1.4 1.0	1.23 0.12 0.07	15 12 15	85.3 10.2 12.7	2 6 1	16 14 18	82 80 81

1.2 Soil sampling and analysis

For the measurement of hourly soil temperature, each study site was equipped with thermistors combined with data loggers (HOBO Pro v2 U23-00x, accuracy: ± 0.1 °C) placed within soil at a 10 cm depth, from fall 2016 until fall 2019. At each site, three 9 m^2 plots were established, where three topsoil samples (A horizon, 0–10 cm depth) were collected each month during the snow-free season, from 2016 until 2019. More specifically, three soil sampling were performed each year in three different periods: within July (sampling period 1), August (sampling period 2) and September (sampling period 3). Each sample consisted of three subsamples that were homogenized by sieving at 2 mm within 24 h of collection and oven-dried at 105 °C in order to obtain the gravimetric water content (GWC). An aliquot of 20 g of fresh soil was extracted with 100 mL K_2SO_4 0.5 M, while 10 g were fumigated using chloroform for 18 h before extraction with 50 mL K_2SO_4 0.5 M. The concentration of dissolved organic carbon (DOC) in not fumigated soil extracts (extractable DOC) was determined with a TOC analyzer (Elementar, Vario TOC, Hanau, Germany) after filtration with 0.45 μm membrane filters. The microbial carbon (Cmicr) was estimated as the difference in extractable DOC between fumigated and non-fumigated samples, corrected using a recovery factor of 0.45 (Brookes et al. 1985). Ammonium (extractable $N\text{-}NH_4^+$) concentrations in soil extracts were measured spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) using a modified Berthelot method based on the reaction with salicylate in the presence of alkaline sodium dichloroisocyanurate (Crooke and Simpson 1971). Nitrate (extractable $N\text{-}NO_3^-$) concentrations in soil extracts were measured spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) using the Greiss reaction (Mulvaney 1996) modified according to Cucu et al. (2014). Total dissolved nitrogen (extractable TDN) in the extracts was measured as reported for DOC. Dissolved organic nitrogen (extractable DON) was determined as the difference between extractable TDN and inorganic nitrogen (extractable $N\text{-}NH_4^+ + N\text{-}NO_3^-$) in the extracts. Microbial nitrogen (Nmicr) was estimated from the difference in extractable TDN between fumigated and non-fumigated samples corrected using a recovery factor of 0.54 (Brookes et al. 1985). For the analysis of total nitrogen and total carbon, soil aliquots were dried at 40° C, milled and then analysed using elemental analysis (Carlo-Erba, Milano, Italy).

1.3 Site-specific climatic-pedoclimatic variables

Each plot was equipped with a datalogger with thermal probes at the soil surface (HOBO Pro v2 U23-00x), measuring hourly temperatures since the beginning of the monitoring, to calculate climatic-pedoclimatic variables and to identify the end of the snow-covered period and the beginning of the growing season. Several site-specific variables were calculated (Table 2): (i) proportion of cumulative raining days from the melt-out day to the sampling day (PRD) (extracted from the AWS data); (ii) snow cover duration (SCD), based on the hydrological year, i.e. from 1 October to 30 September using the daily soil temperature data: when the daily amplitude of soil temperatures remained below 1 °C, the day was considered as a “snow-covered day” (Danby and Hik 2007); (iii) melt-out day of snow (MOD); (iv) duration of soil freezing (DSF); (v) number of soil freeze/thaw cycles (FTC), estimated when the daily mean soil temperature reached values below and above 0 °C (Phillips and Newlands 2011); (vi) mean temperature of soil during the freezing period (MTF); (vii) mean temperature of soil during the snow-free season (MTSF); (viii) intensity of soil freezing (ISF); (ix) gravimetric water content of soil (GWC) estimated at every sampling time (Black, 1965).

Table 2. Selected climatic, pedoclimatic and vegetation variables used in GLMM. * Melt-out day of snow (MOD) and snow cover duration (SCD) are considered pedoclimatic indices since they were calculated from soil temperature data. ** Intensity of soil freezing (ISF) was excluded from GLMM (r >70).

Variables	Term	Definition	Unit
Climatic and Pedoclimatic variables			
Proportion of raining days	PRD	Proportion of cumulative raining days between melt-out day of snow and sampling day	%
Snow cover duration*	SCD	Sum of “snow-covered days” in each hydrological year	days
Melt-out day of snow* or Snowmelt day	MOD	Date of complete snowmelt (indicated as day of the year - DOY)	DOY
Duration of soil freezing	DSF	Cumulative number of days, from October 1 to the melt-out day, when mean daily soil temperature <0 °C	days
Soil freeze/thaw cycles	FTCs	Number of soil freeze/thaw cycles in each hydrological year	number
Mean soil temperature during soil freezing	MTF	Mean daily soil temperature when the soil is frozen, from October 1 to the melt-out day	°C
Mean soil temperature during the snow-free season	MTSF	Mean daily soil temperature between MOD and sampling.	°C
Intensity of soil freezing**	ISF	Minimum soil temperature when soil is frozen	°C
Gravimetric water content	GWC	Soil gravimetric water content measured at each sampling time	%
Vegetation variables			
Salix herbacea Phenophase	SP	Median observed phenophase for each survey date	number
Moisture index	Fm	Soil moisture based on vegetation preferences weighted with Species Relative Abundance	number
Nutrients index	Nm	Soil nutrients based on vegetation preferences weighted with Species Relative Abundance	number
Reaction index	Rm	Soil reaction based on vegetation preferences weighted with Species Relative Abundance	number

1.4 Vegetation and phenology surveys

From summer 2016 to 2019, surveys were carried out to describe the plot vegetation composition and identify the most abundant species. The main plots for vegetation and phenology surveys (close to the soil plots) were 4 m x 4 m squares marked by metal pegs, each set inside a homogenous snowbed pertaining the *Salicetum herbaceae* association. Along the two diagonals of each plot, the vertical point-quadrat method (Daget and Poissonet, 1969) was used to detect botanical composition. At 5-cm intervals plant species touching a steel needle were identified and recorded, for a total of 200 measurements per plot. Since occasional species are often missed, a complete list of plant species found in the plot was recorded. The frequency of occurrence of each species, which is an estimate of species canopy cover, was calculated and converted to species percentage cover (%SC) (Pittarello et al., 2016), i.e. by dividing the frequency of occurrence by 2. To all occasional plant species a %SC = 0.3 was attributed (Tasser and Tappeiner, 2005). The demand of each plant species for moisture level (F), soil pH level (R), and soil nitrogen content (N) was estimated according to the Landolt EIVs (Landolt et al., 2010). Such values range from 1 (low demand) to 5 (high demand). Then, each plant species was associated with the corresponding F, R, and N Landolt value and the mean indexes (Fm, Rm, Nm) were calculated for each plot, separately for each year and site, by averaging species values weighted on their %SC.

Plant phenology was recorded for *Salix herbacea* which not only was the most abundant species across all the plots (%SC ranged from 37% to 73%, being lower than 50% in one single plot), but had an extensive radical mass (Wijk, 1986) when compared with the other species and was since believed to have a greater interaction with soil chemicals. The main plot was divided into 16 adjacent subplots 1 m x 1 m, used as pseudo-replicates, where the phenophase of the most advanced individual was recorded for a minimum of 7 up to 9 times per year throughout the whole growing season (Quaglia et al. 2020). The phenophase was assigned with a visual observation of the individual using an adaptation of the BBCH scale (Hack et al. 1992) fitted for the snowbed plant communities as a reference (Annex 1, Chapter 2). This scale is characterized by a series of progressive integer numbers ranging from 0 to 59, where the tens represent the major phenophases in chronological order (Shoots emergence, Leaves development, Flower buds, Flowering, Fruiting, Seed dispersal) and the units represent the advancement of such phenophases. The variable *Salix herbacea* Phenophase (SP) was calculated for every soil sampling date, as the linear interpolation of the median of the 16 subplots of each phenological survey date.

1.5 Statistical methods

Statistical analyses were performed using R software, v. 3.6.0 (R Core Team, 2019). To assess the relative importance of climatic-pedoclimatic and vegetation variables (i.e. explanatory variables, Table 2), nine Generalized

Linear Mixed Models (GLMMs) were fitted, one for each response variable: N-NH_4^+, N-NO_3^-, DOC, TDN, Cmicr, Nmicr, DON, CNmicr. Before modelling data with GLMMs, a correlation analysis (Pearson coefficient) was performed on explanatory variables to exclude highly collinear variables ($r > |0.70|$) (ISF excluded). Explanatory variables were standardized (*Z*-scores) to allow the assessment of their size effect by scrutinizing model parameters (β coefficients). To account for the repeated measure structure, the soil sampling period, year, and site were considered as crossed random factors. Since all response variables had continuous and positive values ($Y > 0$), models were built both with Gaussian (identity- and log-link functions) and Gamma (log-link function) distributions, then the best fitting one was chosen based on the lowest Akaike Information Criterion (Zuur et al., 2009). For each GLMM, explanatory variables with a non-significant effect on the overall model fit were identified with the '*drop1'* function from 'stats' R package and removed from the model afterwards. Through this procedure, each factor is iteratively removed at a time whilst holding the remaining factors constant and, using an analysis-of-deviance test, a report of the extent to which each factor affects the overall model fit is provided. Model assumptions were checked using 'DHARMa' R package, v. 0.2.7. (Hartig, 2020). Modeling was carried out using the '*glmmTMB'* function from the 'glmmTMB' R package, v. 1.0.1. (Brooks et al., 2017).

2. Results

2.1 Weather Conditions during the Experimental Period

The mean annual air temperature recorded by the AWS in the time-span 2016–2019 (hydrologic years, i.e. 1 October 2015 – 30 September 2019) was -1.5 °C, with mean daily values ranging from a minimum of -21.1 °C (16 January 2017) to a maximum of +15.8 °C (12 June 2018) (Fig. 2). The cumulative liquid precipitation during the snow-free season ranged between 202 mm in 2017 and 380 mm in 2016, with a maximum daily liquid precipitation of 50.0 mm (16 September 2016). The maximum snow depth, measured at the Meteomont station, was equal to 386 cm (13 April 2018). Cumulative snowfall varied between 608 cm in 2017 and 824 cm in 2018. The duration of soil freezing ranged between 0 days in 2017 and 153 in 2018 with a mean soil temperature, during the freezing period, between -0.1 °C in 2019 and -2.0 °C in 2018. The intensity of soil freezing reached a maximum value of -8.78 °C (1 December 2017) (mild/hard freezing, Tierney et al. 2001). The mean soil temperature during the snow-covered season was about 0° C within a range of around 1°C, while during snow-free season it was between around 7 °C in 2018 and 9 °C both in 2016 and 2019.

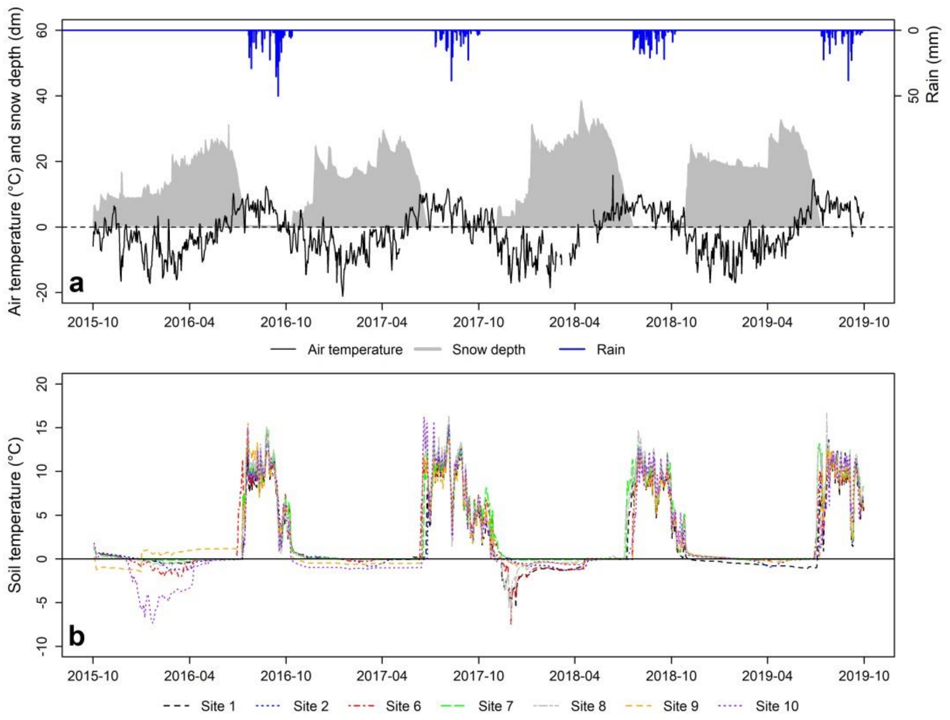

Figure 2. a Air temperature (mean daily values), snow depth and rainfall recorded at the Automatic Weather Station (AWS) from 1 October 2015 to 30 September 2019 **b** soil temperature (10 cm depth, mean daily values) at the 7 study sites.

