Università degli Studi di Ferrara

PH.D. Course

in

Evolutionary Biology and Ecology

In cooperation with:

Università degli Studi di Parma

Università degli Studi di Firenze

CYCLE XXXIII

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ALPINE TUNDRA AND CLIMATE CHANGE: EFFECTS OF SUMMER WARMING AND REDUCED PRECIPITATION ON ECOSYSTEM FUNCTIONS IN A CAREX CURVULA GRASSLAND

Scientific/Disciplinary Sector (SDS) BIO/03

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Years 2017/2021

"…We are entering a period when physical forces, and our reaction to them, will drive the drama on planet Earth"

Bill McKibben, from "It's not science fiction" – a review of Kim Stanley Robinson's "The Ministry for Future", December 17, 2020

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PREFACE

Climate change: a brief note

Scientific research has collected indisputable evidence that climate change is the result of increased greenhouse gas (GHG) emissions following anthropogenic activity that has taken place since the Industrial Revolution in the mid-18th century, with GHG concentrations in the atmosphere rising from 280 ppm to the currently recorded 410 ppm (IPCC, 2013). Such an increase has been particularly marked during the last decade, with an acceleration of the average annual rate of increase of up to 2.2 ppm compared to the 1.5 ppm recorded in the 1990s (source Mauna Loa Observatory, NOAA ESRL).

Consequently, the global mean annual air temperature has risen by 1.5 K compared to the pre-industrial period and has been increasing by 0.2 °C each decade over the last 30 years (IPCC, 2018). The warming trend, however, is not linear and a rapid intensification is currently occurring, the last decade being identified as the hottest on record. A recent report on global climate compiled by more than 500 scientists from over 60 countries, in fact, stated that since 1980 each decade has been warmer than the preceding one and that the 2010-19 period, in particular, witnessed several large-scale heat-wave events worldwide (Blunden and Arndt, 2020).

Global warming, the name that Wallace S. Broecker used in 1975 to address the phenomenon of human-induced increased temperatures (Broecker, 1975), has led to changes in a variety of aspects of the climate system, such as ice melting, increased sea levels, ocean acidification, increased vapour pressure deficit and modified precipitation patterns, with a global intensification of drought and extreme climate events in general (IPCC, 2013). Such trends are predicted to intensify in the future: in particular, scenarios for 2100 show that by then temperatures will be 0.3 - 4.8 °C higher than the period 1986-2005 depending on the rate of carbon emissions (IPCC, 2013); average precipitation will also exhibit substantial spatial variation, with projections for some regions showing an increased yearly amount, while others exhibiting a decrease (IPCC, 2013). These figure, therefore, underline the fact that some areas will be, and already are, more affected than others by climate change. In Polar regions and high-elevation areas, in particular, signs of change are amplified compared to global averages (Pepin et al., 2015; Seddon et al., 2016; Blunden and Arndt, 2020) – see Section 1.1.2 for detailed insights on mountain regions.

As the schematic diagram by Ruddiman (2008) (**Fig. I**) clearly shows, the climate system is made up of a complex network involving a variety of actors, whose interactions determine positive and/or negative feedback which further exert an influence on the initial variations of internal responses. On account of this, ecological research efforts in recent decades have been largely oriented towards an understanding of the degree of variation in the main components of

the climate system, the mechanisms involved, and how the overall functions of ecosystems will be affected by future changes in abiotic conditions.

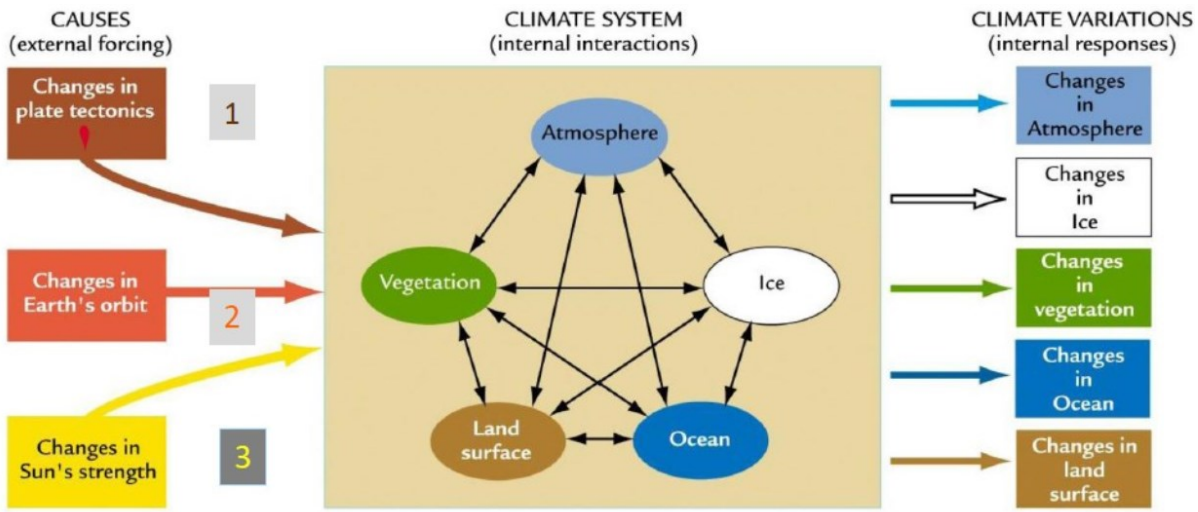


Fig. I: Diagram illustrating the climate system and the main elements constituting it (from Ruddiman, 2008).

Climate extremes: a "new" challenge for ecological research on climate change

Precipitation and heat records have been broken worldwide in recent years (Trenberth et al., 2014; Tollefson, 2015; Zhang et al., 2015; Orth et al., 2016; Kennedy et al., 2018), confirming the output in the 5th report published in 2013 by the Intergovernmental Panel on Climate Change, which stated that frequency and magnitude of extreme events – i.e. drought, heat waves and heavy rainfall events − has increased during the second part of the 20th century (IPCC, 2013). Such trends have also been confirmed in the latest annual reports on the state of climate led by the National Oceanic and Atmospheric Administration National Climatic Data Center (NOAA/NCDC) and the World Meteorological Organization. In particular, the last 5 years have been the hottest ever on record (Blunden and Arndt, 2020; WMO, 2020) and the evidence of recent trends is so overwhelming that a new set of terms – i.e. *climate crisis*, *global heating* − has recently been adopted to refer to the ongoing changing climate conditions (Zeldin-O'Neill, 2019). Such climate extremes, whose effect on ecosystem functions has been widely documented in recent years (e.g. Ciais et al., 2005; Schwalm et al., 2012; Gatti et al., 2014; Li et al., 2019), raise new questions on how this further aspect of climate change will impact ecosystem stability and functions.

It is only in the last decade that researchers have started to look more closely at the effects of climate extremes (Jentsch et al., 2007; Smith, 2011a, b; Reichstein et al., 2013; Bahn et al., 2014; Frank et al., 2015; Sippel et al., 2016; Sippel et al., 2018; von Buttlar et al., 2018), phenomena which, although more localised in space and time, have potentially drastic effects on ecosystem equilibrium and plant performance when compared to "gradual", chronic climate changes (Niu et al., 2014; Germain and Lutz, 2020). Climate extremes can directly or indirectly affect ecosystem

processes, triggering a variety of responses: for instance, reduced plant growth following a frost event represents a direct consequence of the anomalous climate event itself, whereas a pest and pathogen outbreak following drought represents an indirect result of the extreme (**Fig. II** from Reichstein et al., 2013).

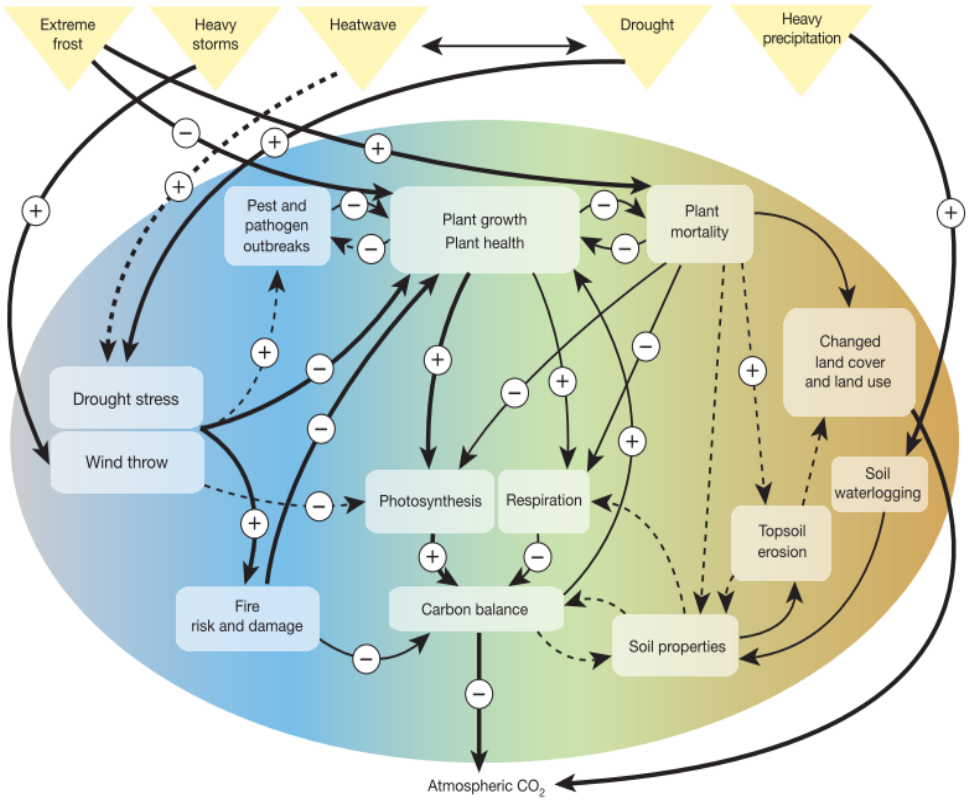


Fig. II: Diagram reported in Reichstein et al. (2013) illustrating the impacts of climate extremes on key variables at the basis of the carbon cycle. The figure highlights the direction of responses (positive or negative), the nature of the causal relationship (direct, full line; indirect, dashed line) and the relative importance of the relationship (arrow thickness).

However, the challenge to ecology research represented by such a complex network of cause-effect relationships is made even more difficult by the definition of "extreme" which is followed when investigating such phenomena (Smith, 2011b). In a report on a set of studies finding no marked ecological response – i.e. alteration of structure and/or functions – outside the range of natural variation to statistically rare climate extremes, Smith (2011b) stresses the need for further investigation into the multiple aspects which may characterise an extreme climate event – e.g. interactions with other types of extreme, altered timings and changed frequencies – in order to understand the mechanisms underlying ecosystem sensitivity to such phenomena.

The aim of the present research project is to play an active role in this context of research by focusing on different aspects of drought, a climate extreme which has considerable implications for ecosystem functions, but is still poorly investigated in alpine regions, one of the most sensitive areas to increased climate variability (Seddon et al., 2016). More specifically, drought is defined from a rainfall perspective, by assessing ecological responses to a variation in the amount of

precipitation and the timing of a dry spell. Soil misture thresholds that limit some key ecosystem responses, such as CO_2 exchange, are also identified and the interaction between drought and a scenario of increased temperatures is also examined.

CHAPTER 1. INTRODUCTION

1.1 STATE OF THE ART

1.1.1 Alpine ecosystems: key aspects and global significance

The present research focuses on ecosystems in the *alpine life zone*, defined by Körner (2003) as the area above the natural high-altitude treeline. Alpine environments represent a particularly interesting open laboratory for studying the effects of climate drivers, on account of the fact that plant communities are exposed to a wide range of naturally-occurring extremes, resulting in sophisticated morphological, physiological and developmental adaptations (Bliss, 1962; Bowman, 2001; Körner, 2003). Plant communities are dominated by slow growing, low statured, perennial plant species whose life cycles are strongly influenced by a variety of abiotic factors. These include: low average temperature and strong thermal seasonal variation; high irradiance; nutrient scarcity; and hydrological dynamics, driven by the interaction of precipitation, wind exposure and landscape position (Körner, 2003; Winkler et al., 2019). Topographic complexity regulates snow accumulation and duration which, in turn, determines the length of the growing season, the water availability during the snow-free period and the amount of nutrients available for plants (Taylor and Seastedt, 1994; Fisk et al., 1998; Litaor et al., 2008). The dominant role that abiotic factors play in shaping plant communities, therefore, makes alpine ecosystems, and cold biomes in general, among the most sensitive terrestrial ecosystems when faced with altered climate conditions (see Section 1.1.2 and 1.1.4).

Considering the global significance of alpine ecosystems − geographical, ecological and economic − the implications of climate change should be of great concern. Alpine ecosystems are, in fact, present in all continents and at all latitudes (Körner, 2003). A recent study mapping the global distribution of alpine ecosystems based on land cover and remote sensing has estimated that these biomes are present in 2.6% of the land surface, excluding Antarctica (Testolin et al., 2020). The alpine life zone in particular is characteristic of mountain areas which, taken together, cover about 12% of the terrestrial land surface (again excluding Antarctica), of which 21.5% is characterised by alpine features (Körner et al., 2011). In addition to their diffusion, the importance of alpine ecosystems is related to their high level of species richness, representing invaluable biodiversity hotspots, home to about 4% of the total plant species present worldwide (Körner, 2003). Such diversity is related to the high degree of climatic zones present, the isolation periods which occurred in the past and the fragmentation of the landscape driven by slope forces (Körner, 1995). It has also been estimated that alpine systems can potentially stock up to 1% of the global carbon pool, when both biomass and soil stocks are taken into consideration (Körner,

1995). Finally, almost 20% of the Earth's human population inhabits these areas and is dependent on its resources – i.e. water, food and energy production (Viviroli et al., 2011; Price and Egan, 2014). In this respect, grasslands provide a series of ecosystem services to human populations, including carbon storage and sequestration, erosion regulation, forage production and food supply, in addition to recreational space (Zhao et al., 2020). Such services are especially important when considering the European Alps (Roilo et al., 2018), an area which are increasingly likely to be negatively affected by extreme climate events.

Over the last decades, cold biomes have undergone significant modifications as a result of climate change (De Boeck et al., 2019; see Section 1.1.2). Moreover, a recently developed vegetation sensitivity index has identified alpine regions as one of the most sensitive areas to climate variability in the last 14 years, together with other regions including the Arctic (Seddon et al., 2016). This index is based on the theory that "systems with a high probability of crossing a threshold experience amplified responses to disturbance and are more sensitive to environmental perturbations" (Seddon et al., 2016). Such an evidence, together with the reasons cited above, makes an understanding of the effects of climate change in alpine ecosystems one of the pivotal tasks of ecological research globally.

1.1.2 Climate in mountain regions: historical trends and predictions

Research on historical climate trends in mountain regions has revealed a greater increase in mean temperatures recorded in these regions during the last century compared to the change observed globally (Beniston et al., 1997; Diaz et al., 1997; Liu and Chen, 2000). For instance, a 2 K increase in the minimum annual temperature was recorded for the Swiss Alps at the end of the 20th century (Beniston et al., 1997). Such a warming trend has been found to be dependent on the elevation, with higher temperatures being recorded at higher elevation sites compared to those in lowland areas (Pepin et al., 2015). As a consequence of warming, water regimes have been modified: a reduction in snow cover and earlier snowmelt have occurred in some areas (Dye, 2002; Keller et al., 2005; Mote et al., 2005); in particular, a 6-day advance in the snowmelt date every 10 years has been recorded in the Northern Hemisphere (Dye, 2002), thus extending the length of the favourable growing season available for high-elevation organisms. Glaciers have also undergone significant reduction over the last decades as a consequence of global warming. A recent estimate on glacier retreat for the Alps has found that mass loss rate between 2000 and 2014 was approx. -1.2% y^{-1} compared to estimates of glacier volume made at the beginning of the 21st century (Sommer et al., 2020).

As far as precipitation patterns are concerned, these have also changed globally (IPCC, 2013) and historical climate data for various mountain areas has revealed increasing evidence pointing to a reduced summer rainfall trend over the last century (Basistha et al., 2009; Yang and Gong,

2010; Brugnara and Maugeri, 2019). However, arriving at an overall view of precipitation trends is a difficult task, especially for mountain regions, due to the fact that mountains generate their own climate on account of the effect that their mass exerts on circulation patterns, precipitation and radiation (Inouye and Wielgolasky, 2003). Even within a single region, such as the European Alps, where the present research was based, trends vary considerably according to the area under consideration, the period and the season (Gobiet and Kotlarski, 2020).