2.2 Interannual variability of site-specific climatic, pedoclimatic conditions and soil C and N forms

During the snow-covered season, the soil temperatures recorded in the study sites were usually close to 0 °C (Figure 2), with an exception in early winters 2015-2016 and 2017-2018. The topsoil daily temperature during the snow-covered season reached a minimum of -7.5 °C in 2017 at site 6, while during the snow-free season it reached the maximum of 16.7 °C at site 8 in 2019 (Figure 2). Considering the PRD variable, in 2016 rainy days were mostly concentrated in September, close to the third sampling, accounting for the 40% of the total liquid precipitation of the snow-free period, while in 2017 rainy days were equally distributed between August and September, during which overall 70% of total rainfall occurred; otherwise in 2018 raining days were slightly concentrated in July, while in 2019 liquid precipitation were quite well distributed across the whole snow-free season. SCD was between 230 (site 8, 2018) and 283 days (site 1, 2016) (Table 3). The SCD was weakly positively correlated with the MOD ($r=0.38$, $p < 0.001$). Earliest MOD occurred at site 6 on DOY 163 (2017) while the latest MOD occurred at site 9 and 10 on DOY 201 (2018) (Table 3). DSF ranged between 0 (site 8, 2019) and 247 days (site 6, 9 - 2018; site 10 - 2017). The maximum number of FTCs (2) was recorded at sites 1 and 10 in 2018 and at site 7 in 2019. Lowest MTF (-2.4 °C) occurred at site 10 in 2016. Lowest and highest MTSF were 2.1 °C and 11.4 respectively at site 10 (2019). ISF was always classified as mild freezing, except for 2 events of mild/hard freezing recorded at site 1,6 and 8 in 2018 (-6.6, -7.5 and -6.8 °C respectively) and at site 10 in 2016 (-7.3 °C) (cf. Tierney et al. 2001). The GWC was between 27 and 28% in 2016 and 2017 respectively, while in 2018 and 2019 it was lower, between 20 and 23% respectively. The lowest GWC (14%) was recorded at site 8 (September 2019) while the highest (44%) at site 9 (September 2017). Overall, during the four years of monitoring, site 7 resulted the driest, with a mean GWC of around 19%, while site 2 and 6 were the wettest with values around 30%.

Table 3. Descriptive statistics for all the variables used in the GLMMs. The units of climatic-pedoclimatic and vegetation variables are reported in table 2. Soil C and N forms are expressed in mg kg^{-1}, except CNmicr. PRD=Proportion of raining days, SCD=Snow cover duration, MOD=Melt-out day, DSF=Duration of soil freezing, FTCs=Soil freeze/thaw cycles, MTF=Mean soil temperature during soil freezing, MTSF=Mean soil temperature during the snow-free season, GWC=Gravimetric water content, DOC=Dissolved organic carbon, TDN=Total dissolved nitrogen, DON=Dissolved organic nitrogen, C/N=Carbon nitrogen ratio, C, N, C:N micr=Microbic C, N and C:N, SP=Salix herbacea Phenophase, Fm=Moisture index, Nm=Nutrients index, Rm=Reaction index

		n	**Mean**		**S.E.**	**Median**	**Min**	**Max**	**Range**
Climatic-pedoclimatic variables									
	PRD	77	0.3	±	0.02	0.3	0.0	0.7	0.7
	SCD	77	261.2	±	1.67	263.0	230.0	283.0	53.0
	MOD	77	184.4	±	1.24	188.0	163.0	201.0	38.0
	DSF	77	167.4	±	7.26	166.0	24.0	247.0	223.0
	FTCs	77	1.1	±	0.04	1.0	1.0	2.0	1.0
	MTF	77	-0.5	±	0.06	-0.3	-2.4	0.0	2.4
	MTSF	77	8.6	±	0.17	8.8	2.1	11.4	9.3
	GWC	77	24.9	±	0.73	23.1	14.3	43.8	29.5
Soil C and N forms									
	N-NH_4^+	77	3.9	±	0.27	3.4	0.6	11.8	11.2
	N-NO_3^-	77	0.5	±	0.04	0.4	0.1	2.0	1.9
	DOC	77	85.1	±	3.88	76.3	29.4	165.1	135.7
	TDN	77	20.2	±	1.24	18.1	4.4	54.0	49.5
	DON	77	15.9	±	1.25	13.3	1.9	50.5	48.7
	Cmicr	77	484.1	±	26.60	445.8	143.0	1166.2	1023.2
	Nmicr	77	36.7	±	2.37	31.4	7.4	86.8	79.3
	C:Nmicr	77	14.5	±	0.57	14.1	5.0	29.4	24.4
Vegetational variables									
	SP	77	46.1	±	1.59	51.0	9.0	59.0	50.0
	Fm	77	3.5	±	0.01	3.4	3.3	3.6	0.3
	Nm	77	2.3	±	0.02	2.4	2.0	2.5	0.5
	Rm	77	2.2	±	0.01	2.2	2.1	2.3	0.2

On the interannual basis (Fig. 3), N-NH_4^+ reached minimum (0.6 mg kg^{-1}) and maximum values (11.8 mg kg^{-1}) in 2017 (site 8) and 2019 respectively (site 9) (Table 3, Fig. 3); the N-NO_3^- content reached minimum (0.1 mg kg^{-1}) values in 2016 (sites 1 and 7) and 2017 (sites 7 and 8), while the maximum value (2.0 mg kg^{-1}) was recorded in 2019 (site 10). DOC content ranged between 29.4 and 165.1 mg kg^{-1}, reached both in 2016 (site 8 and 2 respectively). TDN reached minimum (4.4 mg kg^{-1}) and maximum values (54.0 mg kg^{-1}) in 2017 (site 8) and 2016 (site 6) respectively. DON reached minimum (1.9 mg kg^{-1}) and maximum values (50.5 mg kg^{-1}) in 2019 (site 7) and 2016 (site 6). Cmicr ranged between 143.0 and 1166.2 mg kg^{-1}, reached both in 2017 (sites 8 and 9 respectively), while Nmicr reached minimum (7.4 mg kg^{-1}) and maximum values (86.8 mg kg^{-1}) in 2019 (site 7) and 2016 (site 2) respectively. C:Nmicr reached minimum value (5.0) at site 1 in 2018 and maximum value (29.4) at site 7 in 2019.

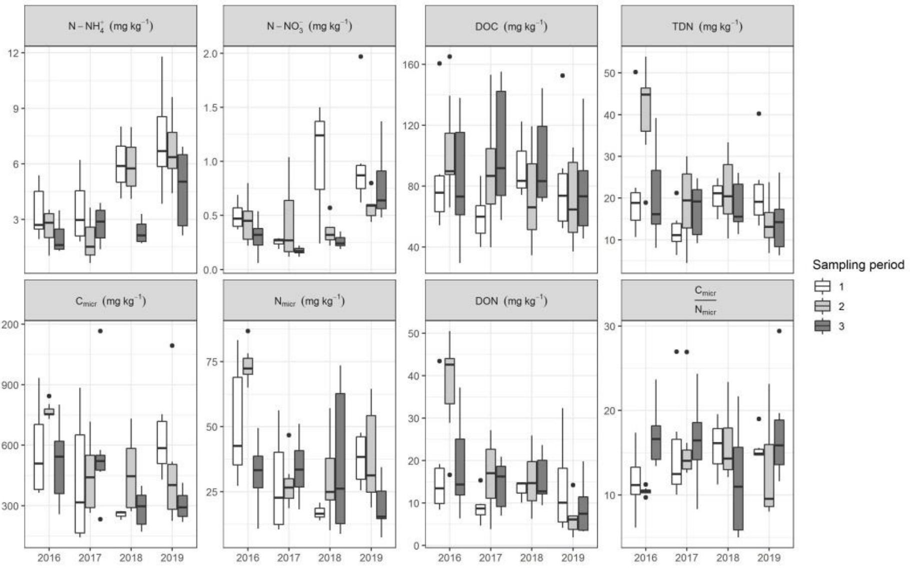

Figure 3. Interannual variability of soil C and N forms in the 7 snowbeds communities. Sampling periods referring to a different moment of sampling, which occurred every year within July (sampling period 1), August (sampling period 2) and September (sampling period 3).

2.3 Phenology dynamics

The growing cycle of *Salix herbacea* showed some variations throughout the monitoring years, with a perceivable shift in the timing of the events. In general, the first phenophases (from emergence to flowering) proceeded in a fast sequence right after the snowmelt, while the late phenophases (fruiting to seed dispersal) occurred at a slower rate (Fig. 4). We registered the earliest appearance of flower buds on DOY 187 and plot 1 during the year 2017, which was generally characterized by a thin spring snowpack and an advanced snowmelt. The latest beginning of flowering was on DOY 216 and plot 6 during the year 2018, with 29 days of delay on the offset of the same phenophase. Median onset of flowering was on DOY 189 in 2017 and 207 in 2018, while it occurred respectively on DOY 204 and 201 in the years 2016 and 2019, respectively. The earliest beginning of flowering occurred on DOY 188 and plot

9 during 2017, while the last one was on DOY 220 and plot 6 during the year 2018, with 32 days of delay on the offset of the same phenophase. Mean onset of flowering was on DOY 191 in 2017 and 212 in 2018, while it occurred respectively on DOY 208 and 206 in the years 2016 and 2019. Beginning of seed dispersal started the earliest on plot 10 during year 2017 (DOY 213) and occurred the last on site 6 during year 2018 (DOY 257) with a maximum 44 days gap. Mean seed dispersal started on DOY 217 in 2017 and 247 in year 2018, while occurred on DOY 241 in 2016 and 234 in 2019.

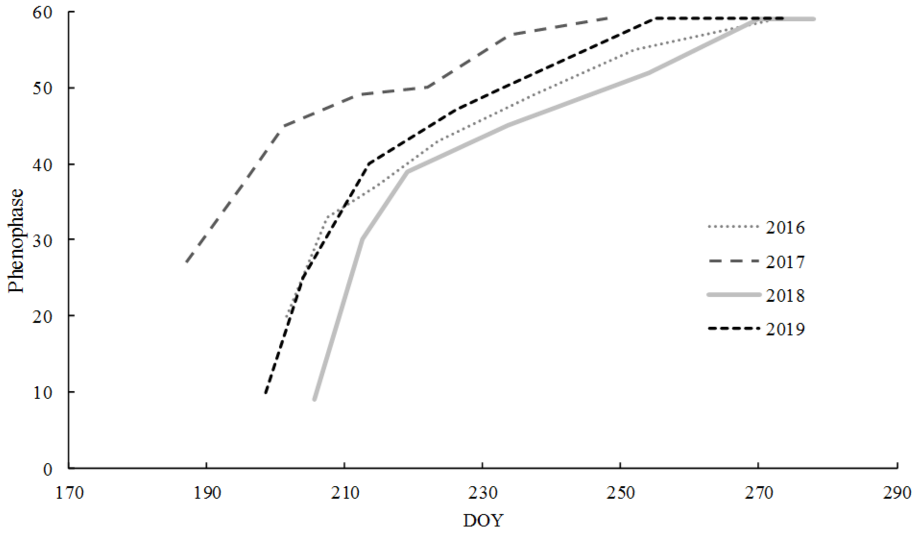

Figure 4. Phenology of the species *Salix herbacea* throughout the four-year monitoring period. Phenophases are indicated on the y axis as a progressive number following the adaptation of the BBCH scale (Hack et al., 1992), while on the x axis Day Of Year is reported as a variable. For every year, the lines represent the median phenophase of the 7 sites in each survey date.

2.4 Influence of climatic-pedoclimatic and vegetation variables on soil C and N forms

GLMMs revealed that several climatic and pedoclimatic variables significantly influenced soil extractable C and N forms in snowbed areas (Table 4), although with a different weight. In particular, considering the ranking of relative importance provided by β coefficients for each soil C and N forms (Table 4), the GWC resulted overall the most important variable, affecting primarily, with a positive relation, extractable DOC, TDN, Cmicr, C:Nmicr. Moreover, it affected, as a second variable, $N\text{-}NH_4^+$ and Nmicr and, as third variable, $N\text{-}NO_3^-$. Also the MOD played a fundamental role, resulting in one of the most important variables, influencing primarily, with a positive relation, extractable $N\text{-}NO_3^-$ and Nmicr. Furthermore, it affected positively, as a second variable, extractable DOC, TDN, Cmicr and as third variable N-NH_4^+. The DSF influenced positively, as a first and third variable respectively, extractable DON and TDN. The climatic variable PRD influenced, with a negative relation, N-NO_3^- (as second variable) and Nmicr (as fourth). Overall, although with a less important ranking, the FTCs affected as second variable, with a positive relation, only C:Nmicr, while the MTSF affected, as third variable with positive correlation, both Cmicr and Nmicr. The SCD played a marginal role, affecting as a third variable only extractable DOC, with a negative relation.

Concerning the vegetation variables, SP and Nm were the most important variables, while the effect of Rm was less pronounced. In particular, SP influenced primarily N-NH_4^+ with a negative relation, affecting also DOC (positive relation) as a fourth variable. Nm influenced negatively as second and fifth variable DON and Nmicr respectively, while Rm affected as a second variable with a negative relation only CNmicr.

Since EIVs are attributed in a scale from 1 to 5 with a 0.5 interval precision, the required variation range for each index significance is 0.5 (Table 1). While Nm's variation is slightly higher, both Rm and Fm have lower variation rates (Rm min = 2.06, Rm max = 2.30, Fm min = 3.29, Fm max = 3.59) and despite Rm's barely significant relation with C:Nmicr, neither one provides other significant interaction with any other measured pedological parameter (Table 4).

Table 4. Ranking of the effect of climatic-pedoclimatic and vegetation variables on soil C and N forms derived from GLMMs. The numbers in bold represent the rank on an arbitrary scale of decreasing importance of the variables for each C and N forms: PRD (proportion of raining days), Snow Cover Duration (SCD), Melt Out Day (MOD), Duration of Soil Freezing (DSF), Freeze/Thaw Cycles (FTCs), Mean soil temperature during soil freezing (MTF), Mean soil temperature during the snow-free season (MTSF), Intensity of Soil Freezing (ISF), and Gravimetric Water Content (GWC), *Salix herbacea* Phenology (SP), Moisture index (Fm), Nutrients index (Nm), Reaction index (Rm) on C and N forms (i.e., 1 is the most important variable, 5 the least). The following number is the ® value derived from the GLMMs and expresses positive (+) or negative (-) relations (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, ^{ns} not significant). The Akaike Information Criterion (AIC) value for each model is reported in the bottom line.

	N-NH$_4^+$	N-NO$_3^-$	DOC	TDN	DON	Cmicr	Nmicr	C:Nmicr
Climatic-pedoclimatic variables								
PRD		**2** -0.30 ***	-0.08^{ns}				**4** -6.38 **	
SCD			**3** -0.12 ***		+0.2^{ns}			
MOD	**3** +0.72 **	**1** +0.49 ***	**2** +0.20 ***	**2** +0.24 ***		**2** +0.14 **	**1** +12.76 ***	
DSF				**3** +0.11 *	**1** +0.23 *			
MTF								
FTCs								**3** +0.11 **
MTSF	**4** +0.29$^{n.s.}$					**3** +0.10 *	**3** +6.81 ***	
GWC	**2** +0.90 ***	**3** +0.22 ***	**1** +0.25 ***	**1** +0.28 ***	+0.13^{ns}	**1** +0.36 ***	**2** +9.60 ***	**1** +0.16 ***
Vegetation variables								
SP	**1** -2.16 ***		**4** +0.09 **					
Fm								
Nm					**2** -0.15 *		**5** -5.13 *	+0.11^{ns}
Rm					+0.2^{ns}		+2.91^{ns}	**2** -0.13 *
Model AIC	255.78	-22.35	712.16	535.30	529.28	1006.55	653.47	456.82

3. Discussion

3.1 Climatic-pedoclimatic drivers on Soil C and N Forms

In the time-span 2016-2019, considering the effect of climatic and pedoclimatic variables on soil C and N forms in our research area, both the snow-covered and snow-free seasons played a fundamental role, strongly influencing the biogeochemical cycles in these high elevation ecosystems. In particular, soil GWC during the snow-free season had a first order control, affecting several soil C and N forms: indeed the greater the GWC, the higher the content of N-NH_4^+, N-NO_3^-, DOC, TDN, Nmicr, Cmicr and C:Nmicr. Our results confirmed the importance of soil water content as one of the main variables favouring microbial activity and the C and N transformations (e.g. Haynes, 1986; Stark and Fireston, 1995; Lipson et al., 1999; Magnani et al., 2017; Pintaldi et al., 2019), both in snow-covered (Larsen et al., 2002) and growing seasons (Lipson et al. 1999). The results are in agreement with those reported by Magnani et al. (2017), who showed a positive correlation between soil water content, microbial biomass and some C and N forms (particularly N-NH_4^+ and DOC), confirming that the magnitude of liquid water is of great importance for the C and N dynamics in these high-elevation ecosystems. In addition, as shown by Lipson et al. (1999), the minimum level of soil microorganisms was found at the lowest level of soil moisture during the growing season, suggesting the importance of soil moisture as a controlling variable on soil microorganisms through a direct osmotic effect or a diffusive effect on the availability of the substrate. Furthermore, the positive relation between GWC, N-NH_4^+ and N-NO_3^-, considering that soil moisture is decoupled from rain (as supported by the lack of correlation between GWC and PRD), may be explained by the contribution of the funnelling effect of rocks (Göransson et al., 2014, 2016), which might channel the N atmospheric deposition (and N snowpack as well) creating nutritional N-hotspots. In addition, the typical concave morphology of snowbed areas may have contributed as well, as the inorganic N forms, following water flows, tend to accumulate in depression (e.g. Biasi et al. 2005, Pintaldi et al 2016).

Among the pedoclimatic variables, MOD had a role of paramount importance as well, influencing significantly, with a positive relation, several C and N forms. In particular, our results indicated that delayed MOD caused an increase in soil N-NH_4^+, N-NO_3^-, DOC, TDN, Nmicr and Cmicr, representing therefore one of the most important abiotic variables in our snowbed areas. The strong effect of MOD on soil C and N could be explained by the interaction of several variables such as the snowpack nutrient input (Filippa et al., 2010; Freppaz et al., 2018), the delayed plant development and nutrient uptake (Cooper et al., 2011) and the related competition dynamics with microbial communities (Legay et al., 2013). As demonstrated by several authors (e.g. Brook and Williams, 1999; Hiltbrunner et al., 2005; Petrone et al., 2007; Filippa et al., 2010; Freppaz et al., 2018, etc.) snowpack is considered a remarkable source of both dissolved inorganic and organic N, therefore the timing of snowmelt defines the start and duration of the growing season, and water and nutrients released from the snowpack influence soil moisture, nutrient status and microbial activity until later in the summer (Freppaz et al., 2018).

The DSF affected significantly with a positive relation DON and TDN. Our results are in agreement with those reported by Pintaldi et al. (2019), although in a subalpine forest catchment, where DSF played a role of paramount importance, influencing with a positive correlation both organic and inorganic N forms. As shown by several authors (Kalbitz et al., 2000; Grogan et al., 2004; Viglietti et al., 2014; Groffman et al., 2011) the DSF can cause an increase in soil organic labile forms, which is mainly attributable to the physical disruption of the litter layer (and/or rootlets) and consequently leaching phenomenon from the organic horizons or even aggregates disruption (Kværnø and Øygarden, 2006) or microbial mortality (Groffman et al., 2001). Furthermore, Austnes and Vestgarden (2009) found that prolonged soil freezing increased soil C and N forms as well, probably partly because of starvation and exhaustion and increased lysis of micro-organisms.

Concerning the climatic variable PRD, our results showed that it influenced significantly with a negative relation both N-NO_3^- and Nmicr: the higher the PRD, the lower the content of N-NO_3^- and Nmicr detected in topsoil during the snow-free season (Table 4). The negative relation between PRD and N-NO_3^- was probably linked with leaching phenomena governed by precipitation (e.g. Egli et al., 2008), as well as with a sort of dilution effect of infiltrating water caused by increasing rain (e.g. Balestrini et al., 2019, Pintaldi et al., 2019). Regarding the relation between PRD and Nmicr, a similar trend was reported by Freppaz et al. (2019), who obtained a negative relation between precipitation and Nmicr. Although not related with the GWC of each plot, it is reasonable to suppose that greater PRD may indicate also a greater soil water content (at least in the short term). Based on this assumption, although in a forest catchment, a negative correlation between soil water content and Nmicr was observed by Pintaldi et al. (2019).

FTC was a weak variable, affecting with a positive relation only the C:Nmicr. This result could be explained by the fact that, during the 4 years of monitoring, a very limited number of FTC was recorded. In addition, snowbed areas are, by definition, characterized by long-lasting snow cover (even 8-10 months, e.g. Hiller et al., 2005) which decouples soil temperature from the extreme cold air temperatures (Körner, 2003). Our result was in contrast with the ones reported by Freppaz et al., (2019), who reported a negative correlation between FTC and microbial C:N ratio, although not significant. Unlike our results, they reported that the duration and the intensity of soil freezing during the snow-covered season were positively related to an increase in the soil and microbial C:N ratio, indicating the possible predominance of fungi, characterized by a higher C:N ratio compared to bacteria. As reported by Lipson et al., (2002), fungi are dominant in winter, resulting better adapted to cold temperatures than bacteria in alpine dry grasslands, with a change in structure and function between winter and summer.

The MTSF influenced significantly only the microbial biomass, affecting with a positive relation both Cmicr and Nmicr, suggesting that increasing soil temperature during the snow free season favoured soil microorganism. These results are partially in agreement to what was found by Magnani et al (2017) in the same area, who reported a positive correlation with Cmicr. The results are consistent with the high levels of sensitivity of soil microorganism to variations in temperatures, especially in high elevation ecosystems (e.g., Bing et al., 2016). Wang et al. (2014) and Rui et al. (2011) too reported an increase of microbial biomass after a soil warming experiment in alpine meadows. Although in a subalpine forest, Pintaldi et al. (2019) as well found that soil temperature affected significantly Cmicr and Nmicr.

Surprisingly, compared to what reported by previous works in the same study area (e.g. Magnani et al., 2017, Freppaz et al., 2019), but not in snowbed areas, which highlighted the strong influence of SCD on several C and N forms, our results indicated that the SCD influenced significantly only extractable DOC. As shown by several authors (e.g., Fisk et al., 1998; Walker, 2000; Edwards et al., 2007), in both alpine and arctic tundra, SCD affects considerably the ecosystem functions and structure, however, the intensity of its effects is still unclear, because it varies among different ecosystems. Concerning DOC, our results agree with those found by Freppaz et al., 2019, who indicated a negative correlation between SCD and DOC. Furthermore, as shown by Magnani et al. (2017), a greater SCD could reduce the average soil temperature during the growing season, with a concurrent decrease of DOC. As the soil temperature usually remained close to 0 °C, it promoted the subnival degradation processes, allowing the progressive depletion of organic substrates caused by microbial respiration (Lipson et al. 2000). Based on the conceptual model proposed by Brooks and Williams (1999), our research area could fall in a transition zone between Zone II and Zone III, where little changes in SCD could have remarkable effects on the soil N and C dynamics (Magnani et al. 2017). However, our results indicate a less pronounced effect of SCD, suggesting that other pedoclimatic variables had a first order control on C and N forms (e.g. GWC and MOD).

3.2 Effect of vegetation on soil C and N forms: plants-soil interaction

Concerning the biotic drivers, the phenology of *S. herbacea* affected significantly with a negative relation $N\text{-}NH_4^+$, while with a positive one DOC. This relationship could be reasonably related to the plant uptake and development: indeed, the plant uptake increases (with increasing phenophases until senescence), reducing $N\text{-}NH_4^+$ in soils, while the DOC content increases, as a result of the release in soils of root exudates (Hütsch et al., 2002). As reported by Hütsch et al. (2002), up to 20% of the photosynthetically fixed C are released by roots into the soil during vegetation period in form of C-rich rhizodepositions, which play several functions both in plant nutrition and soil ecology. Regarding $N\text{-}NH_4^+$, the inorganic nitrogen input from melting snowpack can hold up to 150% of the annual atmospheric nitrogen input in snowbed plant communities (e.g. Bowman 1992, Björk and Molau, 2007, etc.).

Furthermore, N-NH_4^+ is considered the dominant inorganic nitrogen form during the early stage of snowmelt (Mullen et a., 1998), and given that nitrates are more leachable, ammonium represents therefore the main inorganic form in the soils of alpine tundra biome (Makarov et al., 2010). Our results are in agreement with those reported by Makarov et al., (2010) who observed a gradual reduction of N-NH_4^+ during the growing season, attributable to plant and microorganism uptake.

When considering the Nm, its negative relation with DON and Nmicr highlights the vegetation influence on soil nitrogen dynamics. Greater values of the parameter Nm correspond in vegetation surveys to a considerable occurrence of plant individuals with high nitrogen demands, especially the grass *P. alpina*, which is one of the most frequent species in these snowbed communities and, to a lesser extent, the forb *Taraxacum alpinum* (Ten.) DC., which similarly stands as an eutrophic species but is less common. Therefore, we believe that the content of microbial and inorganic nitrogen in the soil is reduced with the increase of eutrophic species in the vegetation composition. Our findings align with many literature sources in confirming the valuable contribution of EIVs for the description of indirect properties (Perotti et al., 2018, Szymura et al., 2014; Delgado and Ederra, 2013). Even though we were not able to confirm the accuracy of more indexes, we argue that the structure of plant composition and the derived nitrogen EIV is a relevant tool to infer the nutrient content in snowbed soils and its fluctuations due to microtopography.

4. Conclusion

In this study, through a 4-year monitoring of soil C and N forms and phenology of 7 homogenous snowbed areas, located at the LTER Site Angelo Mosso Scientific Institute, we investigated the effect of climatic, pedoclimatic and vegetation variables on soil C and N forms. Our results indicated that in these high-elevation microhabitats, among the climatic and pedoclimatic variables, the soil water content, the melt-out day of snow and the duration of soil freezing are the main abiotic factors driving soil C and N dynamics. More specifically, a greater soil water content was related to an increase of N-NH_4^+, N-NO_3^-, DOC, TDN, Nmicr, Cmicr and C:Nmicr, while a delayed melt-out day increased soil N-NH_4^+, N-NO_3^-, DOC, TDN, Nmicr and Cmicr. In addition, the duration of soil freezing played an important role, causing an increase in DON and TDN. Among the vegetation variables, the phenology of *Salix herbacea* represented the main biotic driver, suggesting that the plant-soil interaction played a crucial role, influencing significantly the content of C and N forms in these high-elevation soils.

Our results suggested a strong climate/pedoclimate-dependency in snowbed areas, suggesting that the predicted changes in the amount and timing of snowfall, snow cover duration and melt-out day, combined with increased temperature, will affect their biogeochemical cycles and phenology dynamics. Thus, snowbeds can be considered an ideal natural sentinel to detect the effects of global warming on alpine vegetation and high elevation ecosystems.

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Declarations

All the authors declare that they have no conflict of interest.