On a broad scale, historical precipitation data over a 200-year period revealed an increased precipitation trend in the north-western part of the Alps, while reduced rainfall amounts were revealed in the southern Alps (Brunetti et al., 2006). A more detailed analysis in the central Alps over a 90-year period (1922-2009) effectively found a reduction in the total annual amount of precipitation, although such a decrease was limited to 1.0 -1.5% per decade and was only locally significant (Brugnara et al., 2012). Projection models for the end of the 21st century seem, in fact, to be in line with such a trend. Global warming is forecast to alter water regimes, with decreased summer precipitation (approx. -20%) and higher temperatures (3.3 K) in the south and on the western side of the Alps, always taking into account that a margin of uncertainty in model predictions exists related to the location of this mountain range within the transitional zone of precipitation (Gobiet et al., 2014). Moreover, higher temperatures will, in all likelihood, lead to drier conditions as a result of higher evapotranspiration (Briffa et al., 2009). The return period of heat waves, such as the one that occurred during the summer of 2003, could be as short as 2 years, with an increased frequency of drought episodes (Gobiet et al., 2014) which are predicted to become “a norm rather than an exception” (Calanca, 2007). All these predictions, taken together, will probably lead to (a) a further reduction in both the amount of snow and the duration of snow cover, a forecast which has already been made for some areas of the Alps (Valt and Cianfarra, 2010), and (b) further retreat in glacier cover. In fact, a recent projection regarding Alpine glaciers showed that it is likely that they will have lost between 47% and 52% of their volume by 2050, compared to 2017, a reduction which could lead to a massive loss of glaciers by 2100 in the most extreme warming scenario (Zekollari et al., 2019), with obvious negative effects on water discharge and supply.

The “state of climate” over the last few years seems to confirm such projections. For instance, climate data shows how heat waves and related drought have affected the European continent every summer during the study period of this research. According to the report by Hartfield et al. (2018) Europe recently experienced the five warmest years since 2011, with record-breaking temperatures and extreme drought affecting the Italian peninsula during the summer of 2017. This was also confirmed by a national report which identified 2017 as one of driest years in Italy over the last century (ISPRA, 2018). In the following year (2018), Northern and Western Europe experienced a massive heat wave together with below-average precipitation during the early

summer (WMO, 2019), whereas in 2019 a large area of Europe experienced extreme drought following a dry spring season and summer heat waves (Blunden and Arndt, 2020). While it is well known that continental scale projections do not necessarily reflect local climate conditions, nevertheless the intensification of such events suggests that high-elevation ecosystems, especially in the European Alps, are more likely to undergo extreme climate conditions in the near future.

1.1.3 Alpine climate change: a conceptual framework

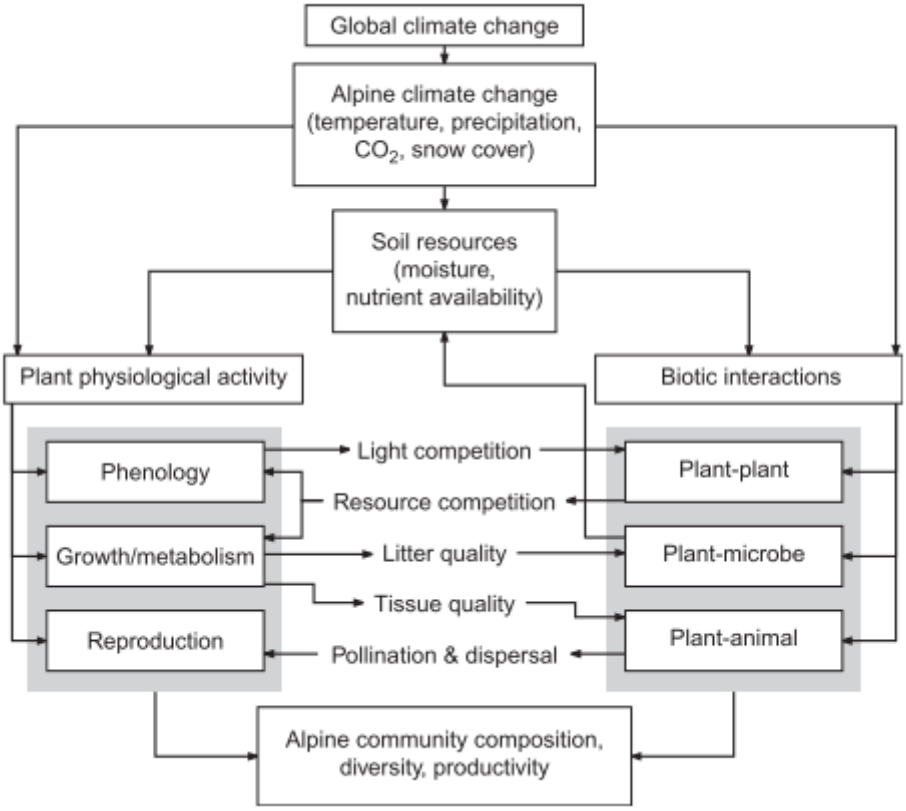


Fig. 1.1: Conceptual diagram of alpine ecosystem responses to climate change produced by Winkler et al. (2019).

Processes in alpine ecosystems are strongly dependent on abiotic factors currently being altered by climate change (see Section 1.1.1). As clearly shown in the conceptual diagram produced by Winkler et al. (2019) (**Fig. 1.1**), modifications in temperature, precipitation, CO_2 and snow cover can directly influence both plant physiological activity and biotic interactions, leading to changes in the composition and structure of the community, and productivity, which ultimately result in modifications of ecosystem functions, including carbon storage and sequestration, erosion regulation, forage production and food supply. For instance, warming can increase the rate of photosynthesis and growth, shift the timing at which diverse phenological stages occur − through anticipated snowmelt and longer growing seasons − and affect species reproductive output within a community. On the other hand, a reduction in precipitation can determine an

overall decrease in plant productivity, alter plant phenology – e.g. anticipated leaf senescence – and cause large changes in the functional and structural composition of plant communities (see Section 1.2). In addition, plant-plant and plant-animal interactions can be modified, regulated by the action of both climate drivers and physiological changes which, in turn, can alter soil microbiota, thus further affecting biogeochemical cycles. Finally, indirect effects can also derive from warming and/or precipitation-related changes of soil moisture and nutrient availability, which act concurrently on each component which regulates ecosystem functions. Such a complex network of interactions, together with the different time scale needed for each process to adjust to new conditions (Shaver et al., 2000), is at the basis of the multiplicity of responses to climate change observed across studies in alpine environments.

1.1.4 The effect of warming on alpine ecosystems

Over the last decades, as a result of global warming, alpine tundra has undergone a series of significant changes in terms of plant diversity, community structure and composition (Pauli et al., 2012; Sandvik and Odland, 2014; Amagai et al., 2018) and overall ecosystem functioning (Gavazov, 2010). In temperature-limited systems such as those found in alpine regions, it has generally been found that warming directly stimulates physiological growth processes, affecting growth and productivity (Jonas et al., 2008; Sandvik and Odland, 2014), richness and diversity (Elmendorf et al., 2012a; Gritsch et al., 2016) and phenology (Carbognani et al., 2016). As reported in the previous section (Section 1.1.3), these responses are the consequence of both direct and indirect effects of a temperature increase, with indirect effects being identified as the most important drivers of change. At the beginning of the 21st century, the conceptual framework provided by Shaver et al. (2000) illustrate that indirect effects would probably lead to even greater changes in ecosystem functions through alterations in the moisture regime, nutrient availability, growing season length and species composition. Studies in subsequent years have confirmed such predictions, especially in alpine ecosystems.

Alpine communities have been found to be strongly affected by the warming-induced reduction of snow cover and anticipation of snowmelt, with communities standing at each extreme of the snowmelt gradient being particularly sensitive (Wipf and Rixen, 2010). Through a change in snow dynamics, warming indirectly influences both the onset and length of the growing season, with the release of water and nutrients available for plants (Haselwandter et al., 1983; Fisk et al., 2001). As a consequence, advanced warming-induced snowmelt has been demonstrated to affect alpine species growth (Galen and Stanton, 1993; 1995; Wipf et al., 2006; 2009) and increase community richness and productivity along natural gradients (Schöb et al., 2009; Carbognani et al., 2012), leading to both short- and long-term shifts in species composition (Carbognani et al., 2014b; Sandvik and Odland, 2014; Amagai et al., 2018). At the same time, it

has also been proven that in cold biomes higher temperatures directly increase microbial community activity, leading to greater decomposition rates (Grogan and Chapin, 2000), increased mineralization (Chapin et al., 1995) and higher nitrogen availability (Melillo et al., 2002). It has also been noted that warming can indirectly impact the decomposition process through shifts in composition, structure and functionality of plant communities and soil biota (Cornelissen et al., 2007). Responses such as these have, ultimately, led to an alteration of carbon stocks and fluxes in both alpine and arctic regions (Virkkala et al., 2018).

The degree to which warming acts on ecosystem processes is, however, modulated by the identity of the species within a community and the level of water available within a particular site (precipitation and soil moisture), which could, therefore, drive ecosystem processes in high-elevation ecosystems differently from what generally observed.

1.1.5 Responses to warming are modulated by water availability

In cold environments, moisture regimes are recognised as modulators of community responses to higher summer temperatures (Walker et al., 2006; Winkler et al., 2016). The meta-analysis carried out by Elmendorf et al. (2012b), regarding warming experiments performed in alpine and arctic ecosystems in the Northern Hemisphere has found that site soil moisture levels partly explain the inconsistency of ecosystem responses across space and time. Another meta-analysis on decomposition rates recorded in warming experiments in cold biomes has also highlighted the important influence of moisture in regulating litter degradation dynamics by limiting decomposition when moisture levels are low (Aerts, 2006).

In alpine systems water is not recognised as a primary limiting factor during the summer, due to snow input and high levels of summer precipitation, but the interaction between precipitation and temperature has been identified as a driver of plant growth response during the growing season (Jonas et al., 2008). The productivity of alpine communities is enhanced either by warming or by an extended growing season length until water becomes a limited resource (Berdanier and Klein, 2011; Liu et al., 2018). In addition, plant functional types appear to respond differently when subjected to water limitations. In fact, results from the manipulation experiment carried out by Winkler et al. (2016) found that forbs were negatively affected by warming in the absence of supplementary watering, while graminoids still exhibited an increase in biomass in heated plots even when water was reduced.

Today, it has been clearly established that the climate on our planet is changing, with precipitation patterns being modified along with temperature increases (see “Preface” and Section 1.1.2). Over recent decades, however, the focus of ecological research on climate change in alpine ecosystems has been on the effects of rising temperature rather than on the impacts of the alteration of precipitation patterns. This is due to the difficulties in making accurate climate

predictions and in setting-up studies of this type (Beier et al., 2012). In the face of growing evidence suggesting the importance of water availability in modulating ecosystem and plant community responses to increasing temperatures (Liu et al., 2009; Zhao and Running, 2010; Elmendorf et al., 2012b; Winkler et al., 2016; Yang et al., 2020a), outcomes from experiments focusing on warming alone could, thus, result as incomplete, not capturing the actual complexity of environmental drivers influencing plant growth and ecosystem functions.

1.1.6 Drought in alpine ecosystems: preliminary research

A large number of studies have been carried out which focus on the effects of precipitation reduction in grassland ecosystems, as highlighted by recent reviews (Knapp et al., 2017a; Hoover et al., 2018). The analysis conducted by Hoover et al. (2018) showed how more than half of the experiments using rain shelters were carried out either in grasslands characterised by a mean annual precipitation below 1000 mm. The review by Knapp et al. (2017a), in particular, further revealed how less than 4% of >200 studies focused on tundra ecosystems, thus confirming what had previously been noted in other syntheses regarding precipitation experiments at the beginning of the last decade (Wu et al., 2011; Beier et al., 2012). Research on drought has, in fact, mostly been conducted in temperate grasslands characterised by a longer growing season when compared to the grassland under investigation in the present study (e.g. Grime et al., 2000; Bloor et al., 2010; Prechsl et al., 2015; Wilcox et al., 2015; de Vries et al., 2016; Wellstein et al., 2017; Ingrisch et al., 2018; Stampfli et al., 2018), whereas little research has been done in high-elevation environments. The reasons for such a limited number of studies are likely to be found in the specific climate features of alpine ecosystems, together with logistical issues related to the particular type of experiment. In many alpine regions, water supplies during the summer are guaranteed by snowmelt input and summer rainfall, which is especially abundant in mid-latitude mountain regions (Körner, 2003). A deficit in rainfall supply, therefore, has not been an issue in many alpine areas until recently. A tendency for ecologists to “focus on the resource or process that is most limiting in their ecosystem” has been identified by Hoover et al. (2018) as one of the reasons explaining the lack of experiments in areas with high levels of annual precipitation (>1000 mm). In addition, it is not only difficult to forecast future precipitation patterns but also technically challenging to set-up and maintain precipitation experiments in such harsh, remote environments (Wu et al., 2011; Beier et al., 2012; Knapp et al., 2017a; Hoover et al., 2018).

The studies that do exist, to a certain degree, all point to the fact that alpine ecosystems could be sensitive to changes in precipitation patterns. Early research carried out in the last century investigated the response of alpine species to water limitations along natural gradients (Oberbauer and Billings, 1982) and in controlled phytotron environment (Peterson and Billings, 1982), revealing a high level of heterogeneity in species sensitivity to drought. None of these

studies, however, directly investigated the effects of experimentally reduced precipitation in the field, so an upscaling of responses at an ecosystem level was lacking. More recently, manipulation experiments have managed to overcome the limitations of observational studies and laboratory-based analysis, allowing an improved understanding of ecosystem processes in the field. Precipitation experiments, which have only recently been carried out in high-elevation environments, have confirmed the vulnerability of alpine ecosystems to drought (e.g. Gilgen and Buchmann, 2009; De Boeck et al., 2016; Schmid, 2017; Zhang et al., 2017; De Boeck et al., 2018b). The number of studies focused on the impact of drought in these environments is still, however, limited and outcomes are controversial: a variety of different experimental approaches characterise these studies (Section 1.2.6) and some important ecological processes (e.g. decomposition) have not been sufficiently studied. In addition, other aspects of drought, such as timing, have, as far as is known, not yet been investigated in alpine grasslands, although studies in other herbaceous ecosystems have illustrated the crucial importance of the moment in which a dry spell occurs to ecosystem processes (Dietrich and Smith, 2016; Zeiter et al., 2016; Denton et al., 2017; Meng et al., 2019). Finally, further knowledge on biological thresholds is needed in order to fully understand if and how alpine ecosystems will be affected by drought (Beier et al., 2012; Estiarte et al., 2016; Knapp et al., 2017b).

1.2 THE COMPLEXITY OF DROUGHT: A SUMMARY

1.2.1 Plant physiological responses to drought and the concept of stress

Water is the key element determining plant strategies on land. It is essential for plant support and structure, nutrient uptake, transport and cooling processes (Lambers et al., 2008). The C-centred paradigm of plant growth (Körner, 2015) suggests that, when experiencing drought, plants put in place a series of complex physiological mechanisms which, ultimately, result in reduced carbon fixation (**Fig. 1.2**). In particular, reduced stomatal conductance leads to a decreased transpiration which, in turn, affects nutrient uptake and cooling, and reduces the rate of photosynthesis (Reddy et al., 2004). The results of these changes may range from anticipated leaf senescence, reduction in leaf area, dry matter accumulation, leaf expansion and tillering to an overall decrease in productivity and water use efficiency – i.e. the ability to fix carbon with a given amount of water (Farooq et al., 2012).

Mechanisms such as those described above take place when plants experience stress. However, in plant ecology the concept of stress is not straightforward, as Lichtenthaler (1996) clearly summarizes in his work on the stress concept in plants. The author provides Larcher's definition of stress (Larcher, 1987), in which the concept is recognised as being characterised by two phases: a first phase in which plants are able to carry out physiological adjustments and

improve their resistance after perturbation; and a second phase where their capacity to survive is limited once thresholds for adaptation are surpassed. Stress is defined by the author as "the state in which increasing demands made upon a plant lead to an initial destabilization of functions, followed by normalization and improved resistance. […] If the limits of tolerance are exceeded and the adaptive capacity is overworked, the result may be permanent damage or even death". Such a definition includes the constructive implications of stress (improved resistance) together with the destructive ones (plant mortality).

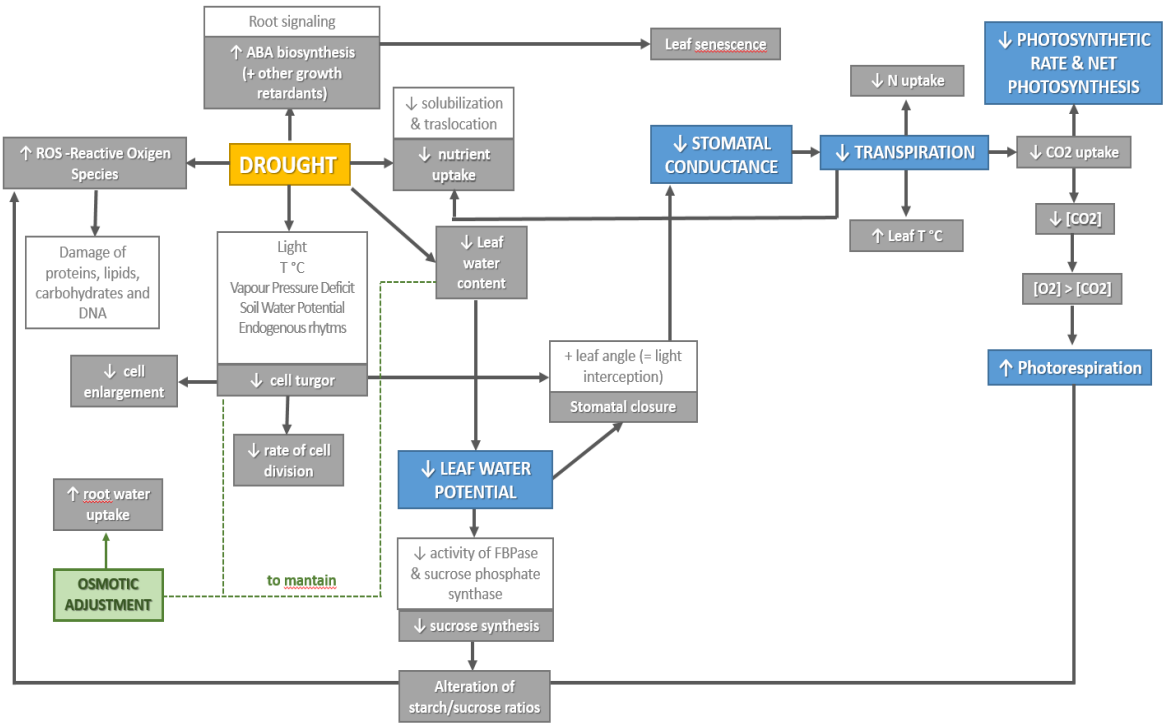


Fig. 1.2: Diagram illustrating main plant physiological responses under drought stress, summarizing mechanisms described in Farooq et al. (2012); Reddy et al. (2004); Lambers et al. (2008); Kirkham (2014); Körner (2003).

Lichtenthaler (1996) extends Larcher's definition to a "dose-dependent matter". Real stress ("dis-stress") – i.e. the set of conditions which have negative consequences on a plant development and survival – is reached when "a certain threshold of a stressor, which can no longer be compensated for by the plant, is exceeded". Dis-stress is anticipated by "eu-stress" – i.e. a mild stress which positively enhances plant physiological activity – with a fluid transition from one state to the other and the position of the threshold depending on both the nature of the stress factors and the plant's identity.

Such a concept of stress implies that (a) stress does not necessarily translate into constraints in plant development, given the ability of plants to readjust to changing environmental conditions, (b) limits exist and are dependent on the nature of the stress factor, its duration and/or intensity ("dose") and the organism's identity ("species"). These considerations underpin the framework of

this study, where the effects of drought depend on the characteristics of the drought imposed (rainfall amount; timing; duration of the dry period) in addition to the intrinsic features of the ecosystem (strong seasonality and community composition).

1.2.2 The "ability to readjust": drought resistance mechanisms

Plants can adopt a series of resistance mechanisms when undergoing drought stress, which include molecular, physiological and morphological adjustments (Farooq et al., 2012). Molecular adjustments include up and down regulation of various gene transcripts and accumulation of stress proteins. Physiological adjustment, on the other hand, includes increased concentration of plant growth substances (e.g. phytohormones), the employment of antioxidant defence systems and osmotic adjustments, which represent the main physiological tolerance mechanism. Osmotic adjustments consist in the accumulation of solutes − both organic and inorganic − which reduce water potential without decreasing water content. This mechanism is fundamental to maintain leaf turgor, improving both stomatal conductance and water uptake by roots. By enabling plants to avoid cell dehydration it, therefore, represents a common strategy shared by plants living in dry environments, or in cold habitats where freezing is the main dehydration risk.

In alpine species, in particular, high solute concentration in cells is common, enabling plants to inhabit cold, high-elevation habitats (Körner, 2003). These species could, therefore, already possess a degree of inherent drought resistance due to adaptation to freezing conditions, thus preventing damage due to excessive water loss. In this respect, an interesting hypothesis was tested for alpine species exposed to natural summer droughts in the high-Andes. Results showed that increased levels of resistance to freezing during the growing season occurred when plants were subject to regular dry conditions (Sierra-Almeida et al., 2016).

Together with physiological adjustments, plants can also adopt a number of strategies based on morphological adaptation, such as drought escape and drought avoidance. Drought escape allows plants to overcome dehydration risks by growing and reproducing in periods where conditions are favourable and is, therefore, typically adopted by species living in areas which experience regular dry periods. This applies to alpine species, where the particularly long snow-covered period characterising high-elevation environments has favoured flora which is mainly dominated by species with freezing avoidance strategies, which could also represent an advantage when coping with drought. Hemicryptophytes are, thus, the most abundant group to be found in alpine ecosystems: this category includes perennial forbs and graminoids, which are characterised by over-wintering buds located at or below the soil surface, and rhizomes and storage organs in the soil. As soon as conditions are favourable these species rapidly develop, completing their entire reproductive cycle (flowering and seed dispersal) in a few weeks after emergence (Körner, 2003).

With regard to drought avoidance, this allows plants to sustain high water level status, achieved through either improved water uptake or decreased water loss. Such a mechanism includes reduced biomass, a high root:shoot ratio, reduced leaf size, reduced stomata number and changed leaf orientation (vertical). Again, alpine species are characterised by a number of features allowing them to cope with water loss, including low stature growth forms (rosette and tussocks in grasslands), a high root: shoot ratio, small leaves which are furrowed or rolled, covered by waxes or having a thick cuticle and abundance of epidermal hairs (Bliss, 1962; Körner, 2003). Such features may guarantee a certain resistance on the part of alpine species when exposed to drought.

The ability to readjust, therefore, provides plants with the capacity to cope with stress to a certain degree. In addition, plants are not only able to acclimate in response to a continuous environmental pressure – through reversible changes of their phenotype or via irreversible morphological change – but they have been recently described has been able to carry out phenotypic adjustments following a past experience of an environmental pressure, hence improving their response to future stress (priming and ecological stress memory; Walter et al., 2011; Niu et al., 2014; Gessler et al., 2020). These capacities further define the degree to which not only individual plants or plants species, but also entire ecosystems, are stable under current and future climate extremes.

1.2.3 Scaling-up to ecosystems: resistance, recovery and resilience

From a functional perspective, the stress concept defined at a plant level can be translocated to a broader level, involving the whole ecosystem and its processes in response to drought. In this case, measures of resistance, recovery and resilience are employed to assess ecosystem stability to climate extremes (e.g. Grime et al., 2000; Lloret et al., 2011; Hoover et al., 2014; Ingrisch et al., 2018). These concepts describe respectively the “ability of individuals and ecosystems to persist and maintain their functioning during a disturbance” (resistance), the “capacity of individuals and ecosystems to return to the undisturbed ecosystem state and functioning following a disturbance” (recovery) and the “capacity of individuals and ecosystems to maintain their functioning in the face of disturbance” (resilience) (Gessler et al., 2020). Resilience, in particular, is determined by both resistance and recovery, with high resilience being the result of high resistance and/or high recovery capacity (Ingrisch and Bahn, 2018). Observational and experimental studies report a high degree of variability in resistance, recovery and resilience of ecological processes to climate extremes (Kreyling et al., 2008; Jentsch et al., 2011; De Boeck et al., 2016; De Boeck et al., 2018b) which is, once again, a result of differences in both ecosystem and stress factor attributes (Hoover et al., 2014). In terms of productivity, for instance, ecosystem stability in response to drought can be related to biodiversity levels (De Boeck et al., 2018a), to

biomass values (Wang et al., 2007), to the land-use (Ingrish et al., 2018) or to the successional stage of the community (Grime et al., 2000). The degree of resistance of a response is also determined by the press or pulse nature of drought (Hoover and Rogers, 2016; De Boeck et al., 2018a): while press events (chronic precipitation reduction) imply long-lasting adverse conditions, with resistance playing an important role due to potential acclimation responses, pulse and more intense drought episodes are more likely to exceed water stress thresholds, with drought resistance mechanisms being surpassed and limited adjustments and recovery being more significant in ecosystem stability (De Boeck et al., 2018a).

1.2.4 "Limits exist": the importance of thresholds

As previously discussed, both Larcher (1987) and Lichtenthaler (1996) recognize, in their definition of stress, that limit exists beyond which plants are seriously damaged, or even die – a "threshold" which depends on the type of stress factor and the nature of the organism experiencing the stress. Concerning ecosystem responses to extreme events, the surpassing of a resilience threshold which places an ecosystem "into an unusual or rare state" defines whether the event is extreme under a functional perspective, thus going beyond the statistical definition of a climate extreme (Smith, 2011b; Frank et al., 2015). For instance, as clearly illustrated by De Boeck et al. (2018a), although characterised by the same return time (statistical extreme), acute drought episodes (pulse) are more likely to exceed water stress thresholds than extended periods (press) of reduced precipitation (**Fig. 1.3**). Thus, these two types of extreme drought may differently impact the same ecosystem.

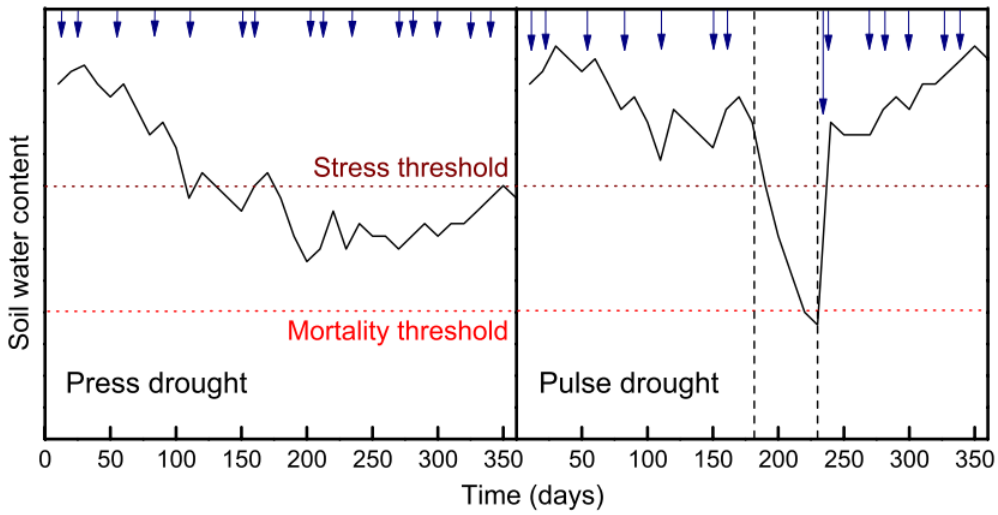


Fig. 1.3: Conceptual illustration by De Boeck et al. (2018a) of the probability of reaching hypothetical soil moisture stress and mortality thresholds under two types of drought (blue arrows): "press" drought (left-hand graph) is characterised by extended periods of precipitation reduction and slow reduction of soil water content, which may fall below the stress threshold, not reaching the mortality threshold; "pulse" drought (right-hand graph) is a short, acute dry spell determining a fast and strong reduction in soil water content, which may reach mortality thresholds.

Having said this, threshold identification in experimental studies is one of the arguments underlying a very interesting open-debate currently taking place in ecological research, which posits the question of whether experiments today should simulate realistic, site-specific climate change projections or push ecosystems beyond their limits (Korell et al., 2020; De Boeck et al., 2020). While a number of scientists advocate the setting-up of studies mimicking realistic climate change scenarios in order to gain a true understanding of ecosystem development in the future (Korell et al., 2020), other scientists maintain that, alongside other objectives, it is necessary for research to "push ecosystem beyond historical and forecast extremes" (De Boeck et al., 2020). The idea underlining this approach is that "the goal of most manipulation experiments is not direct extrapolation of the study's findings, but to improve mechanistic understanding and the representation of biological processes in models, that in turn can predict outcomes under a range of potential future climates" (De Boeck et al., 2020). In this context, knowledge of ecological thresholds may help, for instance, to detect non-linearites in relationships otherwise modelled as linear when including only nominal variability which excludes extremes (Estiarte et al., 2016). Such a consideration is based on evidence provided by Knapp et al. (2017b), who defined a new conceptual model of the total annual precipitation-aboveground net primary production relationship (PPT-ANPP) after data from experiments simulating extreme conditions were included (**Fig. 1.4**). Thresholds define the double asymmetry of the model: on one side showing a saturating response of ANPP in extremely wet years, while on the other a collapse of ANPP in extremely dry years is evident. Knapp et al. (2017b) underline the difficulties of finding such a relationship in temporal (single-site) PPT-ANPP models on account of the extreme variability of ANPP, even under similar PPT amounts, but also because in observational studies recording extreme years is problematic.

Experiments using gradients or testing for extremes are, therefore, necessary to improve our knowledge of biological processes so as to better forecast ecosystem responses to future climate change (Beier et al., 2012; Kreyling et al., 2014; Estiarte et al., 2016; Knapp et al., 2017b).

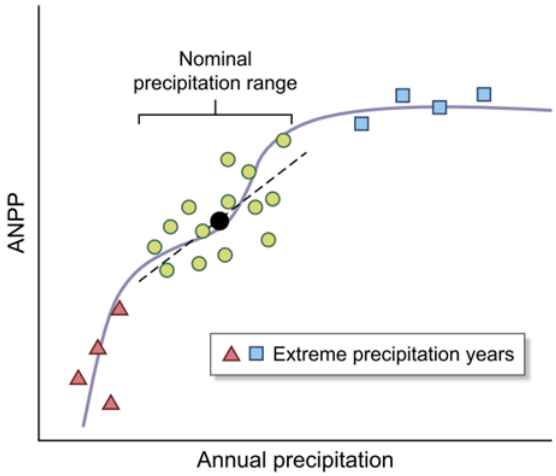


Fig. 1.4: Conceptual model of the PPT-ANPP relationship built by Knapp et al. (2017b) including data from extremely wet/dry years (blue rectangles and red triangles, respectively).

1.2.5 Manipulation experiments: a tool for studying climate change and climate extremes

The present project aims to assess the effects of warming and altered precipitation patterns in an alpine grassland. A manipulation experiment approach was, therefore, followed by artificially changing the relevant climate variables (temperature and precipitation) in order to isolate responses of key ecological processes such as primary production, decomposition and carbon dioxide fluxes. Experiments such as these represent an efficient tool for studying the effects of climate change and especially climate extremes in the natural context (Smith et al., 2011b; Beier et al., 2012), because both the nature and the duration of the climate variable can be controlled, ecological thresholds can be identified and a wide range of ecological responses can be assessed (Smith et al., 2011b). Manipulation experiments can, therefore, improve mechanistic understanding of processes in response to climate extremes (Sippel et al., 2018) and represent an effective complementary method for studying climate change effects by integrating results from observational studies. These studies consist in the analysis of long-term series data, quantifying the degree of change of a target variable from time *t* to *t+1*, which is then related to climate variables recorded within the study area. With regard to climate extremes, although these "opportunistic" observational studies are able to capture the full magnitude of an extreme event, they lack the chance of replication and the control of different aspects characterising the extreme (Smith et al., 2011b). Consequently, manipulation experiments allow researchers to (a) identify ecological responses to a particular climate variable or extreme; (b) control various key aspects of investigated climate extremes (intensity, timing, duration); (c) test interactions across different variables (e.g. drought and heat waves; freezing and drought); and (d) compare responses across

different ecosystems experiencing the same climate conditions – i.e. with the use of common experimental protocols.

Climate manipulation experiments have been widely employed. In tundra ecosystems, experiments have mainly been aimed at testing for snowmelt timing and warming (see Section 1.1.4) whereas, where altered precipitation patterns are concerned, most studies have focused on temperate grassland ecosystems rather than on strictly alpine environments − it is only in the last decade that field studies simulating climate extremes have been performed in alpine ecosystems (see Sections 1.1.6 and 1.2.6).

1.2.6 The multifaceted nature of drought

As stated earlier when considering plant and ecosystem responses to environmental pressure, the features characterising an extreme event are fundamental in driving the response of an ecosystem to precipitation alteration. A large number of precipitation experiments have been conducted worldwide in recent years to test the sensitivity of ecosystems to altered precipitation patterns, simulating different types of drought. The synthesis of Beier et al. (2012) shows the diversity of tested scenarios, including extreme events, precipitation variability and seasonality. Another review by Knapp et al. (2017a) summarizes results from 257 papers in which the type of manipulation carried out across studies is analysed (active or passive method) together with the degree of intensity applied (absolute, relative or matched). Zeppel et al. (2014) illustrate the variety of experiments altering precipitation patterns, focusing on the impacts of extreme precipitation and seasonal changes in precipitation. De Boeck et al. (2018a) shows how duration and nature of a drought (press vs pulse) could partially explain inconsistent biodiversity effects on ecosystem stability (**Fig. 1.3**). Such a variety of experiments and results highlights the multifaceted nature of drought, thus highlighting the necessity to specify the various aspects being analysed when assessing effects of “altered precipitation” (Zeppel et al., 2014). The scheme illustrated in **Fig. 1.5** attempts to summarise the main aspects defining the nature of the stress factor (precipitation alteration), indicating those which are relevant to the present research project.