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Chapter 4: Successional Herbaceous Species Affect Soil Processes in a High-Elevation Alpine Proglacial Chronosequence

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Abstract

The study investigated plant-soil interactions along a proglacial chronosequence in the Italian Alps, with a specific focus on pioneer and grassland species structure and biogeochemical processes, with the aim to evaluate the biotic patterns in ecosystem development. We recorded vascular plant frequencies and the mean diameter of one pioneer and one grassland target species in 18 permanent plots distributed along six different stages encompassing a 170-years chronosequence in the Lauson Glacier forefield (NW Italy). We evaluated the main soil properties and measured the C:N:P stoichiometry in the biomass of pioneer and grassland target species and in the underlying soil. For comparative purposes, we analyzed also bare soils sampled near the sampled plant individuals. Pioneer species number and cover significantly increased 10 and 40 years after deglaciation respectively, while alpine grassland species cover and number peaked only after 65 and 140 years, respectively. Along the chronosequence, soils around the roots of vascular plants were enriched in nutrients, especially around individuals of alpine grassland species, with total organic C contents ranging between 1.3 and 8.9 $g\cdot kg^{-1}$ compared to 0.2 and 3.3 $g\cdot kg^{-1}$ in bare soils. Nitrogen content in bare soils was nearly undetectable, while it increased in the plant-affected soils, leading to a more balanced C:N:P stoichiometry in the oldest stages. The colonization of alpine grassland species started immediately, although species number and cover increased only when the soil acquired sufficient nutrient supply and functionality. Although the ecosystem remained C and N limited, the soil could provide adequate conditions for more competitive species establishment, as confirmed by the increasing number and cover of alpine grassland species. Thus, soil nutrient dynamics were strongly influenced by plants, with a major influence triggered by late-successional grassland species.

Introduction

Throughout the last 170 years, the alpine region has been affected by an enhanced glacier retreat, with the exception of limited periods of glacier advance (Dyurgerov and Meier, 2000; Pörtner et al., 2019). This phenomenon accelerated at historically unprecedented rates during the last few decades, with alpine glaciers that have already lost nearly 50% of their total surface area since 1850 (Paul et al., 2004; Zemp et al., 2006).

Consequently, new ice-free surfaces can nowadays provide the opportunity to investigate primary successions, soil development and emerging patterns of ecosystem structure and functions (Matthews, 1992; Egli et al., 2010).

The glacier retreat modifies the hydrological response and the sediment transport within glacier-covered basins, while the new-ice-free areas are destabilized by paraglacial processes. Mineral substrate left free from ice lacks in biological materials and the net primary production is limited by extreme abiotic constraints, such as low temperatures, intense solar irradiation and strong temperature variations (Stöcklin and Bäumler, 1996; Vitousek et al., 1997; Jones and del Moral, 2009). Receding glaciers open up surfaces for the succession of biota, which protects the released detritus. In turn, the stabilized till supports storage and interception of water and elements that allow plant colonization and soil development (Matthews, 1992; Matthews,1999; Walker and del Moral, 2003). The combination of these abiotic and biotic processes and their interactions change with time since deglaciation, creating unique chronosequences (Egli et al., 2001; Cannone et al., 2008; Eichel, 2019), characterized by a short space-for-time substitution, from young, recently deglaciated debris to the oldest moraines (Pickett, 1989). Time since deglaciation is indeed assumed to be the key driver for ecosystem development, even though local processes at a small scale are not always negligible (Burga et al., 2010; Dümig et al., 2011). One of the typical vegetation dynamics in these environments leads to species replacement along successional stages, from pioneer to early, mid- and late-successional species, where the latter are typical alpine grassland species, above treeline (Eichel, 2019). In order to identify successional trajectories and biodiversity dynamics, the correct attribution of the ecological needs to the species is essential, based on phytosociological findings or species traits. Grouping species in functional pools and evaluating their patterns along a chronosequence can provide a more comprehensive interpretation of vegetation dynamics and an easier tool for comparative community ecology (Caccianiga and Andreis, 2004). Additionally, the study of species traits, such as demographic structure of target species belonging to different successional stages (i.e., pioneer or late-successional species) or Competitor, Stress-tolerator, Ruderal (CSR) strategies (Grime, 2006), can allow deep insights into the reproductive mechanisms or the community interactions such as facilitation (Caccianiga et al., 2006; Tešitel et al., 2014).

The progressive plant colonization of high-altitude environments leads to soil formation, including mineral weathering (Mavris et al., 2010; Bernasconi et al., 2011), changes in microbial communities (Nemergut et al., 2007; Jumpponen et al., 2012), carbon (C) accumulation and nutrient cycling, especially nitrogen (N) and phosphorus (P) (Egli et al., 2006; Bernasconi et al., 2011). The combination of pedogenic processes with the C:N:P stoichiometry in the soil- microorganisms-plant system can furnish key information for understanding the ecological relationships between plant communities and nutritional requirements. In particular, pioneer communities can play an essential role in the kick-off of soil biogeochemical processes, while late-successional ones exert a stronger influence on nutrient dynamics (Matthews 1992). It should be further noticed that, in high altitude primary successions, the effect of single herbaceous species may be easily identified, because of the low and discontinuous vegetation cover. Bonanomi et al. (2016), for instance, described the effect of single plants of *Silene acaulis* on soil C and N contents along an altitudinal gradient, proving its beneficial effects compared to soil without vegetation.

Soil nutrient dynamics and ecosystem development may in turn drive the C:N:P stoichiometry in photosynthetic tissues (Sardans et al., 2012; Zhang et al., 2018). If C and N generally remain the limiting factors in alpine ecosystems (Körner, 2003), in proglacial environments, the short spatial-temporal span can lead to faster changes in C:N:P patterns in plant species, although nutrient limitation and co-limitation in these environments are still poorly understood. However, a comprehensive approach which encompasses functional species pool cover and successional single species dynamics with soil biogeochemistry still remains largely unknown in high-altitude proglacial chronosequences.

Based on these considerations, we hypothesized that the succession and the related actions of plant species occurring in a short spatial-temporal span since deglaciation play a pivotal role for soil formation and development, leading to favourable ecological conditions for progressively hosting more competitive species. Thus, this study aimed at evaluating pioneer and late-successional grassland species patterns and their role in soil development and nutrient biogeochemistry along a high-altitude proglacial chronosequence in the western Italian Alps (Lauson glacier). To deeply understand the soil-plant interplayed relationship, we further investigated the role of two herbaceous single species in nutrient dynamics, i.e., the pioneer *Saxifraga oppositifolia* L. subsp. *glandulifera* Vacc. (hereafter *Saxifraga*) and the late-successional *S. acaulis* (L.) Jacq subsp. *bryoides* (Jord.) Nyman (hereafter *Silene*).

1. Materials and methods

1.1 Study Area

The study was carried out in the proglacial foreland of the Lauson glacier, a small glacier with a 0.23 km^2 surface and 700 m length (Smiraglia and Diolaiuti, 2015; Figure 1), located within Gran Paradiso National Park (SAC/SPA IT1201000) in the upper Cogne Valley (Aosta Valley Region, North-Western Italy). The considered proglacial foreland was between the glacier terminus in 2016 and the frontal moraines left around 1820 during the Little Ice Age, located at 3,030 and 2,750 m a.s.l., respectively. The linear distance between the two moraines is 1,300 m, with a surface of about 0.65 km^2. The climate of the Cogne Valley is endalpic, with mean annual precipitation of about 700 mm and mean annual temperature of +4.1°C (mean values of Valnontey and Lillaz weather stations, at 1,700 m a.s.l.). The proglacial foreland is usually covered by snow between October and mid-July, and it has mean annual temperatures between −1 and −3°C (Mercalli and Berro, 2003). The soils are Eutric Skeletic Regosols (IUSS Working Group WRB, 2015), characterized by homogeneous texture and parent rock composition (mainly paragneiss with small quantities of amphibolites, Le Bayon and Ballevre, 2006), with disturbances limited to cryoturbation, solifluction and a weak water erosion. The vegetation is mainly composed of sparse individuals of alpine pioneer and grassland species, such as *S. oppositifolia*, *Artemisia genipi* Weber ex Stechm., *S. acaulis* and *Poa alpina* L. Out of the proglacial area, the climax vegetation is alpine grassland, dominated by *Carex curvula* All. The potential treeline in the area is around 2,350 m a.s.l. (Pecher et al., 2011). Climax soils under the climax alpine prairie are Skeletic Umbrisols or Skeletic Dystric Cambisols (IUSS Working Group WRB, 2015; D'Amicoet al., 2020a). We retraced the retreat of the proglacial terminus since 1820 until 2016 by analyzing historical field surveys carried out by the Istituto Geografico Militare in 1820, 1882, and 1931 and by performing photointerpretation of Regional Technical Maps (1975), ortophotos (1999, 2005, 2012), and SPOT satellite images (2009) (SctGeoVdA, 2020). A six stage chronosequence was then identified, encompassing a temporal range of 170 years and a spatial distance of 1,000 m (Figure 1), with the lowest limit at 2,850 m a.s.l. (Table 1), ca. 500 m above potential treeline (according to Pecher et al., 2011). Half of the stages were located within 200 m from the 2016 glacial terminus in order to deeply examine the early phases of the primary succession, i.e., the ones occurred in the last 40 years.

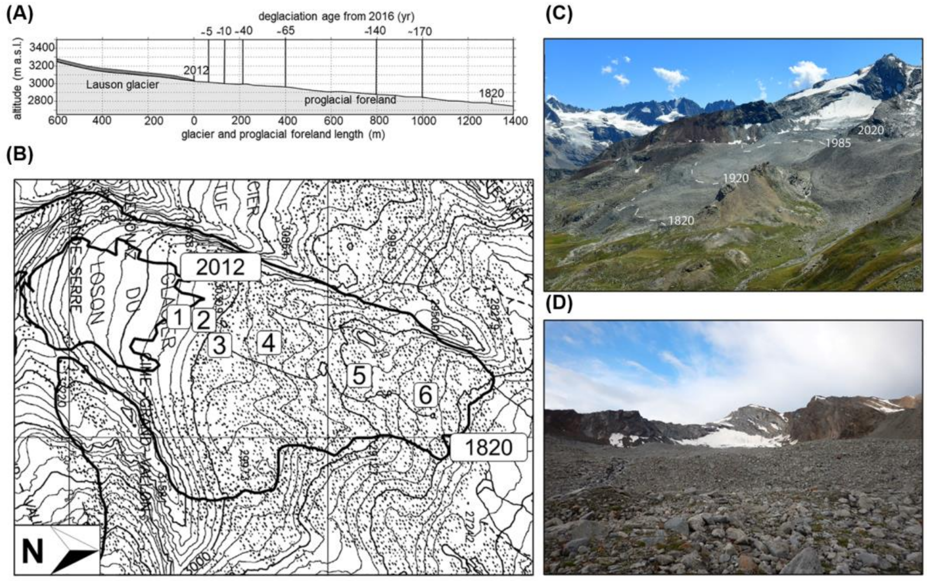

Figure 1. Representation of the Lauson glacier foreland: elevation profile (A) including

the limits of the reconstructed glacier retreat (1820–2012) and the age from deglaciation of the six stages of the chronosequence; Regional Technical Map (B) with the positions of the stages of the chronosequence and the glacier terminus in 2012 and 1820; photo of the study area (C) with drawing of four known historical glacier terminus; photo of the oldest stage (D) deglaciated for ca. 170 years.

Table 1. Description of the identified chronosequence stages.

Chronosequence stage	**Deglaciation age in 2016** (yr)	**Distance from glacier terminus in 2016** (m)	**Altitude** (m a.s.l.)
1	~5	~35	3025
2	~10	~90	3015
3	~40	~180	3000
4	~65	~350	2970
5	~140	~800	2885
6	~170	~1000	2850

1.2 Vegetation Surveys

Three permanent quadrat plots (5 m × 5 m) were placed at each chronosequence stage, avoiding areas visibly disturbed by water erosion or deposition processes. Plant species composition was then described following the vertical point-quadrat method (Daget and Poissonet, 1971) at every node of a 25 cm × 25 cm grid (accounting for a total of 441 sampling points). At each point, all vascular plants were identified at species or subspecies level, while cryptogams were pooled in an aggregated group. A complete list of all occasional species occurring within the plot but not found at the nodes was recorded as well, following the same approach as the phytosociological one (Braun-Blanquet, 1932). The species cover was calculated as its frequency of occurrence across the plot divided by the total number of sampling points. A percentage cover of 0.1% was attributed to all occasional species not found at the nodes but within the plot (Tasser and Tappeiner, 2005). Taxonomic nomenclature followed the new checklist of the Italian vascular flora (Bartolucci et al., 2018). Plant diversity of vascular plants was assessed for each plot in terms of species richness and Shannon- Wiener index (Magurran, 1988). Moreover, we associated a phytosociological optimum (at class level) to each vascular plant species according to Aeschimann et al. (2004) in order to identify different functional pools of species, which are characterized by similar ecological needs. We identified two functional species pools, corresponding to different vegetation successional stages: 1) pioneer species (belonging to *Thlaspietea rotundifolii* class) and 2) alpine grassland species (belonging to *Caricetea curvulae, Carici rupestris-kobresietea, Elyno-seslerietea, Molinio-arrhenatheretea, Nardetea strictae,* and *Salicetea herbaceae* classes). Following this approach, adopted in many different alpine contexts (e.g., Pittarello et al., 2016; Moris et al., 2017; Perotti et al., 2018), we computed species number and cover for each functional species pool in the different plots. Additionally, we studied the population structure of two target species, *Saxifraga* and *Silene*, chosen according to the criteria of abundance and ubiquity along the chronosequence. *Saxifraga* was selected as the pioneer species, with a phytosociological optimum corresponding to *Thlaspietea rotundifolii* (Aeschimann et al., 2004). Silene was chosen as an alpine grassland species, with a phytosociological optimum corresponding to *Caricetea curvulae* (Aeschimann et al., 2004). For both target species, we determined the diameter (mean value of the 2 min and max perpendicular diameters, in cm) of all individuals found in each vegetation plot, as a proxy of individual age (Benedict, 1989). We then assessed the population structure by calculating the frequencies of occurrence (number of individuals in 100 m^2) of each 1 cm diameter class.

1.3 Soil and Plant Tissue Analyses

A soil profile close to each vegetation plot was opened and described following the FAO guidelines (FAO, 2006). Soil samples were then collected from each genetic horizon, air- dried and sieved at 2 mm. Soil pH was measured potentiometrically in deionized water (soil:water 1:2.5). Total carbon (TC) and nitrogen (TN) were determined by dry combustion with an elemental analyzer (NA2100, CE Instruments, Rodano, Italia). Carbonate content was assessed by volumetric analysis. The content of total organic carbon (TOC) was determined by difference between TC and carbonate-C.

Amorphous iron oxides (Feo) were extracted with an ammonium oxalate solution, buffered at pH 3 (Schwertmann, 1964), while total pedogenic iron oxides (Fed) were determined by the dithionite-citrate-bicarbonate extraction (Mehra and Jackson, 2013). Two forms of phosphorus were assessed: total phosphorus (TP), determined following Bowman (1988), and bicarbonate extractable phosphorus (Pav), assumed as readily available phosphate for plants (Olsen, 1954). The ammonium acetate extraction method (pH 7) was adopted to determine cation exchange capacity (CEC) (Chapman, 1965); basic cations (Ca, Mg, K) were then measured by Atomic Absorption Spectrophotometry (AAS, Perkin Elmer, Analyst 400, Waltham, MA, United States).

To evaluate the influence of vegetation on soil properties, in each plot three replicates of *Saxifraga* and *Silene* individuals with the largest diameters were sampled, as well as the underlying soil at 0–10 cm depth. For comparative purposes, three soil replicates without vegetation, close to the eradicated plants, were also collected at each stage.

The eradicated individuals of *Saxifraga* and *Silene* were placed in sealed polyethylene bags, immediately stored at 4°C in a portable refrigerator, and transported to the laboratory, where green stems and leaves were separated. Aboveground green tissues (i.e., stems and leaves) were dried, grounded to 0.5 mm and analyzed to assess total C, N, and P contents following the above-mentioned procedures.

Soil samples were separated in two aliquots: one was air-dried, sieved, and analyzed to determine TOC, TN, TP, and Pav as described above. The other aliquot was processed within 24 h from sample collection, in order to measure microbial biomass C (Cmicr) and N (Nmicr). In particular, a 30 g aliquot was extracted with 100 ml K_2SO_4 0.5 M, while a 10 g aliquot was fumigated with chloroform for 18 h before extraction with 50 ml K_2SO_4 0.5 M. The DOC concentration in non-fumigated soil extracts was determined with a TOC analyser (Elementar, Vario TOC, Hanau, Germany) after filtration with 0.45 μm membrane filters). The same procedure was applied to fumigated samples; the difference in DOC between fumigated and non-fumigated samples, corrected by a recovery factor of 0.45, was Cmicr (Brookes et al., 1985).

In order to measure Nmicr, ammonium (extractable N-NH4+) concentrations in soil extracts were determined spectrophotometrically (U-2000, Hitachi, Tokyo, Japan) by the method described by Crooke and Simpson (1971). Nitrate (extractable N-NO3−) was determined following Cucu et al. (2014). Total dissolved nitrogen (extractable TDN) in the extracts was determined as reported for DOC. Dissolved organic nitrogen (extractable DON) was determined as the difference between extractable TDN and inorganic nitrogen (extractable N-NH4+ + N-NO3−). Microbial nitrogen (Nmicr) was then calculated as the difference in extractable TDN between fumigated and non-fumigated samples, corrected by a recovery factor of 0.54 (Brookes et al., 1985). All analyses were carried out in triplicate.

1.4 Statistical Analyses

The differences among the six stages of the chronosequence in terms of the considered vegetation variables (i.e., cryptogam and vascular plant cover, the two plant diversity indices, and species number and cover for the two functional species pools) were tested performing a one-way ANOVA. To investigate the variations within the population structures of *Saxifraga* and Silene along the chronosequence we performed a PERMANOVA for each species on the frequencies of diameter classes among stages. The similarity index was calculated using Euclidean distance method and 9,999 permutations were set. The soil property changes triggered by *Saxifraga* and *Silene* were analyzed with two separate one-way ANOVAs. The same analysis was carried out on chemical properties (i.e., TOC, TN, TP, C:N, and N:P) of *Saxifraga* and *Silene* tissues, among stages and between the two plant species. For univariate tests, assumptions of normality and homoscedasticity were checked with Shapiro-Wilk’s and Levene’s tests, respectively and, in case of assumption violation, logarithmic and square-root transformations were applied to variables prior to perform the ANOVAs. Whenever normal distribution or homoscedasticity did not occur, even after transformations, we performed the non-parametric Kruskal-Wallis test. Tukey’s and Dunn’s post-hoc tests were adopted for one-way ANOVA and Kruskal-Wallis, respectively, in case of significant differences.

In order to describe the interactions between soil properties and vegetation cover along the chronosequence, two matrices were arranged: 1) a soil property matrix, with TOC, TN, TP, Pav, Cmicr, and Nmicr of soil samples without vegetation, and 2) a vegetation matrix, including the covers of cryptogams, total vascular plants, pioneer species, and alpine grassland species. A Mantel test was used to calculate the correlation between the soil and vegetation matrices. A preliminary detrended cross-correlation analysis (DCCA) was performed to assess the lengths of gradients (Ter Braak and Smilauer, 1998). The DCCA revealed the presence of short (linear) gradients (<4 standard deviations) so we performed a redundancy analysis (RDA) between the two matrices (Ter Braak and Smilauer, 1998). Univariate analyses were carried out with SPSS 24 (SPSS, 2016), while PERMANOVA and Mantel test analysis with PAST 3.15 (Hammer et al., 2001) and RDA with CANOCO 4.5 (Ithaca, NY, United States); significance was set at $p < 0.05$.

2. Results

2.1 Vegetation

A total of 65 vascular plants, belonging to 19 botanical families, were recorded within the 18 plots. The most abundant species were *Saxifraga bryoides* L., *Cerastium uniflorum* Clairv., *Silene acaulis* subsp. *bryoides, Poa alpina* L., and *Saxifraga oppositifolia* subsp. *glandulifera*. No species belonging to the Fabaceae family were found in the study area. Five years since deglaciation, seven species colonized the siliceous parent material of the Lauson foreland and the pioneer *Saxifraga oppositifolia* was the most widespread in terms of both frequency and cover. Vegetation cover increased significantly along the chronosequence, for both cryptogams and vascular plants (Figures 2A,B). In particular, vascular species richness increased from values close to 0 at 5–10 years since deglaciation to 24% at the 170 years stage (Figure 2B). Species richness and Shannon-Wiener index increased along the chronosequence as well (Figures 2C,D).

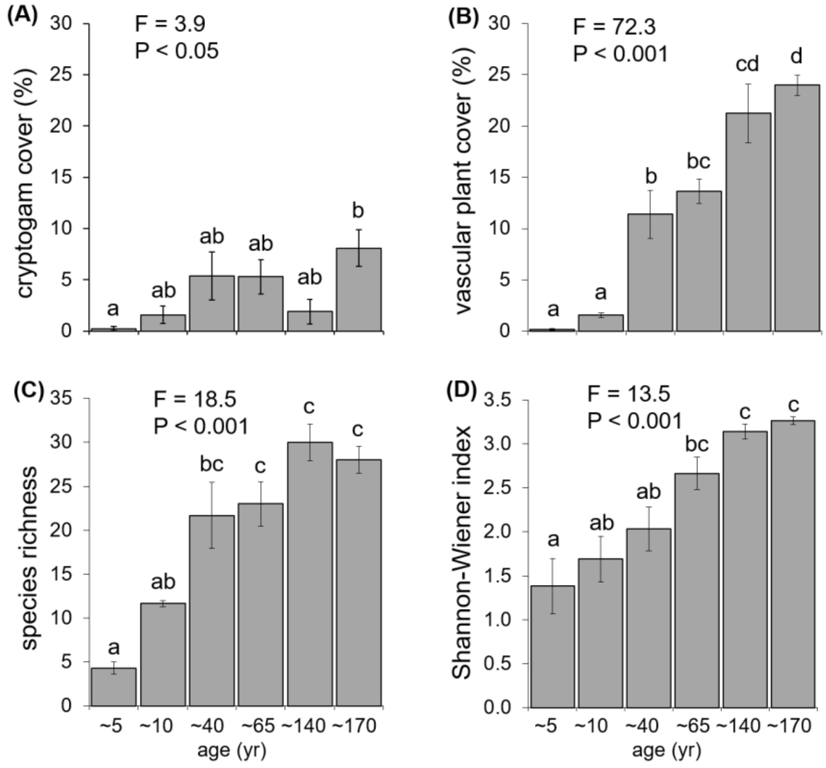

Figure 2. Plant species cover ((**A**), cryptogams; (**B**), vascular plants) and diversity indexes ((**C**), species richness; (**D**), Shannon-Wiener index) along the six stages of the chronosequence. Different letters indicate significant differences ($p < 0.05$) among stages according to Tukey's HSD test.

The number of species belonging to both functional species pools increased along the chronosequence as well (Figures 3A,B). In particular, pioneer species number significantly increased already after 10 years and, even if not statistically significant, tended to decline at older stages, while alpine grassland species number showed a slower but progressive increase along the whole chronosequence. Species cover significantly differed among stages for both pioneer and alpine grassland species, reaching the highest values since 40 and 65 years after deglaciation, respectively (Figures 3C,D).

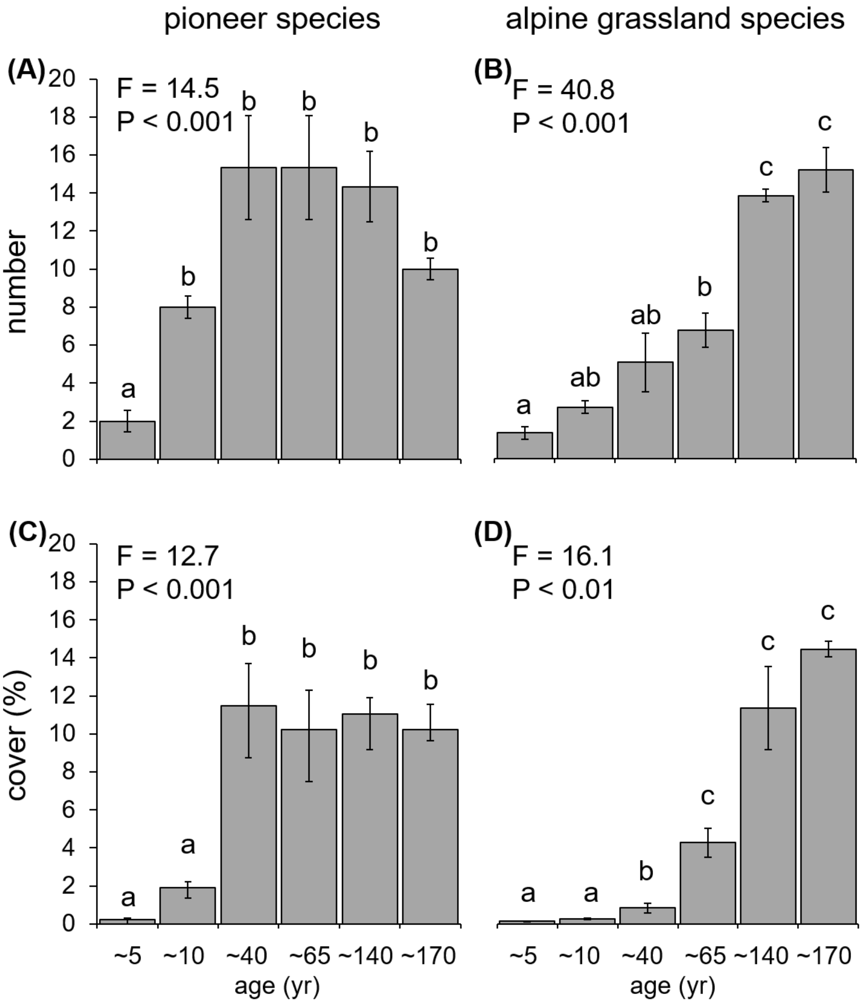

Figure 3. Species number and cover for the two functional species pools along the six stages of the chronosequence: pioneer species (**A**,**C**) and alpine grassland species (**B**,**D**). Different letters indicate significant differences ($p < 0.05$) among stages according to Tukey's HSD test.

Overall, we studied 1931 plant individuals for the population structure analysis, 775 were *Saxifraga* (observed in 14 plots out of 18) and 1,176 *Silene* (observed in 12 plots out of 18).

In the 5 years deglaciated plots, many *Saxifraga* individuals (ca. 70 in 100 m^2) were counted, with the highest frequencies in the 1 cm diameter class (Figure 4A). The highest number of individuals and cover was found in plots deglaciated for 40 years, with plants occurring in all diameter classes up to 25 cm and abundance of large individuals (mean diameter 13 cm and several individuals with diameter over 30 cm). This species almost disappeared in plots deglaciated for longer times. Conversely, no individuals of *Silene* were found within the first deglaciation stage, while it sparsely colonized young moraines only after 10 years (Figure 4B). The species became more frequent after 40 years (mainly with small individuals) and spread on the 65 years-old areas, with diameter classes up to 19 cm constantly present. In the last two stages, the frequencies of regenerating individuals (1 and 2 cm classes) doubled and large individuals (45 cm class) were detected.