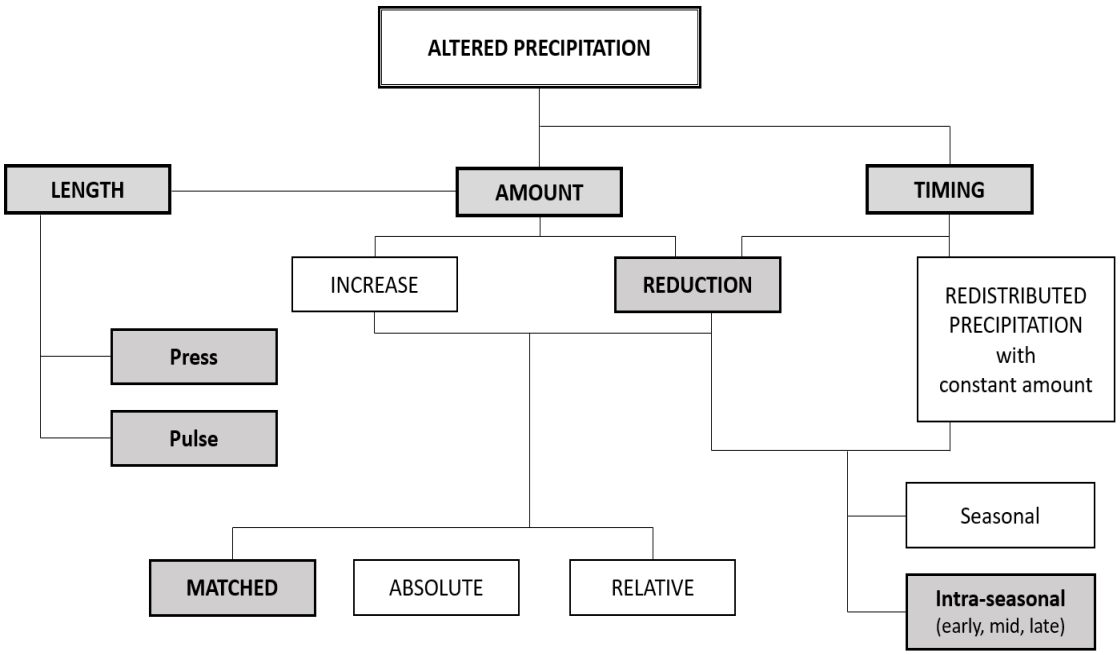


Fig. 1.5: Schematic diagram showing the different aspects which characterise altered precipitation patterns; bold, grey elements indicate precipitation attributes that have been considered in the present research project. Experiments altering precipitation patterns can modify the amount of precipitation and the time of its occurrence: there can be an increase or decrease of either an absolute amount (e.g. -200 mm), a relative quantity (e.g. -50%) or an amount matching historical records or future scenarios (Knapp et al., 2017a); timing can consist of either the redistribution of precipitation without modifying the total amount (Zeppel et al., 2014) or of the period in which a dry spell occurs which, at the same time, also reduces the total amount (e.g. Denton et al., 2017). Ecological responses to altered precipitation timing can be either tested across seasons (e.g. spring vs summer) or within different moments of the same season (early, mid and late summer). Depending on the length and the frequency of precipitation, an event can either be "press" – i.e. an extended period of chronic precipitation alteration – or "pulse" – i.e. a short, acute period of absence or marked increase of precipitation (Hoover and Rogers, 2016; De Boeck et al., 2018a).

In the present study, the following attributes of precipitation were considered: length, amount and timing. Two separate experiments were conducted: in one, a chronic, seasonal rainfall reduction (press) was imposed on the ecosystem in the field, decreasing the amount of rain during the summer by a percentage matching the site-specific extreme derived from historical climate records; in the other, a different intra-seasonal timing of a short, acute dry spell (pulse) was investigated by carrying out full rainfall exclusions in two different periods of the growing season (early and mid-season). This type of experiment was carried out under controlled conditions (mesocosm) and in the field, responding to the need of focusing on other aspects of precipitation alteration, rather than concentrating solely on the amount. Timing of precipitation events during the growing season (dry spells in this case) could, in fact, be crucial in determining the magnitude of drought impact on ecosystems processes (Jentsch et al., 2007; Knapp et al., 2008; Wu et al., 2011; Reichstein et al., 2013; Sippel et al., 2016; 2018).

1.3 RESEARCH OBJECTIVES

The main objective of the present research project is to widen the geographic and ecological extent of drought experiments in order to gain a better understanding of how drought sensitivity varies across different terrestrial ecosystems (Hoover et al., 2018). Specifically, the effects of increased summer temperatures and altered precipitation patterns were assessed on a typical alpine ecosystem (*Carex curvula* grassland), located in the Italian Rhaetian Alps (approx. 2700 m a.s.l.). Manipulation experiments were carried out both in the field and in the lab, the latter by performing a mesocosm experiment. In detail, rainfall alteration consisted of manipulations to test: (a) the effects of a site-specific, historically based precipitation reduction (in the field); and (b) the impact of dry spells occurring at different times during the growing season (in both lab and field). The lab experiment was carried out in order to detect critical thresholds for two key ecosystem processes: ecosystem respiration and gross ecosystem production. Investigations on the effects of altered climate variables were mainly carried out at an ecosystem level, with analysis focused on three main processes underlying energy flow and matter cycle: net primary production, litter decomposition and CO_2 fluxes.

Part of the research (Study I, Chapter 4; Study II, Chapter 5) was carried out within the framework of two international research networks: the International Drought Experiment (IDE), or Drought-Net, and the International Tundra Experiment (ITEX). Drought-Net aims to assess the differential sensitivity to drought of terrestrial ecosystems and identify the mechanisms underlying the patterns observed; long-term drought conditions are simulated by imposing a site-specific precipitation reduction, by means of precipitation shelters, according to historical climate records (Knapp et al., 2017b). Synthesis of results obtained through the Drought-Net network are only just becoming available (e.g. Henry et al., 2018). The ITEX network, on the other hand, investigates the impacts of warming in Arctic and alpine tundra using passive Open Top Chambers. Active since the 1990s, this network has gathered important evidence on the impact of increased temperatures in cold biomes, thanks to short- and long-term experiments carried out in 13 different countries (e.g. Elmendorf et al., 2012b).

Part of the present research, therefore, presents data which was collected according to shared protocols regarding the type of manipulation to be applied and the variables of interest to be measured. In this way, collected data will overcome limits due to the variety of methodologies employed to analyse a single variable when synthesising results on a global scale (Knapp et al., 2017a; Henry et al., 2018).

The current research consists of four studies, which are briefly presented below:

Study I (Chapter 4): The effects of two years (2018-2019) of artificial warming and drought on net primary production and plant cover were evaluated. Primary production, in particular, represents a key variable which could reveal the degree of resistance of an ecosystem to changing abiotic factors. The study assessed the extent to which estimated cover values of the most common *taxa* and aboveground net primary production at different levels of organization – total vascular species, plant functional types and species – were affected by treatments. Effects of manipulations on root responses and biomass allocation patterns, including total root production and variation across the vertical soil profile, were also investigated. Differences in primary production across years, including the pre-treatment year of 2017, were also analysed.

Study II (Chapter 5): Litter decomposition was analysed, assessing mass loss responses to a single-year (2019) summer warming and reduced precipitation treatments. The role seasonality plays in litter quantity and quality was also assessed to estimate the contribution that a long snow-covered season might make to the stability of decomposition processes under altered climate conditions during the summer. Both standard litter (teabags) and native litter were used: green and rooibos tea were employed to analyse the effects of altered temperature and precipitation on summer decomposition, whereas indigenous litter was employed to assess the natural variability of litter quality present in the investigated alpine grassland and verify the degree to which potential decomposition using teabags reflected the decomposition dynamics of indigenous litter.

Study III (Chapter 6): The role of drought timing – early- and mid-season drought – on the target alpine grassland was investigated in a mesocosm experiment on grassland monoliths collected in the field. In particular, the extent to which rates of CO_2 fluxes – i.e. net ecosystem exchange, gross ecosystem production and ecosystem respiration – were affected by dry spells occurring during different moments of the growing season was assessed. A further full-season drought treatment was simultaneously carried out to detect the soil moisture and temporal thresholds that trigger different rates of CO_2 emission and assimilation. Direct estimates of productivity were also gathered at the end of the study period to gain a more detailed picture of plant growth under diverse conditions.

Study IV (Chapter 7): The role of drought timing – early- and mid-season drought – on CO_2 fluxes of the target alpine grassland (already assessed in the lab, Study III) was investigated in a complementary field study carried out in summer 2020. Indirect estimates of productivity,

together with measurements of morphometric traits and chlorophyll fluorescence for the major species, were also obtained to gain a more detailed picture of plant growth under diverse treatments.

The study site and the investigated community, common to all the experiments, is illustrated in Chapter 2. The experiment shared by Study I and Study II is presented in Chapter 3, together with the relative climate data.

CHAPTER 2. STUDY SITE AND TARGET COMMUNITY

2.1 STUDY SITE

The research area is located in the Italian Rhaetian Alps, on an east-facing slope situated at approx. 2700 m a.s.l. in the high Gavia valley (Stelvio National Park), approx. one km from the Gavia Pass (46°21'13.79" N; 10°29'23.59" E) (**Fig. 2.1**). Cold winters and precipitation concentrated in the summer and autumn characterise the temperate-continental climate of the Ortles-Cevedale Massif (Albertini, 1955) which encompasses this area. At a more detailed spatial resolution (1 km), mean annual rainfall and mean annual temperature amount to 1150 mm and −1.4 °C, respectively (WorldClim data interpolation, Hijmans et al., 2005 in Carbognani et al., 2014a), with continuous snow-cover for most of the year and a consequent brief snow-free growing season of about 3-4 months (June-September/October). The present study is carried out in a typical sedge-dominated *Carex curvula* primary alpine grassland, which represents the climax vegetation within the alpine vegetation belt (Pignatti and Pignatti, 1958). The studied community develops on siliceous bedrock and Inceptisols represent the main soil type (Hiller et al., 2005; data for Swiss Central Alps), with observed depths in the experimental area reaching 20-30 cm. With regard to climatic, geological and geomorphological features a comprehensive description can be found in Carbognani (2011), Carbognani et al. (2014a) and Bernareggi (2016). General characteristics of the plant community, together with specific aspects pertaining to the study site are described in more detail below.



Fig. 2.1: Site location (on the left) and *Carex curvula* grassland within the study site (on the right).

2.2 TARGET COMMUNITY

2.2.1 *Carex curvula* grasslands: distribution and features

In the upper glacial valleys of European mountain ranges, *Carex curvula* grasslands represent one of the main landscape features. In the Alps, in particular, they are extremely diffuse, representing the typical grassland type within the alpine life zone (Reisigl and Keller, 1990; **Fig. 2.2**).

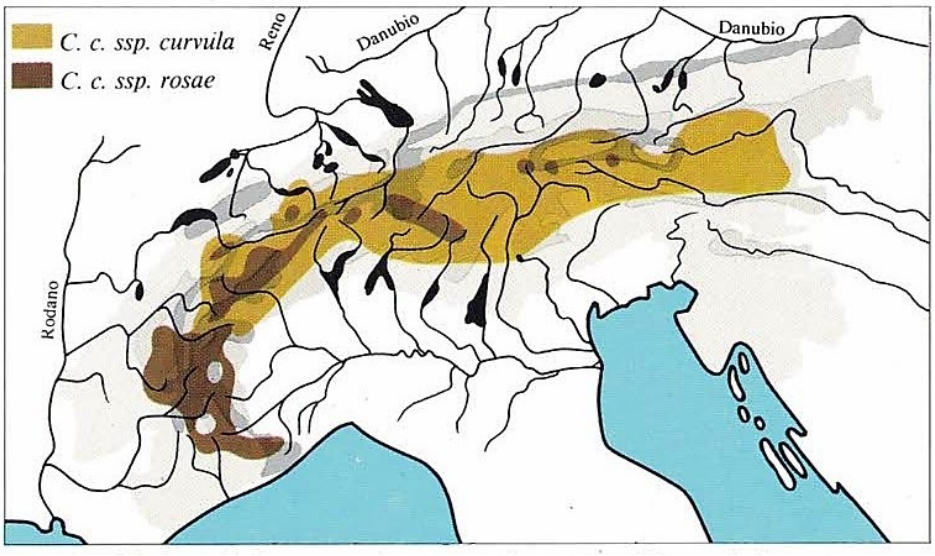


Fig. 2.2: Distribution of the two main subspecies characterising *Carex curvula* grasslands in the Alps, with *C. curvula ssp. curvula* and *C. curvula ssp. rosae* being present on siliceous and calcareous bedrocks, respectively (figure from Reisigl and Keller, 1990).

These herbaceous formations can be found in central Europe's main mountain chains (Pyrenees, Alps, Carpathian Mountains, Balkans) (Reisigl and Keller, 1990). They can extend over a wide area on moderately steep slopes and represent the alpine climax above 2400 m, growing on siliceous bedrocks and acidic soils with pH values amid 4.3 – 5 and where there is permanent winter snow cover (Grabherr, 1989; Reisigl and Keller, 1990; Wagner and Reichegger, 1997).

Carex curvula grasslands are easily identified by the presence of the sedge *Carex curvula* All., defined by Grabherr (1989) as the keystone species regulating grassland functions. This particular sedge dominates the community by forming dense, homogeneous mats [up to 4400 shoots m^{-2} (Erschbamer et al., 1994 in Busch, 2001)] which, on a micro-topographic scale, are interrupted in those areas where snow cover extremes limit its growth. In other words, *C. curvula* growth and development is hampered where snow cover is not continuous during the winter – i.e. on snow-free, wind-blown areas (such as ridges) – or where snow accumulates and determines extremely short growing seasons – i.e. in snowbeds (Reisigl and Keller, 1990; Wagner and Reichegger, 1997).

On account of the features characterising the dominant species, *Carex curvula* grasslands represent particularly stable plant communities. *Carex curvula* is, in fact, a slow-growing species, with low migration capacity due to a rate of horizontal expansion equal to 0.4 – 1 mm every year (Grabherr, 1989; Steinger et al., 1996). Both leaves and tussocks of this particular sedge are long-lived. Regarding the leaves, they have a life span of up to 3 years, as observed by Erschbamer et al. (1995), who described this species as an "evergreen overwintering", following the definition by Sørensen (1941); each sprout is, in fact, reported to live up to 10 years, bearing 4 leaves (2 new and 2 from the precedent season) enclosed in a sheath of dead leaves (Reisigl and Keller, 1990). Although long-lived, *Carex* leaves turn brown early during the growing season as a result of the action of a fungal infection by the ascomycete *Clathrospora elynae* Rabh. (Erschbamer et al., 1995). Such a phenomenon contributes to the formation of standing dead material, which in turn regulates the microclimate and may represent an effective windbreak, as suggested by Körner (2003). With regard to whole tussocks, de Witte et al. (2012) estimated a genet of *Carex curvula* to be 4100-5000 years old, whereas Steinger et al. (1996) estimation of a clone sampled in the central Alps was around 2000 years. The authors of this study also observed that this particular sedge can form quite large monoclonal patches, covering up to 1 m^2. Although clonal multiplication plays an important role in the biology of the species, sexual reproduction also occurs, with flowering taking place as soon as the snow melts (Wagner and Reichegger, 1997); having said this, however, germination of new individuals is reported to be an extremely rare event (Reisigl and Keller, 1990).

Among the few remaining *taxa* occurring together with *Carex curvula*, lichens also represent a consistent part of the aboveground living biomass, reaching up to 64% of total dry mass (Grabherr et al., 1978 in Grabherr, 1989). However, both abiotic conditions and biotic filtering through root competition contribute to the growth limitation of other species, as suggested by Grabherr (1989): root biomass represents a large fraction of the total biomass – i.e. the aboveground : belowground biomass ratio equals 1 : 18 – which is mainly produced by *C. curvula*, mostly located in the upper soil layers and consisting of material from preceding years.

2.2.2 The *Carex curvula* grassland in the Gavia Valley: field site description

The *Carex curvula* grassland investigated in the present research can be ascribed to the alliance *Caricion curvulae* Br.-Bl. in Br.-Bl. & Jenny 1926 (Biondi and Blasi, 2015). For a comprehensive description of the main features characterising the grassland in the Gavia Valley, the descriptive results derived from the pre-treatment survey conducted in 2017 on the 20 plots (1 m^2) of the warming and precipitation reduction experiment presented in Study I and Study II are hereby reported.

The pre-treatment survey revealed the presence of 26 vascular species in the areas selected for the experiment, 8 of which are present in all monitored plots, 4 are present in over half the plots and the remaining 14 species are unevenly distributed, being present in less than half of the surveyed areas (**Table 2.1**). Mosses and lichens are present in all plots: in particular, among mosses, *Polytrichum juniperinum* Hedw. is one of the most common species, while *Cetraria islandica* (L.) Ach. makes up most of the lichens observed. Few other vascular species with a scattered distribution can also be found in the study site, but were not recorded within the surveyed plots: *Nardus stricta* L.*, Geum montanum* L.*, Vaccinium gaultherioides* Bigelow and *Loiseleuria procumbens* (L.) Desv.

When grouping species on the basis of morphological and physiological similarities, in line with the concept of Plant Functional Types (PFTs) (e.g. Elmendorf et al., 2012a; Winkler et al., 2016), seven PFTs were identified within the grassland: sedges, grasses, forbs, shrubs, rushes, mosses and lichens. Although sedges, rushes and grasses could be further grouped into graminoids, in the present research the distinction will be maintained given their different responses to increased temperature and reduced precipitation reported in the literature (e.g. Klanderud, 2008; Liu et al., 2018).

As far as species richness is concerned, the mean number of vascular species per square meter in the target community is equal to 12.6 ± standard deviation (SD) 1.6, with a minimum and maximum number of 10 and 15 species per plot, respectively; half of plots have a species richness of 11-14 species.

Table 2.1: List of vascular species present in the target community, with relative ID code used as a reference for each species, plant functional type (PFT), % frequency (Freq) and % cover (mean ± standard deviation for frequency >1) within the 20 experimental 1 m^2 permanent plots; records refer to the 2017 pre-treatment survey. The list is reported according to a descending frequency and cover order. Species names are standardised following Pignatti (1982). The * highlights species that were found only in one plot.