According to PERMANOVA results, the distribution frequency of individual diameters differed among the chronosequence stages for both target species (Figure 4).

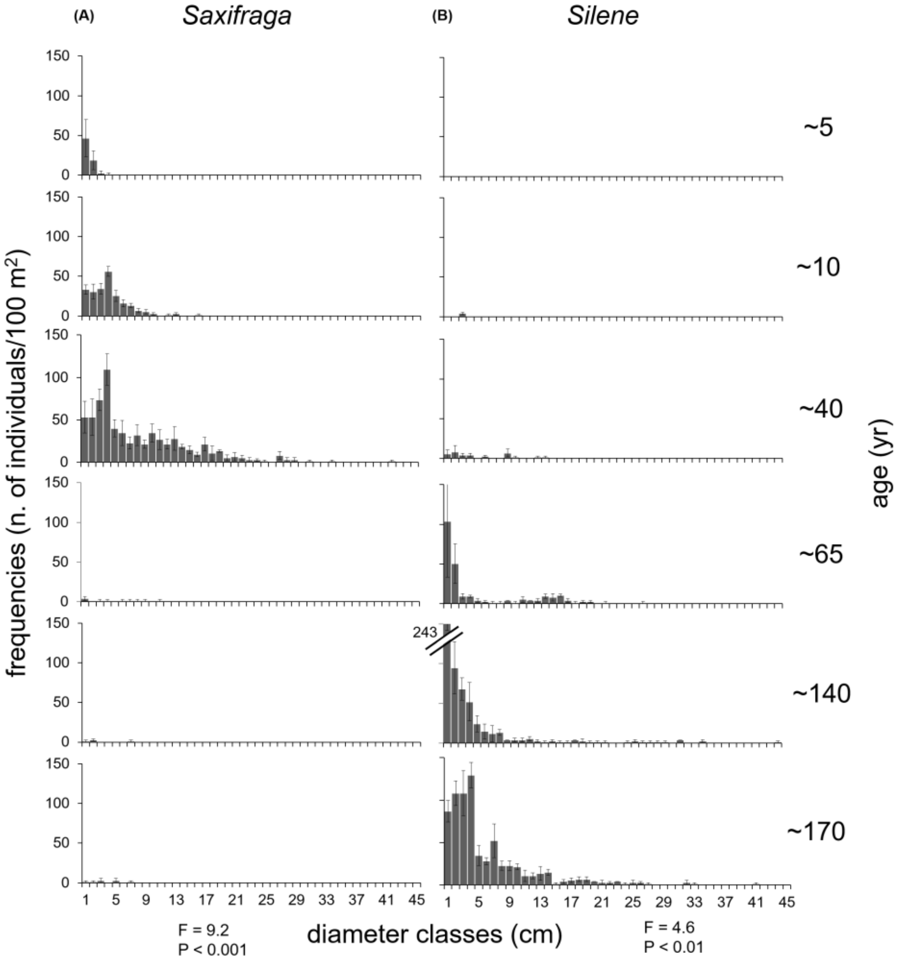

Figure 4. Population structure (distribution frequencies of individual diameters) in each stage of the chronosequence for the two target species Saxifraga oppositifolia L. subsp. glandulifera Vacc. (**A**) and *Silene acaulis (*L.). Jacq. subsp. bryoides (Jord.) Nyman (**B**). *Silene* individuals were not found in the first stage (~5 years) and are not represented. F and p indicate the results of PERMANOVA analysis among stages and within species.

2.2 Soil Development

According to the WRB classification (IUSS Working Group WRB, 2015), the soils of the first and third stages of the chronosequence were classified as Eutric Skeletic Regosols, while the others as Eutric Skeletic Regosols (Turbic). Soil chemical properties are shown in Table 2. We observed two to five genetic horizons in the soil pits of each stage, including A, AC, CA, and C horizons, with soil depths (A + AC or CA horizons) ranging from 15 to over 40 cm. Soil pH values ranged from 8.9 (C horizon deglaciated for 10 years) to around 6.0 in the upper horizon of the 170 years old stage. pH values decreased below 7.0 only in the surface horizons of the 65 years-old stage. Despite the high pH values, no carbonates were detected. Total organic carbon (TOC) was always greater in surface than in deep soil horizons and increased along the chronosequence, with a maximum content of 26.2 g kg^{-1} in the A1 horizon deglaciated for 170 years. Organic horizons were never detected. Total nitrogen (TN) was below the limit of quantification in soils up to 40 years since deglaciation and in all C horizons of the chronosequence (except the 140 years-old one), while the greatest contents were detected in A horizons, ranging between 0.2 and 1.5 g kg^{-1}. As a consequence, the TOC: TN ratio varied between 8 (at 40 years) and 17 (at 170 years). Younger soils (i.e., up to 40 years) showed higher Fe_o concentrations than older ones and both Fe_o and Fe_d were higher in surface horizons than in deeper ones. The Fe_o:Fe_d ratio, which indicates the ratio between poorly crystalline Fe (hydr)oxides and pedogenetic Fe (hydr)oxides, decreased along the chronosequence with the lowest value in the soils deglaciated for 170 years, in A2 and AC horizons (0.4 g kg^{-1}). Total phosphorus (TP) ranged between 593 and 776 mg kg^{-1} without recognizable age or depth trends, whereas the available form (P_{av}) was 0.5 mg kg^{-1} in the youngest soil stages and reached 4 mg kg^{-1} in the A horizons of the 170 years-old stage. Cation-exchange capacity (CEC) ranged between 0.89 $cmol_{(+)}$ kg^{-1} in the C horizon of the 65 years-old stage and 10.1 $cmol_{(+)}$ kg^{-1} in the oldest topsoil (Table 3). Base saturation (SATB) was greater in younger stages, ranging between 67% and 100% in the first 40 years-old stages. In the oldest stages, it was generally lower (except for A horizon from the 65 years-old stage with 83%) with a minimum value of 23.7% in the AC horizon of the 140 years ice-free stage. Calcium ion was the main element saturating the exchange complex, both in terms of concentration and ratio, especially in deeper soil horizons (Table 3).

Table 2. Chemical properties of soil pits along the six stages of the chronosequence (identified by the age from deglaciation). pH; TOC, total organic carbon; TN, total nitrogen; Fe_d, pedogenic iron oxides; Fe_o, iron amorphous oxides; Fe_o:Fe_d, iron pedogenic-to-amorphous ratio; TP, total phosphorus; P_{av}, bicarbonate extractable (available) phosphorus.

Age	Horizon	Depth	pH	TOC	TN	TOC:TN	Fe_o	Fe_d	Fe_o:Fe_d	TP	P_{av}

yr		**cm**		**g kg^{-1}**	**g kg^{-1}**		**g kg^{-1}**	**g kg^{-1}**		**mg kg^{-1}**	**mg kg^{-1}**
~5	**CA**	0-5/10	8.5	0.3	0	-	1.6	1.9	0.8	592.9	0.8
	C	5/10-35+	8.3	0.0	0	-	1.9	2.0	1.0	671.4	0.5
~10	**CA**	0-3	7.7	0.3	0	-	0.8	1.6	0.5	643.2	0.7
	C	3-15+	8.9	0.2	0	-	1.3	1.7	0.8	700.0	0.5
~40	**A**	0-3	7.1	1.6	0.2	8.0	1.4	2.8	0.5	727.8	1.1
	CA	3-14	7.4	0.2	0	-	1.7	2.3	0.7	775.6	0.9
	C	14-20+	7.3	0	0	-	1.8	2.3	0.8	705.6	0.7
~65	**A**	0-4	6.2	8.1	0.6	13.5	1.2	2.7	0.4	713.5	3.5
	CA	4-15	6.9	0.1	0	-	1.0	1.8	0.5	699.6	1.4
	C	15-22+	6.9	0.1	0	-	0.5	1.1	0.5	691.9	0.7
~140	**A**	0-5/8	6.2	14.1	0.9	15.7	1.1	2.7	0.4	688.4	3.4
	AC	5/8-18/20	6.8	0.7	0.1	-	0.9	1.9	0.5	751.2	0.9
	C	18/20-40+	7.4	0	0	-	0.9	1.6	0.5	605.9	0.7
~170	**A1**	0-3	6.0	26.2	1.5	17.5	1.0	2.5	0.4	724.4	4.0
	A2	3-10	6.0	11.3	0.8	14.1	0.8	2.0	0.4	728.6	2.8
	AC	10-15	6.4	0.5	0	-	0.7	1.7	0.4	683.4	0.8
	AC/C	15-25/30	7.1	0	0	-	0.7	1.7	0.4	648.7	0.7
	C	25/30-40+	7.4	0.5	0	-	0.7	1.7	0.4	609.9	0.6

Table 3. Cation-exchange capacity and base saturation of soil pits along the six stages of the chronosequence (identified by the age from deglaciation). CEC, cation-exchange capacity; Mg_{ex}, magnesium exchangeable cations; Ca_{ex}, calcium exchangeable cations; K_{ex}, potassium exchangeable cations; SATB, base saturation; satMg, magnesium saturation; satCa, calcium saturation; satK, potassium saturation, Ca:Mg, calcium-to-magnesium ratio; Mg:K, magnesium-to-potassium ratio.

Age yr	Horizon	CEC $\mathrm{cmol_{(+)}\,kg^{-1}}$	$\mathrm{Mg_{ex}}$ $\mathrm{cmol_{(+)}\,kg^{-1}}$	$\mathrm{Ca_{ex}}$ $\mathrm{cmol_{(+)}\,kg^{-1}}$	$\mathrm{K_{ex}}$ $\mathrm{cmol_{(+)}\,kg^{-1}}$	SATB %	sat Mg $\mathrm{g\,kg^{-1}}$	sat Ca $\mathrm{g\,kg^{-1}}$	sat K $\mathrm{g\,kg^{-1}}$	Ca:Mg	Mg:K
~5	**CA**	-	-	-	-	-	-	-	-	-	-
	C	2.00	0.11	1.23	0.20	77.00	5.50	61.50	10.00	11.18	0.55
~10	**CA**	1.29	0.05	0.89	0.04	75.61	3.54	69.05	3.02	19.51	1.17
	C	1.53	0.07	3.93	0.10	100.00	4.58	87.14	6.54	56.14	0.70
~40	**A**	-	-	-	-	-	-	-	-	-	-
	CA	1.18	0.07	0.73	0.05	72.33	5.96	62.12	4.25	10.43	1.40
	C	1.67	0.09	0.92	0.10	66.69	5.51	54.98	6.20	9.98	0.89
~65	**A**	2.67	0.18	1.94	0.11	83.36	6.79	72.51	4.07	10.69	1.67
	CA	2.05	0.07	0.76	0.10	45.52	3.27	37.15	5.09	11.34	0.64
	C	0.89	0.04	0.44	0.05	59.31	4.34	49.47	5.50	11.41	0.79
~140	**A**	7.36	0.26	2.36	0.12	37.23	3.49	32.04	1.69	9.18	2.06
	AC	4.11	0.09	0.84	0.04	23.67	2.28	20.42	0.97	8.96	2.34
	C	1.36	0.07	0.66	0.09	60.29	5.15	48.53	6.62	9.43	0.78
~170	**A1**	10.08	0.34	2.16	0.18	29.40	2.78	25.21	1.41	6.31	1.88
	A2	7.13	0.19	1.26	0.10	24.31	1.63	21.81	0.87	6.63	1.88
	AC	0.99	0.06	0.53	0.06	65.54	6.17	53.78	5.60	8.72	1.10
	AC/C	0.96	0.05	0.54	0.06	68.00	4.98	56.60	6.41	11.36	0.78
	C	1.08	0.04	0.59	0.06	64.50	4.13	55.25	5.13	13.39	0.80

2.3 Soil and Plant Interaction

Chemical composition in the topsoil layers sampled under *Saxifraga* (SaxS), *Silene* (SilS) and in the corresponding non-vegetated soils (BareS) significantly differed within the same age and among stages (Figure 5). The comparisons among all the three target soils were possible only at the third and fourth stages, where we found individuals of both *Saxifraga* and *Silene* with adequate development. Total OC showed a lag time in the first stages and then increased, especially with *Silene* establishment, reaching 6–8 g C kg^{-1} after 140 years, whereas the C content in the non-vegetated soil reached no more than 4 g C kg^{-1} (Figure 5A). Total N followed the same trend with more pronounced differences between the vegetated and non-vegetated soil samples (Figure 5B). At the 170 years-old stage SilS showed the highest N content. The stoichiometric TOC:TN ratio did not differ among target soils nor among stages, except for a higher value of SilS compared to BareS at the fifth stage (Figure 5C). Microbial C and N in vegetated and non-vegetated soils was comparable in the youngest stages and then significantly increased along the chronosequence in SaxS and SilS (Figures 5D,E). Total phosphorus content did not show significant trends in the three series of soils, while an increasing nutrient availability along the chronosequence, due to the presence of the two species, was observed (Figures 5F,G). Lower P_{av} concentrations were found in BareS if compared to SaxS at 10 years since deglaciation and to SilS at 140 and 170 years since deglaciation, respectively.