ID	Species	PFT	Freq (%)	Cover (%)
CC	*Carex curvula* All.	Sedge	100	48.4 ± 9.1
AP	*Alchemilla pentaphyllea* L.	Forb	100	39.1 ± 16.1
PA	*Poa alpina* L.	Grass	100	2.5 ± 2.1
LH	*Leontodon helveticus* Mérat	Forb	100	2.4 ± 2.4
PH	*Phyteuma hemisphaericum* L.	Forb	100	2.1 ± 1.3
SP	*Soldanella pusilla* Baumg.	Forb	100	15.3 ± 9.1
LA	*Leucanthemopsis alpina* (L.) Heyw.	Forb	100	1.7 ± 2.1
AR	*Agrostis rupestris* All.	Grass	100	0.9 ± 1.1
EM	*Euphrasia minima* Jacq. ex DC.	Forb	85	0.1 ± 0.1
PG	*Primula glutinosa* Wulfen	Forb	65	0.8 ± 0.8
GS	*Gnaphalium supinum* L.	Forb	55	0.1 ± 0.1
SH	*Salix herbacea* L.	Shrub	50	1.3 ± 1.7
LM	*Ligusticum mutellina* (L.) Crantz	Forb	35	23.9 ± 11.6
OD	*Oreochloa disticha* (Wulfen) Link	Grass	30	2.5 ± 3.7
PV	*Polygonum viviparum* L.	Forb	30	0.6 ± 0.8
PAU	*Potentilla aurea* L.	Forb	25	2.0 ± 1.6
AV	*Avenula versicolor* (Vill.) Lainz	Grass	25	1.0 ± 1.2
FH	*Festuca halleri* All.	Grass	15	1.8 ± 1.9
HA	*Homogyne alpina* (L.) Cass	Forb	10	3.7 ± 4.6
TA	*Taraxacum alpinum* (Hoppe) Hegetschw.(aggr)	Forb	10	0.6 ± 0.5
SPR	*Sibbaldia procumbens* L.	Forb	10	0.2 ± 0
AA	*Anthoxanthum alpinum* Love et Love	Grass	10	0.1 ± 0.1
PC	*Phleum commutatum* Gaudin *	Grass	5	0.3
HP	*Hieracium piliferum* Hoppe *	Forb	5	0.2
PK	*Pedicularis kerneri* D. Torre non Huter *	Forb	5	0.2
LAP	*Luzula alpinopilosa* (Chaix) Breistr. *	Rush	5	0.1
MOSS	-	Mosses	100	8.5 ± 7.4
LICH	-	Lichens	100	8.7 ± 9.2

A redundancy analysis (RDA) was performed on species and non-vascular plant's cover (vascular species, mosses and lichens) to evaluate the degree of difference between blocks and treatments prior the beginning of experimental manipulations. The RDA indicated that the variation in cover was significantly explained by blocks ($F_{4,15}$= 2.8886, p=0.001), with distinct composition between the first two blocks (B1 and B2) and the rest (B3, B4 and B5), mainly driven by the presence of *Ligusticum mutellina* (LM) (**Fig. 2.3**). Composition within blocks, on the other hand, was quite homogeneous (with the exception of B1) and no initial difference across treatments emerged from analysis.

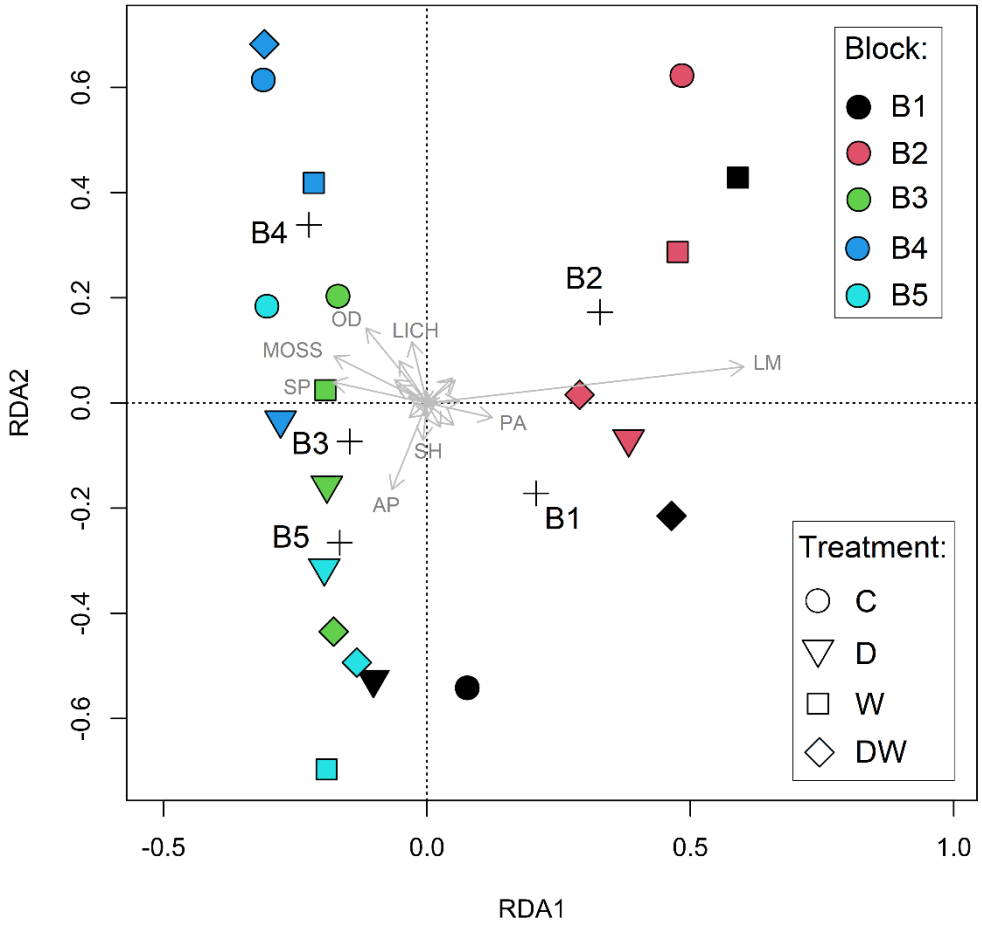


Fig. 2.3: RDA plot showing variations in cover among treatments and blocks during the pre-treatment year 2017; cover of vascular species, mosses and lichens was visually estimated at the peak of the growing season in areas then assigned to the four treatments (from 2018). Each treatment was replicated five times (B1-B5) following a random block design [treatments: control (C), drought (D), warming (W), drought + warming (DW)]. The first axis (RDA1) explains 20.49% of the variance in cover, while RDA2 explains 4.27%. For clarity, only the 8 *taxa* which vary the most across plots were reported (ID code of *taxa* defined in Table 1). Collinearity among predictors was checked with the variance inflation factor (VIF), with a cut-off value of 3; the significance of the model and of predictors was assessed through permutation tests; RDA was carried out with the R package vegan (Oksanen et al., 2019).

Together with frequency, visually estimated cover (%) (**Table 2.1**) and aboveground phytomass (hereafter, biomass) values (g m^{-2}) (**Fig. 2.4**) estimated on the 1 m^2 permanent plots in 2017 show how the community is mainly dominated by two species: the sedge *Carex curvula* (**Fig. 2.3**) and

the forb *Alchemilla pentaphyllea.* The former constitutes 48.4 ± SD 9.1% of the absolute total cover within the experimental plots and gives the largest contribution in terms of vascular plants biomass (43.6 ± SD 6.6%); the latter is the second species in order of contribution, with 39.2 ± SD 16.1% cover and 34.1 ± SD 9.1% biomass. Overall, these two species account for approx. 80% of the whole community biomass. Methods for cover and ANPP estimates are reported in Chapter 4 (Section 4.3.3).

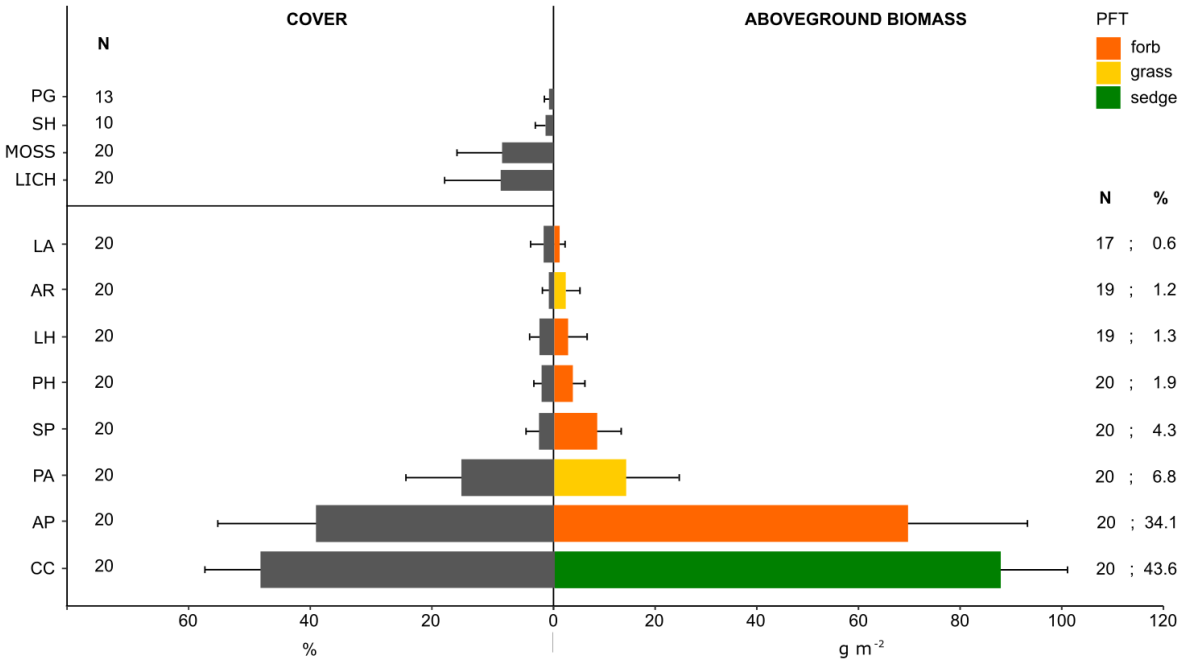


Fig. 2.4: Mean visually estimated cover (%, left panel) and aboveground biomass (g m^{-2}, right panel) of vascular plant species, mosses and lichens recorded in the 1 m^2 permanent plots during the pre-treatment year 2017. Error bars represent standard deviation and species ID is reported in Table 1. "N" stands for sample size from which estimates were obtained. The "%" on the right indicates the mean percentage each species represents of the total aboveground biomass. The relative plant functional type (PFT) for each species is shown. Data is reported according to an ascending order of biomass and only for those species/groups present in more than half of the sampled areas (≥ 10), with cover and phytomass values > 0.5%.

In general, average aboveground biomass production (taking into account only vascular species) is equal to 202.2 ± SD 23.3 g m^{-2} (N=20; **Fig. 2.5**), a similar value to that reported for other grasslands located in the Austrian Alps (Schäppi and Körner, 1996) or, from a wider perspective, to the primary production during a 105-128-day long growing season, quantified in Reisigl and Keller (1990) as 100-160 g m^{-2}.

When roots are taken into account, data shows that most of the biomass within the grassland is located belowground, with a mean value along the 0-30 cm vertical profile estimated to be equal to 783.6 ± SD 225.6 g m^{-2} (N=16; **Fig. 2.5**; root biomass estimation calculated from cores gathered within the plots in 2017; see Chapter 4, Section 4.3.4 for methodology followed). When the vertical profile is divided into three 10-cm sections (0-10 cm, 10-20 cm and 20-30 cm), data shows how the majority of roots (72.9 ± SD 7.2%) are to be found within the upper part, while the

mid and deeper soil profiles host 17.5 ± SD 6% and 9.6 ± SD 6% of the total root biomass, respectively.

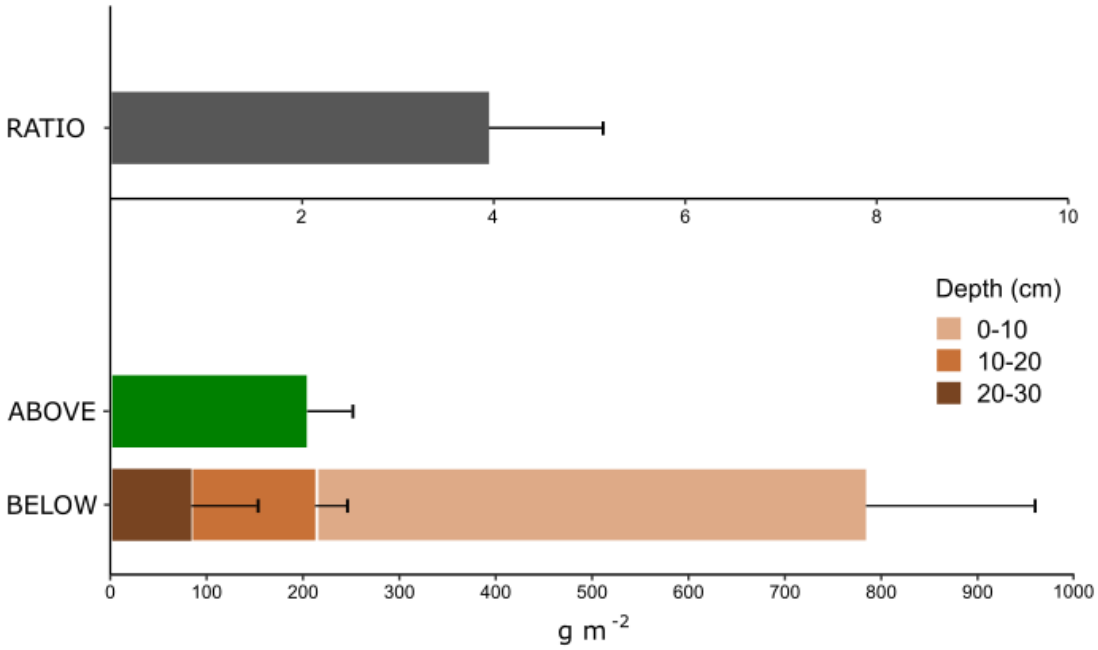


Fig. 2.5: The bottom panel illustrates the mean aboveground biomass (g m^{-2}) of vascular plant species (N=20) (above) and of roots in the 0-30 cm soil profile (N=16) (below) recorded in the 1 m^2 permanent plots during the pre-treatment year 2017. The average root biomass for each of the 3 investigated soil profiles (depth) is also shown. The upper panel indicates the mean root:shoot ratio obtained from belowground : aboveground biomass (N=16). Error bars represent standard deviation.

It needs to be specified, however, that soil depth distribution is not homogeneous across the grassland, since in a number of cases the bedrock prevents it reaching the 10-20 cm and 20-30 cm profile. For descriptive purposes, therefore, results of the actual distribution of belowground biomass have been corrected, for use in the present chapter, with a value obtained from soil depth essays carried out in each plot in 2017. In detail, the procedure consisted in inserting a metal pin into the soil in the area surrounding the plot for a definite number of times (between 3 and 7), noting down the number of times each of the three layers was reached. Subsequently, the ratio of the number of times where the soil depth was actually reached to the total number of depth essays within each plot was calculated. The value obtained was then applied to the final dry mass of roots relative to each plot and soil depth in order to account for the heterogeneity of soil depth within the study site. Such a correction, however, was not applied when analysing belowground production response to treatments (see Chapter 4, Section 4.3.4) so as to be able to test manipulation effects while avoiding the introduction of bias in the data. See **Table 2.2** reported below for major details on the 2017 belowground biomass data.

Table 2.2: Average root biomass (g m^{-2}) in 1 m^2 permanent plots during the pre-treatment year 2017 before (Pre) and after (Post) multiplying for correction value (Corr V, mean ± standard deviation) derived from soil depth essays carried out within plots. Values are reported for each soil profile (Depth) and for each future treatment (Trt), encoded for temperature (ambient =T, warming +T) and precipitation (ambient =P, reduction -P) manipulations carried out during experimental years 2018 and 2019. The standard deviation of root biomass is also reported (SD).

Depth (cm)	Trt	Temp	Prec	Corr V	Pre	Post	SD
0-10	C	=T	=P	1 ± 0	603		64
	D	=T	-P		624		197
	W	+T	=P		513		37
	DW	+T	-P		544		311
10-20	C	=T	=P	0.8 ± 1.4	137	115	40
	D	=T	-P	0.7 ± 0.2	181	131	31
	W	+T	=P	0.8 ± 1.8	194	151	58
	DW	+T	-P	0.8 ± 1.2	159	122	29
20-30	C	=T	=P	0.7 ± 0.3	135	105	77
	D	=T	-P	0.5 ± 0.2	215	119	66
	W	+T	=P	0.4 ± 0.2	172	68	77
	DW	+T	-P	0.5 ± 0.4	80	39	18

Finally, when the total biomass is taken into consideration, the mean root:shoot ratio (roots for 0-30 profile and shoot representing aboveground biomass of vascular species) is equal to 3.9 ± SD 1.2 (**Fig. 2.5**). When looking exclusively at the yearly production of fresh material, the ratio is reduced to approx. 1.5 on average (see Chapter 4, Section 4.4.5), confirming that most of the material belowground comes from old, standing roots characterised by slow turnover rates (see Chapter 5, Section 5.4.3).