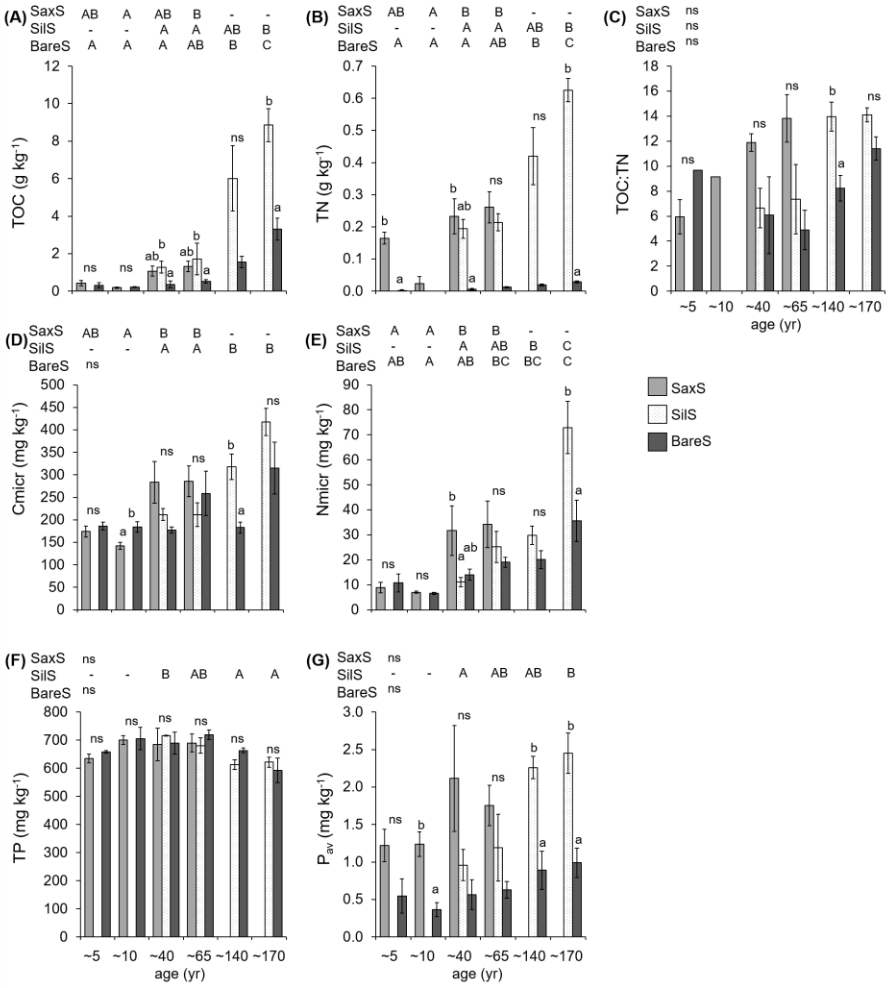

Figure 5. Chemical properties of target soils along the six stages of the chronosequence. SaxS, soil under individuals of *Saxifraga oppositifolia* L. subsp. *glandulifera* Vacc.; SilS, soil under *Silene acaulis* (L.) Jacq subsp. *bryoides* (Jord.) Nyman; BareS, bare soil; TOC, total organic carbon (A); TN, total nitrogen (B); TOC:TN, total carbon to nitrogen ratio (C); Cmicr, microbial carbon (D); Nmicr, microbial nitrogen (E); TP, total phosphorus (F); Pav, bicarbonate extractable (available) phosphorus (G). Different letters indicate significant differences ($p < 0.05$) among stages within each target soil (uppercase letters) and among target soils within each stage (lowercase letters) according to Tukey's HSD test. ns, $p \geq 0.05$.

In plant tissues, a decreasing trend occurred in P and N with time for *Saxifraga* and *Silene*, respectively, leading to a corresponding significant N:P and C:N increase (Figure 6). No differences were found between the two target species for any of the considered chemical features at the third and fourth stages, i.e., where both species were found.

A significant correlation was detected between the soil and vegetation matrices by Mantel test (r 0.45, $p < 0.001$), highlighting that chronosequence stages with similar soil properties had similar vegetation covers. Significant correlations between soil and vegetation variables were observed (Figure 7), explaining 74.2% of the distribution fitting with the first axis and 2.8% with the second axis.

Cryptogam cover appeared weakly associated with soil properties, as highlighted by the arrangement of the vector relatively to the soil ones. Pioneer species cover showed an intermediate trend between cryptogam and vascular plant covers, this latter being strongly related to TN, C_{micr}, and N_{micr}. Alpine grassland species cover was, instead, strongly associated with TOC and P_{av}. The only variable arranged in the left part of the bi-plot was TP, showing a contrasting distribution compared to most of the other variables. The chronosequence stages separated well along the two axes of the bi-plot. More specifically, the first two stages were at the opposite of both soil and vegetation features, while the two intermediate stages were mostly related to the covers of cryptogam and pioneer species and the oldest ones related to more developed soils (in terms of TOC and P_{av}) and to late- successional species.

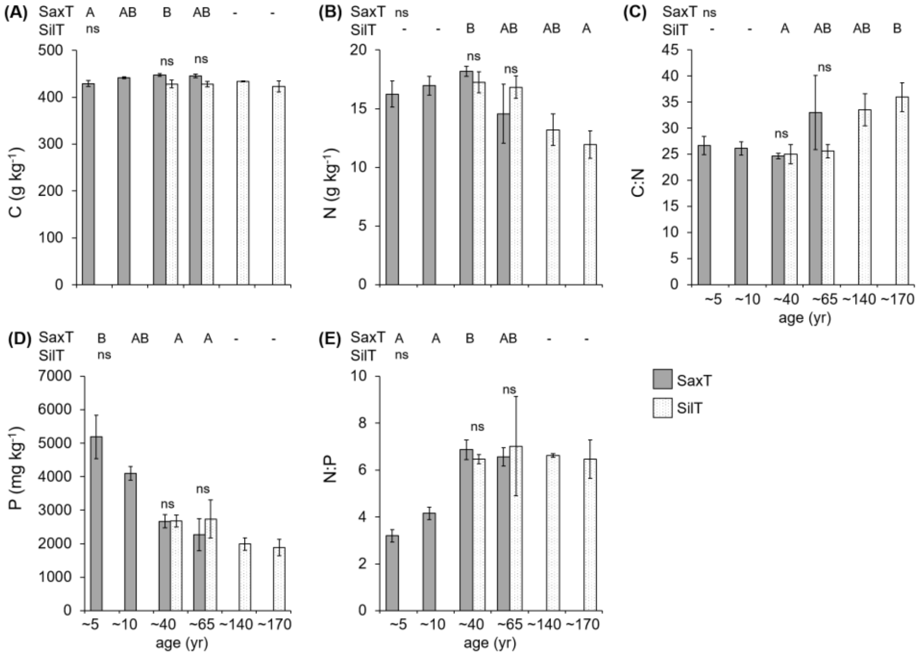

Figure 6. Chemical properties of plant tissues for the target species along the six stages of the chronosequence. SaxT, Saxifraga oppositifolia L. subsp.

glandulifera Vacc. L. tissues; SilT, Silene acaulis (L.) Jacq subsp. bryoides (Jord.) Nyman tissues; C, carbon (A); N, nitrogen (B); C:N, carbon to nitrogen ratio (C); P, phosphorus (D); N:P, nitrogen to phosphorus ratio (E). Different letters indicate significant differences ($p < 0.05$) among stages within each target species (uppercase letters) and between target species within each stage (lowercase letters) according to Tukey's HSD test. ns, $p \geq 0.05$.

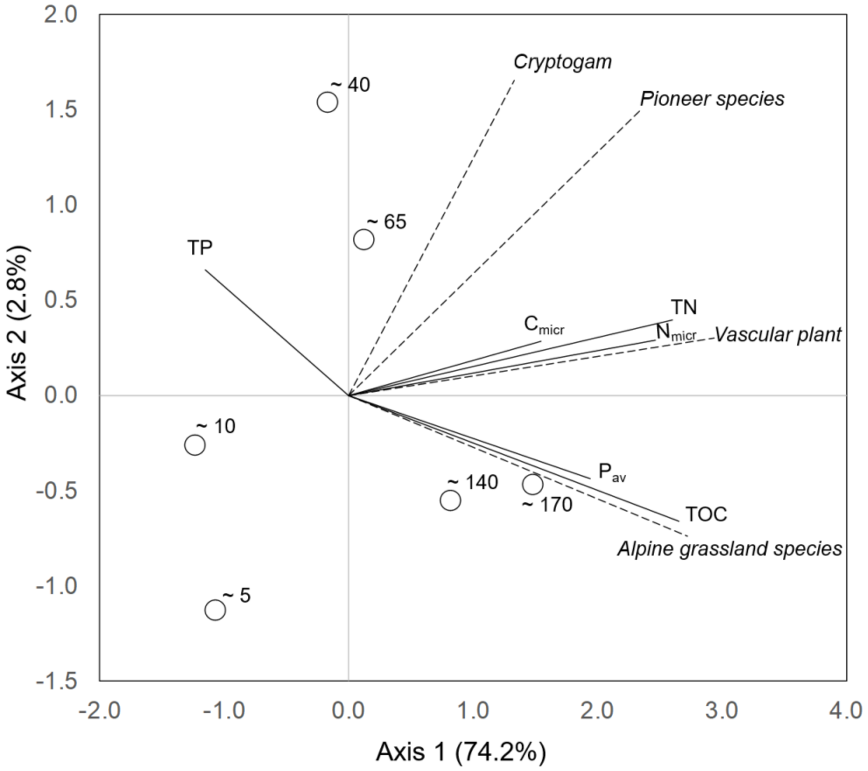

Figure 7. RDA ordination bi-plot showing the distribution of the six chronosequence stages (represented by the centroids of the three replicates per stage) and the relationships between soil properties (solid lines) and vegetation covers (dashed lines). TOC, total organic carbon; TN, total nitrogen; Cmicr, microbial carbon; Nmicr, microbial nitrogen; TP, total phosphorus; Pav, bicarbonate extractable (available) phosphorus. The variance explained by each axes is provided in brackets.

3. Discussion

The univariate and multivariate analyses highlighted a clear pattern of progressive soil development and vegetation successional dynamics throughout the six stages of the Lauson proglacial foreland, at increasing ages from deglaciation. More specifically, the colonization patterns along the chronosequence of the foreland by vegetation, considering the large difference in elevation between the lower limit of the study area and the potential treeline (500 m), was extremely rapid in the first forty years following deglaciation, in terms of both species richness and cover. An average of four and twelve different species were able to colonize the Lauson proglacial till after 5 and 10 years since deglaciation, respectively. This early process was more rapid than the one observed in the Austrian Alps by Tscherko et al. (2005) and Raffl et al. (2006), in study areas located around the potential treeline (study areas at 2,300–2,450 m a.s.l., with a potential treeline at 2,400 m a.s.l. according to Pecher et al., 2011). Conversely, the studied area was comparable to the results obtained by Burga et al. (2010) in Switzerland, in a proglacial area located at 1900–2,100 m a.s.l., i.e., completely below the potential treeline, which is located at 2,450 m a.s.l. (Pecher et al., 2011). Similar results were also obtained by Cannone et al. (2008) in the Italian Alps, in a study area located at 2,700–2,850 m a.s.l., 300–450 m above the potential tree line, located at 2,400 m a.s.l. (Pecher et al., 2011), and by Matthews and Vater (2015) in southern Norway (study area at 1,420 m a.s.l., 400 m above the local tree line). In all these environments, substrate stability, together with the ability of seeds to reach colonization sites, created a suitable environment for the establishment of pioneer species. The lack of seeds, stated by several studies as a fundamental limiting factor (Chapin et al., 1994; Jones and del Moral, 2009), probably was not the major constraint for plant colonization in the young Lauson foreland, likely because of the short distance between bare sites, freshly released by the glacier, and plant communities growing on nearby slopes, which acted as seed sources. We suggest that, under the studied conditions, other factors, such as drought, frost and cryoturbation may have played a major role limiting plant establishment, according to Erschbamer and Caccianiga (2016). These factors are naturally interrelated with proglacial micro-topography and with the occurrence of protected microsites, important also for nutrient accumulation. In the Lauson chronosequence, after 65 years since deglaciation, vegetation cover increased due to the additive contribution of the alpine grassland species that partially replaced pioneer ones, contributing to an overall rise in vascular plant cover. Despite the increase in species richness, the vegetation cover was very scarce even after 170 years since deglaciation. At the last stage of the chronosequence, cryptogams and vascular plants indeed occupied only 34% of the surface, while Schumann et al. (2016) recorded mean vegetation cover values of 70% in 123 years-old stages from 16 proglacial forelands in the Alps (located between 2,000 and 2,600 m a.s.l.).

This difference may be related to the complete absence of trees and shrubs within and around Lauson foreland, which is far above the treeline.

The dynamics observed for pioneer and alpine grassland species were confirmed by the population structure of the two target species, *Saxifraga* and *Silene*. In particular, *Saxifraga* showed a typical pioneer behavior, spreading over the study area since the very first stages of the chronosequence and drastically reducing its cover after 65 years, while the more nutrient-demanding *Silene* firmly established only after (40-) 65 years of ice-free development, indicating a complementary successional pattern. Nevertheless, both species showed an uneven-aged demographic structure, with higher frequencies in the smallest diameter classes and lower frequencies in the largest ones. *Saxifraga* and *Silene* seem indeed to perform ruderal strategies (Grime, 2006), which implies a great amount of seed production, resulting in the abundance of seedlings and showing fast-colonization ability, rather than a stress tolerant behavior, as it can be expected in extreme environments such as glacier forelands. The results obtained by our demographic approach, indicating a huge investment in plant reproduction, align with the findings of many authors in proglacial environments, where ruderal strategies allow for plant colonization in the early successional stages (Tscherko et al., 2005; Caccianiga et al., 2006; Gobbi et al., 2010; Erschbamer and Caccianiga, 2016).