Taken as a whole, these descriptive results indicate that the target grassland reflects assemblages typically found in humid, high-alpine conditions, where snow persistence is prolonged, leaving a 4-month snow-free growing period for plant development (Reisigl and Keller, 1990). This is confirmed by the presence of *Ligusticum mutellina*, *Primula glutinosa*, *Leucanthemopsis alpina, Gnaphalium supinum* and *Salix herbacea*, together with patches where mosses are particularly abundant. However, it needs to be clarified that, on a more detailed scale, these species are unevenly distributed (**Table 2.1**, **Fig. 2.3**), depending on topographically-driven snow persistence, wind exposure and, hence, the availability of water. Small tufts of *Oreochloa disticha* are present in drier patches, together with lichens which, in these grasslands, are usually found to be more abundant in wind-exposed areas (Reisigl and Keller, 1990), whereas *Ligusticum mutellina* is present on the areas of the site with higher soil moisture.

A further consideration relates to the contribution of *Carex curvula* and *Alchemilla pentaphyllea* within the community. The overall structure of the grassland could be shaped by the

specific traits of these two dominant species, strongly determining the degree of resistance and resilience to climate change of the ecosystem as a whole.

CHAPTER 3. EXPERIMENT DESIGN AND CLIMATE DATA FROM STUDY I AND STUDY II

The following chapter aims to present the experimental design characterising two studies carried out in the field (Study I in Chapter 4, Study II in Chapter 5). A short introductory paragraph will define drought as it is employed in this research project. There will then be a description of the experiment design, followed by a summary of microclimate data gathered from the experiment in both studies. This chapter does not include the experiment designs on drought timing carried out in controlled conditions (mesocosm; Study III) and in the field (Study IV). These are presented in each of the relevant chapters (see "Table of contents").

3.1 DEFINITION OF DROUGHT

The present study responds to the call for manipulation experiments designed to investigate effects on ecosystem functions as a result of the alteration of single environmental factors and/or a combination of these (Beier et al., 2012). Before illustrating the experiment design underlying the *in situ* studies presented in Chapter 4 and 5 of this thesis, the definition of "drought" employed in the present research needs to be clarified, since a variety of definitions are available in the literature which imply different meanings derived from the same term (Gilbert and Medina, 2016; Slette et al., 2019).

In the present research, drought is defined from the perspective of precipitation, by which a period of absence or marked rainfall deficiency is intended. In particular, the type of drought applied – or "reduced precipitation" – falls into the category "differs from normal" identified by Slette et al. (2019), where "drought is quantified in the context of site history and refers to conditions that differ from normal".

Water deficit imposed in the current experiment is equal to the statistically extreme deviation in annual precipitation relative to long-term records. This accords with the IDE protocol, based on the fact that perceived "extreme" climate conditions differ among ecosystems, each of which is characterised by unique historical climate variability (Knapp et al., 2015b; 2017a; Henry et al., 2018). Rain-shelters were, therefore, built so as to reduce rainfall amount during the growing season by a percentage based on the 1st percentile of a long-term climatic record, specific to the target site.

The extreme rainfall reduction calculated for the Gavia study site during the summer period (June-September) was equal to 43%, based on climate records gathered by two nearby weather stations, belonging to the *Provincia Autonoma di Trento, Dipartimento Protezione Civile e Infrastrutture, Servizio Prevenzione Rischi, Ufficio Previsioni e Pianificazione*: Careser Diga

(46°25'21.5'' N, 10°41'56.1'' E; 2600 m a.s.l.) and Passo Tonale (46°15'47.9'' N, 10°36'39.8'' E; 1780 m a.s.l.) (**Fig. 3.1**). Both stations are approx. 15 km from the study site and historical climate data for each station used to calculate precipitation reduction refers to the periods 1930-2017 (Careser Diga) and 1923-2004 (Passo Tonale). Calculations of extreme site-specific recipitation reduction were done using the "precipitation manipulation tool" provided by the IDE network (www.drought-net.colostate.edu) (**Fig. 3.2**).

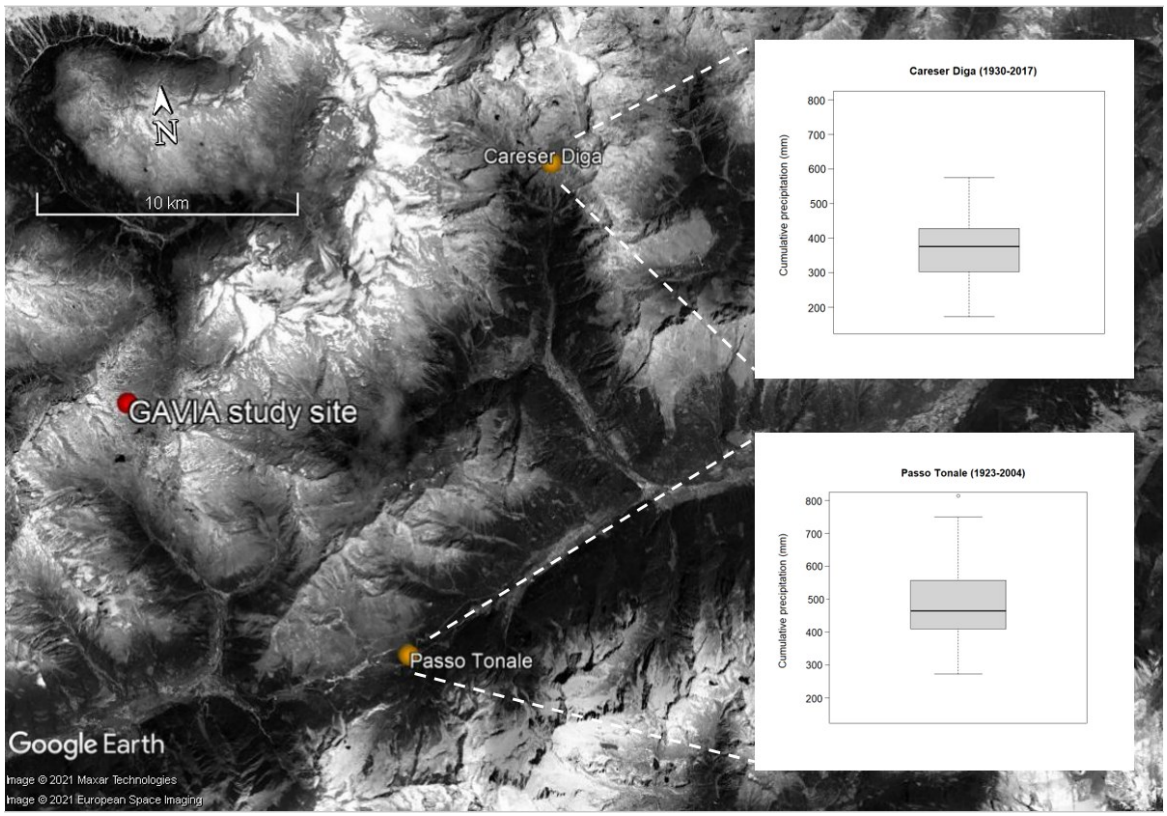


Fig. 3.1: Location of the two weather stations (Careser Diga and Passo Tonale) used for computing the 1st percentile site-specific precipitation extreme. The position of each station relative to the Gavia study site is indicated. On the left, the rainfall distribution during the growing season (June-September) recorded at each station and the period to which the time series refers are reported.

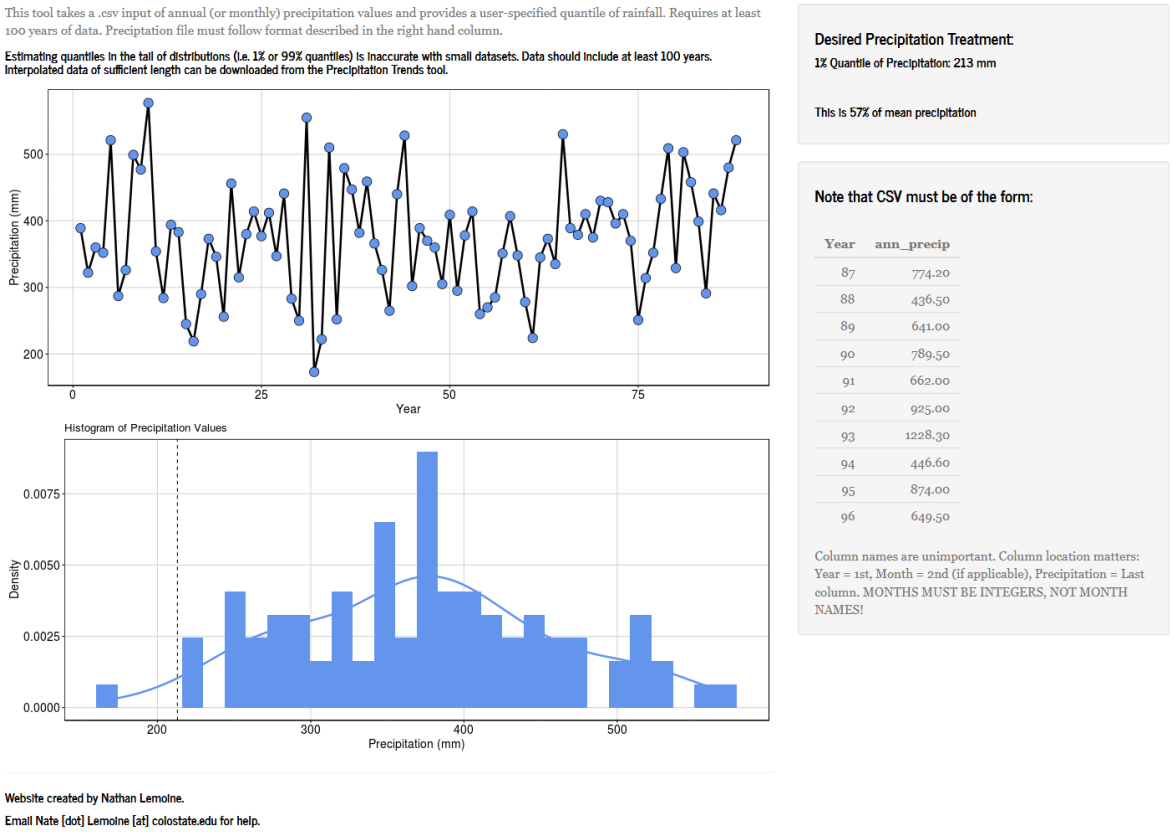


Fig. 3.2: Output of the "precipitation manipulation tool" provided by the IDE network (www.drought-net.colostate.edu) used to calculate 1st percentile of rainfall amount to define site-specific extreme precipitation reduction applied to the Gavia study site. Figure shows calculations using historical precipitation data (1930-2017; 88 years) for the growing season period (June-September) gathered by the Careser Diga weather station (2600 m a.s.l.).

3.2 EXPERIMENT DESIGN

In the present research, Study I (Chapter 4) and Study II (Chapter 5) present data deriving from a factorial experiment made up of 4 treatments, including a control (**Fig. 3.3**), applied to permanent plots of 1 m^2. The experiment sticks to a randomized complete block design, with five replicates for each treatment (**Fig. 3.4**).

The drought treatment (D, **Fig. 3.3**) was imposed by using 3.0 m × 3.5 m removable rain-out shelters placed over the permanent plots, which were situated in a central position to avoid margin effects. The structure was built in order to passively reduce each rainfall event by a fixed percentage (Yahdjian and Sala, 2002), consisting of aluminium frames sustaining ten rows of 3 mm thick, V-shaped, UV-transparent sheets of poly-methyl methacrylate (PMMA). Artefact effects on microclimate were minimized by placing the shelters at approx. 1 m above the surface of the canopy. Trenching, using plastic material, was put in position upstream of each drought treatment to prevent incoming water flow.

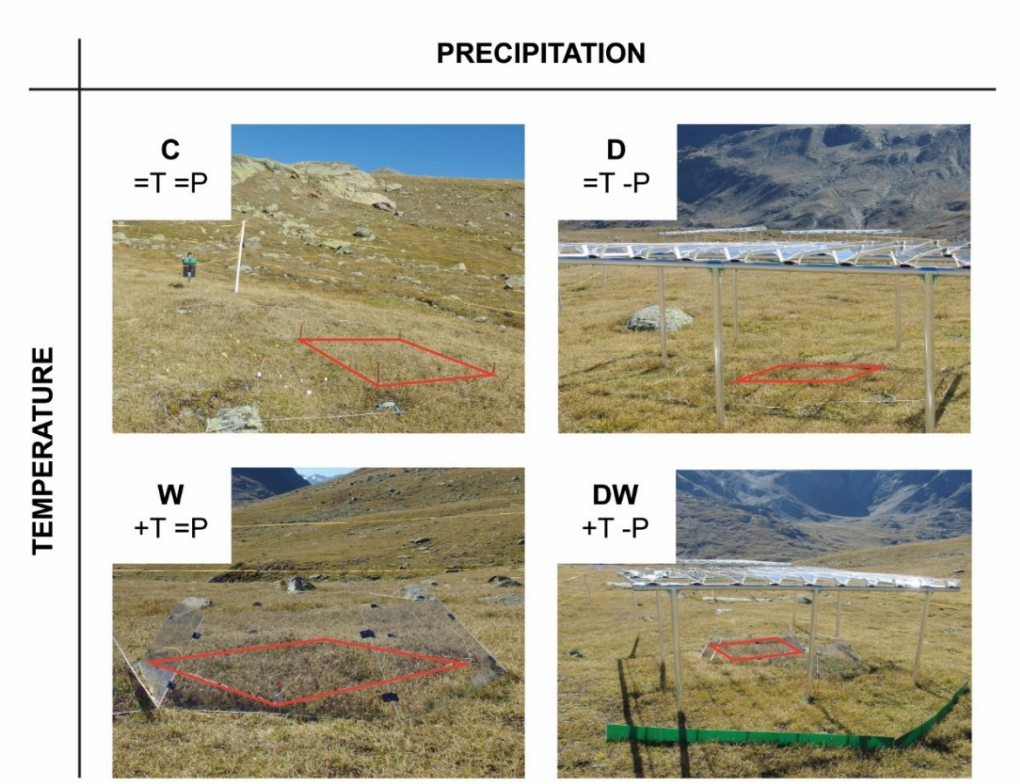


Fig. 3.3: Scheme showing the four manipulation treatments, including control, applied following a factorial approach with a single and combined manipulation of temperature and precipitation: C – Control, no experimental manipulation; D – Drought, reduction of ambient precipitation (-43%); W – Warming, increase in temperature (c. 1.5 K); DW – combination of D and W treatments. Red lines indicate the 1 m^2 permanent plots monitored during the study period.

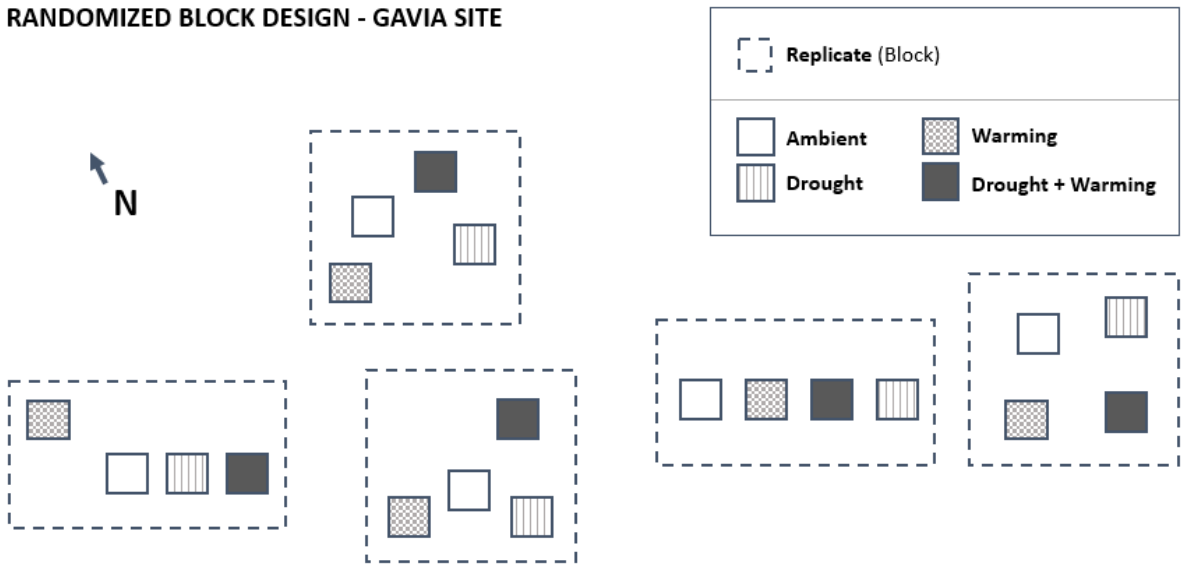


Fig. 3.4: Experiment set-up scheme, with the three climate treatments, together with controls, carried out following a randomized block design; the actual spatial distribution of blocks and plots is illustrated.

The warming treatment (W, **Fig. 3.3**) was simulated following the ITEX protocol (Henry and Molau, 1997). Hexagonal Open Top Chambers (OTCs), 34 cm high and made of 3 mm thick, UV transparent sheets of PMMA, were placed on top of the plot. OTCs transmit visible wavelengths, while blocking infrared ones (Marion et al., 1997), and are able to increase the soil and air temperature by an average of 1-3 K; the amount of solar radiation and its duration determines the magnitude of the warming effect (Bokhorst et al., 2013).

Precipitation reduction and increased temperature (DW, **Fig. 3.3**) were simulated concurrently by placing OTCs under the rain-out shelters. Trenching was also put in position for DW.

Rain-out shelters and OTCs were set up at the beginning of each growing season, after snowmelt in June, and removed at the end of the summer in September; the entire area was fenced during the experimental period to prevent cattle grazing at the site, which generally occurs in the first two weeks of August. The experiment set-up and length were, therefore, tied to the natural onset of the growing season, which differed over the two years of the present research: snowmelt in 2018 occurred on 5 June, and that of 2019 on 21 June. The second study year, therefore, was characterised by a 16-day delay in snowmelt compared to 2018 which, per ~100 day growing season (3 months), is a 16% reduction in the time available for alpine plants to complete their seasonal cycle. Time between snowmelt and site set-up, together with instrument installation, also differed between years (**Table 3.1**) for logistical reasons – i.e. road access to Gavia Pass – while removal of shelters, OTCs and instruments occurred at the end of September (25/09/2018 and 24/09/2019).

Table 3.1: Dates of snowmelt, beginning of treatments and instrument installation in 2018 and 2019.

	2018	**2019**
Snowmelt	5 June	21 June
Start treatments	15 June	01 July
Instrument installation	21-22 June	21 June - 06 July

3.3 MICROCLIMATE

3.3.1 Microclimate measurements

To evaluate treatment effects on microclimate and inter-annual variability in climate conditions, measurements of air temperature and relative humidity, and soil temperature and moisture were recorded hourly for each treatment (2 replicates) during each growing season. Canopy temperature (°C) and relative humidity (%) were obtained by placing sensors (EHT

Temperature/RH Sensor, Decagon Devices, Inc.) 5 cm above the soil surface. The resulting data allowed the subsequent calculation of mean daily vapour pressure deficit (VPD, kPa), following Jones (1992) − R package "plantecophys" (Duursma, 2015); mean daily temperature and mean daily relative humidity were used for VPD computation.

Soil temperature was recorded at -5 cm and -8 cm (HOBO Pendant® Temperature/Light 64K Data Logger, Part # UA-002-64) during the entire growing season for both years. In 2019, soil temperature measurements were also carried out at the soil surface level (0 cm) and at -15 cm to monitor microclimate in the decomposition experiment (Study II, Chapter 5).

Soil moisture was monitored hourly by measuring soil volumetric water content (VWC, %; WaterScout SM100, Spectrum Technologies, Inc.) using probes placed horizontally into the soil at -5 cm and -15 cm in two plots per treatment, and at -25 cm in two plots per the C and D treatments. To allow for the stabilisation of sensors in the surrounding soil, only data recorded in 2019 is reported. Prior to calculating the mean daily values for each sensor, missing hourly data due to logging failure (approx. 4% of the total) was interpolated by means of linear regression using data from sensors located in the same treatment and depth which showed the best fitting. Then, for each sensor, daily mean VWC values were calculated, based on hourly data, and averaged by treatment and depth. Finally, VWC values recorded in control plots at the beginning of the 2019 growing season – i.e. 9 days after snowmelt and first day of manipulations – were set as the baseline level for all treatments and depth (-5, -15 and -25 cm). Consequently, at a given depth, all treatments had the same initial value at the beginning of the season, assuming there were similar soil conditions due to recent snowmelt input (or field capacity) and no influence deriving from experimental manipulations.

To quantify shelter effects and performance, together with a site-specific climate characterisation, the following additional climate data was logged every hour, both under and outside the rain-out shelters, throughout the growing season: air temperature and relative humidity at approx. 20 cm above the ground (Spectrum WD1650); total precipitation (mm) (Rain Collector, Decagon Devices, Inc.); photosynthetic active photon flux density (PPFD, µmol m^{-2} s^{-1}); and solar radiation flux density (W m^{-2}) (Spectrum Technologies, Inc.). Unfortunately, due to malfunctioning of the site weather station, 2018 and 2019 values of precipitation outside the shelters, together with SR and PPFD (the latter lacking only in 2018) in ambient conditions derive from instruments installed in an experimental snowbed site situated approx. 1 km from the target grassland.

3.3.2 Effects of treatments on microclimate

In the present section, a comprehensive view of treatment effects on microclimate during the entire manipulation period in the two experimental growing seasons is presented. It should be

noted that treatment effects on climate are further reported in Study I and Study II. In Study I, however, data refers to only the 50 days preceding the point intercept date, to homogenise differences in climate across the 2 years due to differences in the length of the growing season and timing of instrument installation (**Table 3.1**). In the present section, the whole data is reported with a descriptive purpose (no statistical analysis), illustrating (a) shelter effects on precipitation, PPFD and SR and (b) treatment effects over the season in terms of air temperature and VPD, soil temperature and VWC.

Precipitation, PPFD and SR

As far as precipitation is concerned, data revealed a high rainfall frequency during the entire treatment periods, with 61% and 47% of total recorded days being rainy in 2018 and 2019, respectively, and an average of 3 consecutive rainy days in both experimental growing seasons (**Fig. 3.5**). Shelters effectively reduced precipitation by a percentage that varied between the two study years: in ambient conditions, the cumulative rainfall amount was 407 mm in 2018 and 347 mm in 2019, whereas underneath the shelters total rainfall was 239 mm (-41%) in 2018 and 232 mm (-33%) in 2019.

In both years, the precipitation reduction was just slightly above the 1st percentile of 213 mm computed using data from the highest weather station, Careser Diga (**Fig. 3.6**). Therefore, the drought treatment reduction roughly corresponded to the statistical extreme based on the site-specific historical record, although the data also revealed that the percentage reduction varied from one year to another. In particular, the percentage reduction recorded in the 2019 drought treatment was lower than that recorded the previous year. This might be the result of a mismatch between ambient and drought data gathered in different sites, or it might reflect the intrinsic characteristics of rainfall events: panels constituting the roof of each shelter may be less efficient under heavy rainfall, with the intercepted raindrops potentially bouncing within the space between each band, thus reaching the area under the shelter. In addition, strong wind during rainfall events could be a further factor explaining the less effective precipitation reduction recorded in 2019. Unfortunately, the malfunctioning of the site's weather station impeded gathering the data necessary to test such a hypothesis.

Shelters were found to have minimal effects on PPFD and SR. When considering PPFD, comparison of values measured within the same site (2019) showed that shelters reduced daily values by as much as 17% compared to ambient conditions, with mean daily values equal to 370 ± standard deviation (SD) 126 µmol m^{-2} s^{-1} under shelters vs 447 ± SD 149 µmol m^{-2} s^{-1} in ambient (**Fig. 3.5**). For SR, daily mean values recorded underneath the shelters were the same as those in ambient conditions in 2018 (203 ± SD 80 W m^{-2} under shelters vs 206 ± SD 73 W m^{-2} in ambient), while exhibiting reduced mean values (8%) in 2019 (190 ± SD 69 W m^{-2} vs 205 ± SD 79 W m^{-2})

(data not shown). It should be noted, however, that SR data was also characterised by a mismatch between ambient and drought data gathered in different sites in the same way as precipitation data (see Section 3.3.1).

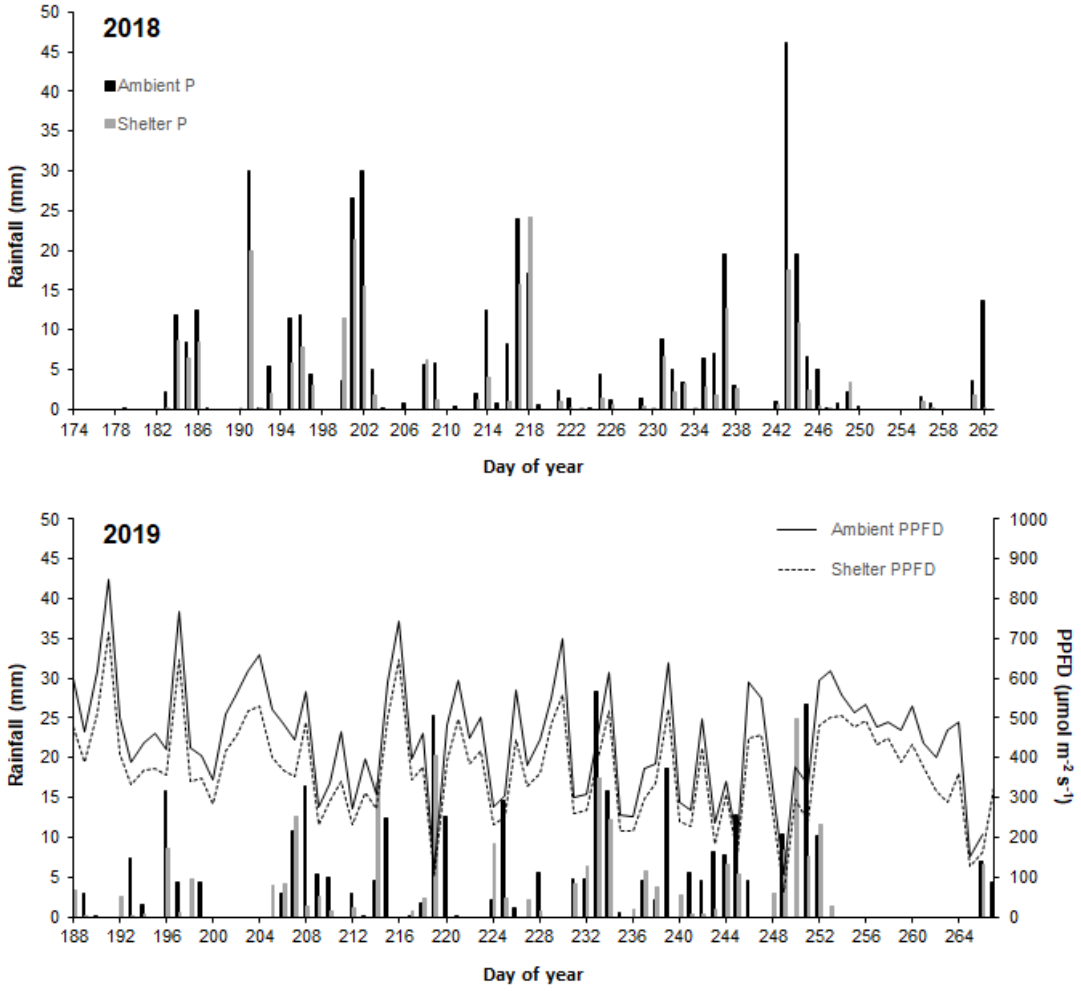


Fig. 3.5: Daily rainfall (P, mm) recorded in 2018 (23 June – 19 September) and 2019 (7 July – 24 September) in ambient conditions and under shelters. The daily average photosynthetic active photon flux density (PPDF, µmol m^{-2} s-1) is also reported for 2019. It should be noted that rainfall values for ambient conditions refer to records gathered at a nearby experimental site, located approx. 1 km from the target grassland.

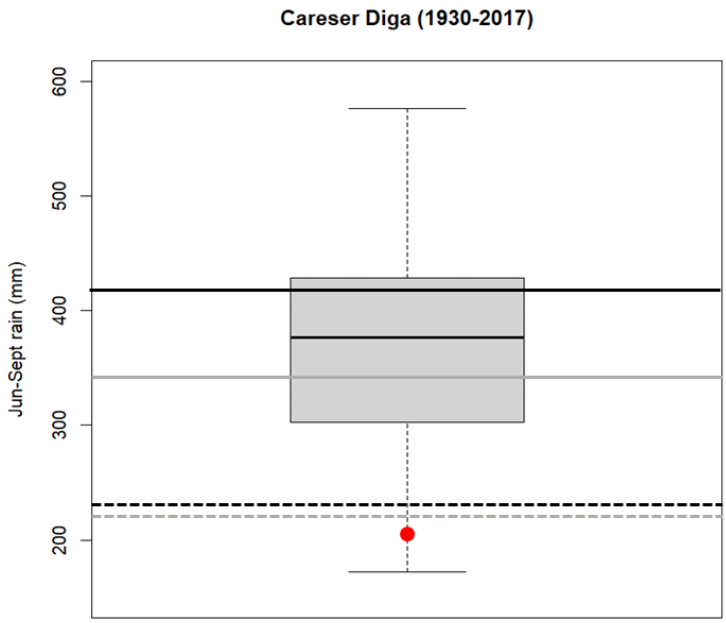


Fig. 3.6: Cumulative rainfall (mm) distribution for the June-September growing season recorded by the weather station Careser Diga, from which the historical data was used to compute the site-specific extreme precipitation reduction simulated within the experiment (88-year precipitation series, 1930-2017). The red point indicates the 1st percentile of the precipitation record; for the two treatment years 2018 (black lines) and 2019 (grey lines), full lines indicate ambient cumulative rainfall measured within the study site, while dashed lines refer to cumulative rainfall recorded under the shelters.

Air temperature and VPD

Precipitation manipulation did not alter either air temperatures above the canopy – measured at 20 cm from the soil surface – or VPD, both exhibiting similar mean daily values across the season, both under shelters and in ambient conditions.

As far as air temperature is concerned, in 2018 mean daily values above the canopy recorded in controls was equal to 8.0 ± SD 2.5 °C (vs 7.9 ± SD 2.5 °C in D), while in 2019 values were equal to 7.8 ± SD 3.0 °C (vs 7.6 ± SD 3.0 °C in D) (**Fig. 3.7**). At the canopy level (5 cm), average temperature was generally 2 K higher than that recorded at 20 cm for both years (**Fig. 3.7**). Furthermore, mean canopy temperatures differed across treatments, with the highest values recorded in OTCs. Under the single drought manipulation, temperature was 0.5 K higher than controls, whereas in W the increase ranged from 1.5 K (2018) to 1.7 K (2019) and was highest in DW, ranging from 1.7 K (2018) to 2.0 K (2019).

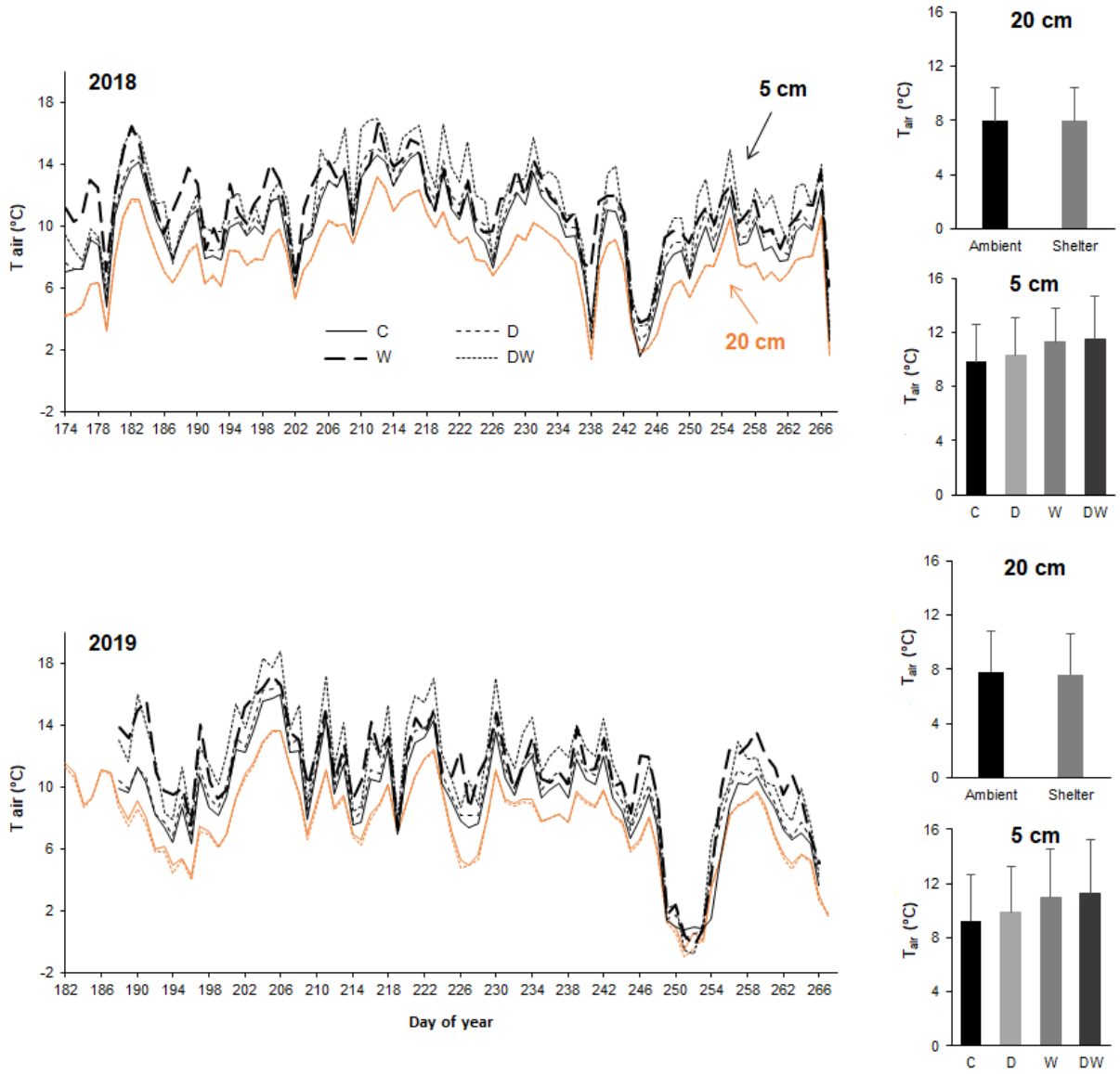


Fig. 3.7: Daily air temperatures (°C) recorded above the canopy (20 cm, orange) and at canopy level (5 cm, black) in 2018 (23 June – 24 September) and 2019 (1 July – 23 September at 20 cm; 7 July – 22 September at 5 cm). Temperatures at 20 cm refer to ambient conditions and shelters, whereas temperatures at 5 cm refer to the 4 treatments [C – control; D – drought; W – warming; DW – drought + warming]. Left-hand graphs illustrate daily values during the growing season. Right-hand graphs illustrate average daily values ± standard deviation over the entire season for each year.