Frequency variations in diameter classes were statistically significant for both species throughout the entire chronosequence even if a complete transformation of the demographic structure, from uneven-to even-aged, was not reached in the Lauson glacier foreland.

A clear pattern of a primary succession was observed in both functional species pool dynamics and target species population structure, with an increase in alpine grassland species number and cover over time, and a simultaneous decline in pioneer species. Overall, considering the limited vegetation cover and the partial plant community substitution among successional stages, the Lauson chronosequence time span (170 years) was not sufficient for the establishment of a stable alpine grassland climax community (highlighted also by the *Silene* demographic structure), supporting the 500 years-minimum age required for *Carex curvula* All. climax type stated by Andreis et al. (2001).

Although all soils found in the Lauson glacier foreland referred to the WRB group of Regosols, we observed clear soil-forming processes such as a weak mineral weathering (evidenced by the Fe_o and Fe_d values) and leaching processes (evidenced by pH and SATB decrease), mostly guided by the soil organic matter build- up with time since deglaciation. The decline observed in pH followed the typical pattern of the proglacial chronosequences (Messer, 1988; Frenot et al., 1998; Anderson et al., 2000); it was however much slower than in proglacial areas located at lower elevations (e.g., D’Amico et al., 2014), in which the overall ecosystem productivity was enhanced by higher temperatures and the larger input of plant litter accelerates soil acidification. In our study, soil pH was very high in the recently exposed sediments. As no carbonates were detected, the high pH was probably related to the initial weathering of silicates, which could lead to a release of base cations, able to increase pH values in very coarse-grained soils. In young ice-free till (ca. 5–10 years from deglaciation), not yet vegetated, these processes can lead to very high pH values because of the lack of colloidal buffer surfaces, as deduced by the very low clay content (<2%) and the related scarce cation exchange capacity (max 2 $\mathrm{cmol}_{(+)}\ \mathrm{kg}^{-1}$ in CA horizon in the 5 years old material) (Celi et al., 2013). Moreover, the influence of seasonal glacial runoff cannot be excluded, possibly bringing high Ca and K loads, regardless of bedrock (Anderson et al., 1997, 2000) and thus increasing soil pH. High concentrations of K in glacial melt waters, derived from leaching of interlayer cations from biotite, were observed in many works (Drever and Hurcomb, 1986; Stallard, 1995). However, soil acidification occurred over time, likely induced by the increasing vegetation cover and the related release of carboxylic acids through root exudates and/or acidic compounds formed during organic matter decomposition, as well as by organic acids released by bacteria and fungi (Brunner et al., 2011). The leaching of base cations, due to the lack of exchanging surfaces, could contribute as well to the pH decrease (Burt and Alexander, 1996; Darmody et al., 2005; Bernasconi et al., 2011).

In addition to climatic stress such as drought, frost and cryoturbation, nutrient limitation plays a fundamental role in controlling plant establishment and emphasizes the role of safe- sites (sensu Harper et al., 1961), where water fluxes increase moisture and nutrient accumulation, enabling the vegetation to survive. Glacial runoff, which represents an important input of nutrients and base cations, could have facilitated the uptake by the early-successional species such as *Saxifraga*, despite the very harsh substrate of the recently deglaciated debris (Göransson et al., 2016). In the Lauson foreland, we indeed found an optimal base saturation for plant establishment in the first 40 years since deglaciation, with Ca:Mg:K stoichiometric ratios suitable for plant nutrition. Despite the decreasing pH along the terrain age gradient, cation-exchange capacity (CEC) raised, likely as a consequence of organic matter accumulation rather than formation of secondary minerals having higher CEC compared to the unweathered parent material (Bernasconi et al., 2011).

Total nitrogen levels were extremely low and were detectable only in the more organic matter-rich and developed upper horizons. This result highlighted that N was one of the major limiting factors of the Lauson soil-vegetation system due to the lack of N-fixing plants (Körner, 2003; Göransson et al., 2016) and to a possible, moderate contribution of N-fixing free-living microbes (Duc et al., 2009) and lichens such as *Solorina crocea*. Göransson et al. (2016), in the wetter Damma proglacial chronosequence in Switzerland, found an exponential increasing trend of TN during the first 137 years of soil development and argued that the main N input was probably due to atmospheric deposition (assumed to be approximately 5–10 kg ha^{-1} yr^{-1}). Although in nearby areas (Indren Glacier, Lys Valley, Monte Rosa Group), the atmospheric N deposition was similar, being 6.6 kg ha^{-1} yr^{-1} in the last few years (Colombo et al., 2019), the smaller N inputs in our soils were associated with lower mean yearly precipitation because of the isolated position of the Lauson proglacial area, surrounded by high mountains. Thus, our findings suggest that in the Lauson foreland, similarly to areas with low N-deposition like Glacier Bay in Alaska or Franz Josef Glacier in New Zealand (Chapin et al., 1994; Menge and Hedin, 2009), the role of atmospheric deposition was less important in N cycling. Furthermore, Göransson et al. (2016) found, particularly at early stages, that N inputs exceeded plant uptake, and low P levels limited plant colonization more than low N levels. In the entire Lauson chronosequence, soil TP contents ranged between 600 and 700 mg kg^{-1}, which represent a favourable range for P cycling and plant establishment (Yang et al., 2013). With time, the release of protons and carboxylic acids caused the dissolution of P-bearing minerals, slightly contributing to feed the available P pool and then plant uptake (Celi et al., 2013). In the older, more structured, and more functional soils, with increased cover of alpine grassland species, P biocycling became particularly evident. Particularly at the fourth and sixth stages of the chronosequence, the upper soil horizons became enriched in TP content. This depth trend can be attributed to plant root exploration and uplift of P from the deeper soil horizons to the surface, through deposition of plant residues. However, compared to other proglacial chronosequences in the Alps, with similar time span, the pedogenic processes remained at an incipient stage, likely because of the lower litter inputs (D'Amico et al., 2014): the cold temperatures characterizing the Lauson forefield and the associated processes (i.e. short growing season, cryoturbation, root damages and slow decomposition rates) slow down plant colonization, which in turn reduces TOC accumulation and N availability; the low N availability, in a low-N deposition site, in turn, slows down plant colonization, creating a negative feedback which inhibits ecosystem development.

The present study provided novel information concerning the differing function of pioneer and late-successional herbaceous species in affecting soil biogeochemistry in recently deglaciated debris. Indeed, the comparison among soils sampled under plants (SaxS and SilS) and non-vegetated soils (BareS) highlighted the pivotal role of vegetation in soil development and nutrient mobilization, along the whole chronosequence, from the very early stages to the latest ones. *Silene* performed a greater soil conditioning than *Saxifraga* in terms of TOC and TN accumulation and development of microbial biomass even within the early stage time points of soil development where both plant species occurred. This may be due to the different growth forms of the two target species, as the cushion species *Silene* may exert more enhancing effects on soil formation than the prostrate species *Saxifraga* (Bonanomi et al., 2016). The drastic decline of *Saxifraga* after 65 years in the chronosequence, likely induced by the competition with more nutrient-demanding species, suggested a facilitation process for the establishment of other plants, linked to the several microsites enriched in organic matter and nutrients. After 140 years, soil development and functionality are proved by higher TOC contents in BareS, as a result of organic matter translocation and mixing processes, which led to even higher concentrations than those in SaxS after 65 years. Microbial and fungal processes could have contributed to this TOC and N enrichment as well. Nitrogen content increased significantly in BareS with time, as observed also in soil pit horizons. Nonetheless, unlike processes involving organic matter, TN resulted in very low concentrations even in the oldest stages, representing the major limiting factor for more nutrient-demanding plant species. Where measurable, the TOC:TN ratio was between 8 and 17.5, thus in the normal range for high-altitude grassland and tundra soils (e.g., D'Amico et al., 2014, 2015). The increase in both microbial C and N associated to the target species (and more generally to vascular species cover) along the chronosequence proved again the strong biotic impact in nutrient accumulation compared to abiotic processes, such as atmospheric deposition. In particular, the enhanced microbial biomass and activity associated to the increased concentration of C and N under the target species may have exerted a positive impact on enzyme activity and therefore on microbial N fixation. Similarly, available P content in BareS was very low, but both target species enhanced its concentration along the chronosequence confirming the priming effect of vegetation on P biocycling.

In the youngest stages (ca. 5–10 years since deglaciation) the observed accumulation of P in *Saxifraga* tissues was probably a consequence of N limitation: under drastic limiting conditions the accumulation of nutrients in tissues has been demonstrated as a possible plant physiological response (Simon et al., 2017). This may be further confirmed by the quite high C:N ratio in the herbaceous tissues, especially for *Silene* (Hobbie et al., 1998). In older stages, where organic matter and N became more abundant, P content in *Saxifraga* tissues fell to more typical values (Richardson et al., 2004). However, N:P in plant tissues was very low, along the whole chronosequence, and far from the value of 12, a threshold beyond which plant development is P-limited, such as in highly developed soils (Wardle, 2004) or in particular P-limited systems. For instance, in the Damma proglacial chronosequence (N:P ratios in the aboveground vegetation >19, Göransson et al., 2016) the main P-containing mineral was the slowly weathering fluoroapatite (Bernasconi et al., 2011), while the Verra Grande forefield (D'Amico et al., 2020b) developed on serpentinite and almost devoid of P bearing minerals.

4. Conclusion

This work provided novel results on individual plant species trajectories and related soil development through successional stages of a proglacial chronosequence. Despite the extreme conditions for plant establishment, the

Lauson proglacial foreland allowed a remarkable early (5–10 years) and species-rich colonization, attained by pioneer species in the available safe micro-sites. Carbon seemed to be the major constraint to microbial development together with N, which also hampered plant colonization. However, nutrient requirements for pioneer plants were met, likely thanks to the water fluxes funneled between rocks. The alpine grassland species colonization started indeed immediately, although species number and cover pronouncedly increased only when the soil reached sufficient nutrient supply and functionality. Then, after about one century of ecosystem development since deglaciation, soil conditions appear suitable for the establishment of more competitive vegetation, typical of alpine grassland communities. Nonetheless, the improving effect on soil triggered by *S. oppositifolia* was already evident on quasi-inert substrates, free from ice for a limited time, highlighting the relevant role of biotic factors in stimulating the initial phases of soil formation. However, more biomass-productive species, such as *S. acaulis*, performed a greater soil conditioning than *Saxifraga*, even within the early stage time points where both plants were present, becoming progressively more expressed with time. Therefore, C:N:P stoichiometry in proglacial areas is plant-influenced, and the impact on nutrient dynamics is related to functional species pool, with a major role played by late- successional grassland species.

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Chapter 5: Conclusion

We are used to perceive the function and shape of high-alpine biomes as steady through time: indeed, due to elevation and harsh conditions, variations usually occur at a slow pace. Nevertheless, Climate Change bears the potential to alter the balance of the finely-tuned mountain environment in a matter of decades, a speed rate which has never been witnessed before. Such biomes are the result of complex relations between soil and vegetation, which are tightly interlaced in a mutual exchange of substances and a continuous shaping of the environment.

The response of plant communities to new climatic and pedoclimatic conditions might depend on the plasticity of the species and their ability to reconfigure their growing cycle to match it with a longer and hotter season. Regardless of functional pool or optimum preferences, the majority of the snowbed species proved sensitive to temperature and snowmelt in the timing of their growing cycle, while some of the species showed unidentified patterns. The availability of nutrients and the life cycle of soil microbiota, which are influenced by temperature and precipitation dynamics, might also be a key factor for plant development and reproduction timing. While it is certain that the C and N dynamics in the snowbed microhabitat are strongly influenced by climatic and pedoclimatic variables such as meltout day and duration of soil freezing, we assessed on the other hand that N supplies in the soil are conditioned also by the the key species *Salix herbacea*. We argue that, among our variables, the phenology of said species was the main biotic driver and had a strong influence on nitrogen pools in the soil. There are still uncertainities on the thriving of high-alpine species, nonetheless this biome is with no doubt undergoing some major changes in the near future. Some plant species will have the chance to take advantage of new temporal niches, flowering earlier in spring or extend the seed dissemination period longer in the late summer. Other less opportunistic species, once failed to endure in the competition, might undergo a gradual fragmentation of their distribution and possibly disappearance. Moreover, species might profit also from the seizing of new spatial niches. Indeed the colonization of barren areas, such as recently deglacialized moraines, represents a crucial step for soils too, with their development strictly connected to the progressive succession of more complex plant communities. Our findings highlight how the C:P:N stoichiometry of proglacial soils is higly dependent on the presence of plants, especially late-successional grassland species.

To shed more light on the future of the alpine environment, the study of plant phenology and interdependent plant-soil dynamics is still a relevant tool. By providing insight on the application of phenological surveys, we hope to have highlighted the pratical aspects of this method and possibly suggested some new tools, such as the adapted BBCH scale. Our method of registering plant phenology proved accurate and time-efficient, and might offer advantages in the fieldwork. We also wish to stress the importance of considering Alpine biomes as a complex system where the vegetation dynamics act on soils and get influenced in return. We believe that coupling data of plant phenology with thoroughly collected information on soil C and N forms could advance our understanding of their mutual influence and the functioning of plant life cycles in the alpine biomes. With this thesis, we aimed at emphasizing the state of the art of these well-known topics with a new focus on their mutual relations. Since alpine ecology is a compound field, where the final result is often derived by the combination of multiple factors, further research should be encouraged, with the objective of encompassing neglected variables in order to achieve a broader view on the functioning of high-elevation biomes.