In 2018, mean daily VPD measured above the canopy (20 cm) showed equivalent values outside and under the shelters, with mean daily values in both controls and shelters being equal to 0.43 ± SD 0.10 kPa (**Fig. 3.8**). In 2019, mean VPD under the shelters was slightly higher (0.44 ± SD 0.11 kPa) compared to controls (0.37 ± SD 0.11 kPa), but such a difference was already evident in the period between instrument installation and shelter set-up (data not shown) and, therefore, cannot be attributed to the rain-out structure. At the canopy level, VPD across treatments was slightly higher under shelters in both experimental years, with the highest values in DW during the entire season (**Fig. 3.8**). The difference, however, was very small when average values were considered, with VPD in DW being 0.30 ± SD 0.11 kPa (2018) and 0.31 ± SD 0.14 kPa (2019) in

comparison with average VPD in C at 0.25 ± SD 0.10 kPa (2018) and 0.26 ± SD 0.11 kPa (2019). In both study years, air dryness at the canopy level was lower compared to that recorded above, which could be due to the modulating effects of the dense mat produced by *Carex curvula* on microclimate, which may prevent excessive water loss (Körner, 2003).

Soil temperature

When soil temperature at the soil surface level (0 cm) is considered, average daily values measured in controls were 0.8 K higher than those recorded at the canopy level in 2019 (**Fig. 3.9**). Differences in soil temperature across treatments were similar to those observed for canopy air temperature, although the difference compared to C was greater, with a higher average daily value in D (+1.2 K), in W (+3.4 K) and DW (+4.0 K) compared to that recorded in C (10.0 ± SD 3.2 °C). Belowground, at -5 cm, there was less difference across treatments. Average soil temperatures in D plots were 0.4 K and 1 K higher in 2018 and 2019, respectively, compared to those recorded in C (10.6 ± SD 1.7 °C in 2018 and 10.8 ± SD 1.6 °C in 2019). With regard to the warming treatments, OTCs alone increased soil temperature at -5 cm by 1.1 K in 2018 and 1.2 K in 2019 compared to C, while in combination with drought the increase was equal to 1.0 K in 2018 and 1.7 K in 2019. Average soil temperatures measured at -8 cm in controls across the season were similar to those recorded at -5 cm for both years. At -15 cm, the deepest layer investigated, in 2019 average soil temperatures were by 0.2 K lower compared to -5 cm in controls (9.4 ± SD 1.9 °C) and the DW treatment was the warmest, being on average 1 K higher compared to C.

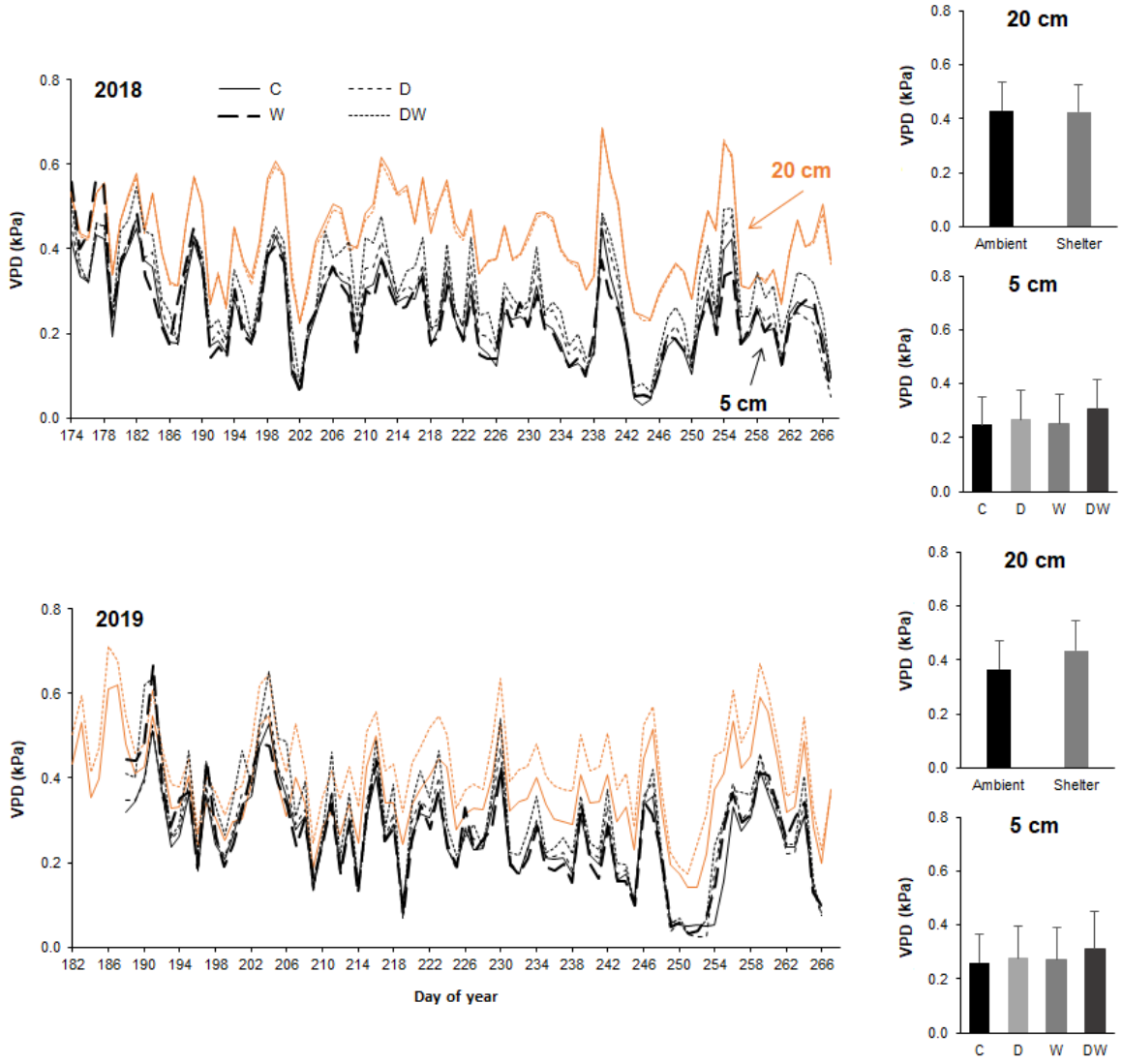


Fig. 3.8: Daily vapour pressure deficit (VPD, kPa) recorded above the canopy (20 cm, orange) and at canopy level (5 cm, black) in 2018 (23 June – 24 September) and 2019 (01 July – 23 September at 20 cm; 7 July – 22 September at 5 cm). Temperatures at 20 cm refer to ambient conditions and shelters, whereas temperatures at 5 cm refer to the 4 treatments [C – control; D – drought; W – warming; DW – drought + warming]. Left-hand graphs illustrate daily values during the growing season. Right-hand graphs illustrate average daily values ± standard deviation over the entire season for each year.

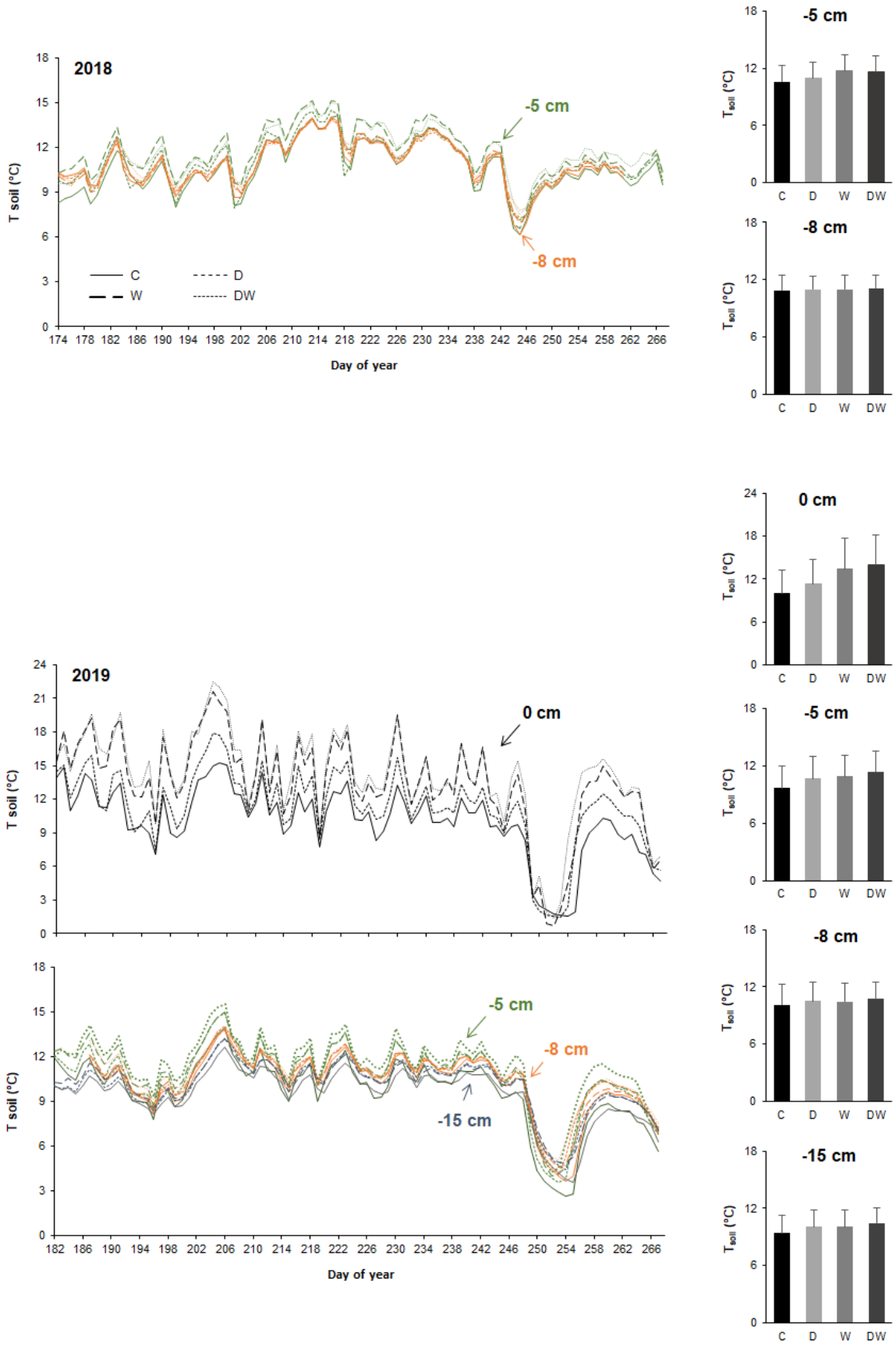


Fig. 3.9: Daily soil temperature (°C) recorded in the 4 treatments [C – control; D – drought; W – warming; DW – drought + warming]: in 2018 at -5 cm (23 June – 24 September) and -8 cm (23 June – 19 September); and in 2019 at 0 cm (soil level), -5 cm, -15 cm (01 July – 24 September) and -8 cm (6 June – 24 September). Left-hand graphs illustrate daily values during the growing season. Right-hand graphs illustrate and each depth average daily values ± standard deviation over the entire season for each year.

Soil moisture

With regard to soil moisture (VWC), trends were consistent with precipitation events within the site, with the largest differences across treatments in the upper soil layer (-5 cm) (**Fig. 3.10**). Data revealed that values were slightly reduced under the precipitation treatment, and that the difference was more evident in the upper layers, while gradually reducing as soil levels became deeper. In particular, daily VWC recorded at -5 cm in D and DW were on average 3% and 4% lower than those recorded in C, respectively. The single warming treatment also exhibited 4% lower VWC compared to C, which could be due to an OTC side effect reported in studies in alpine and polar sites (Bokhorst et al., 2013).

Regarding VWC at greater depths (-15 and -25 cm), values were similar between treatments. Moreover, the deeper the soil, the lower the VWC, with -15 cm and -25 cm showing on average 3% and 10% less VWC compared to the near-surface soil layer, with mean values for each depth equal to 29 ± SD 2% (-5 cm), 26 ± SD 2% (-15 cm) and 19 ± SD 2% (-25 cm).

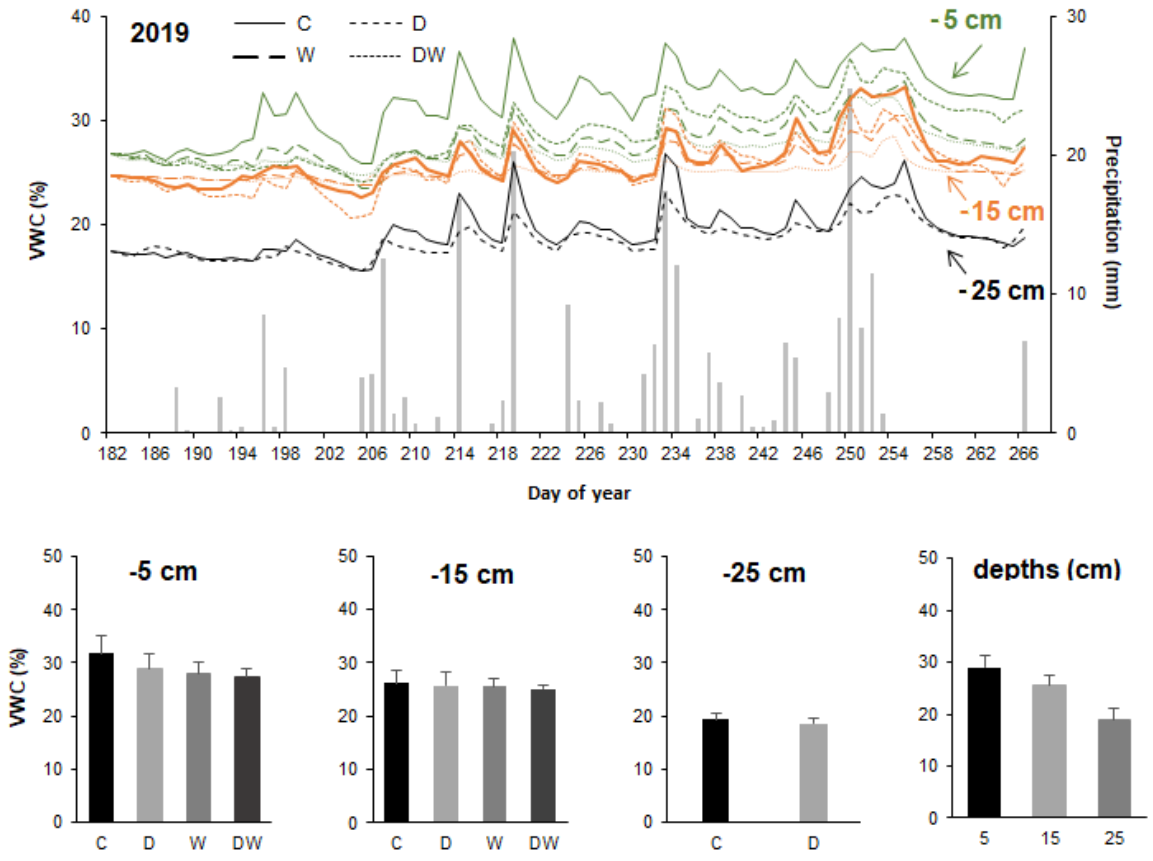


Fig. 3.10: Daily volumetric water content (VWC, %) recorded at three depths (-5, -15 and -25 cm) in 2019 (01 July – 23 September) for each treatment [C – control; D – drought; W – warming; DW – drought + warming]. Top graph illustrates daily values during the growing season together with precipitation (mm) recorded under the shelter. The following 4 graphs illustrate average daily values ± standard deviation for each depth and each treatment over the entire season; average values across the three depths are also reported.

When assessing overall shelter effects on VWC, including both D and DW, data showed that the reduction compared to controls was greater in the near-surface layer (-5 cm) compared to the next deepest one (-15 cm) and that VWC fluctuations were higher, with SD 98% at -5 cm and SD 0.69% at -15 cm (**Fig. 3.11**, top graph). It appeared, therefore, that under reduced precipitation VWC was more stable in the deeper layer, with fluctuations observed at -5 cm probably the result of a more intense drying and re-wetting cycle. On the other hand, when assessing the effect of OTCs compared to controls on VWC, there were no differences in reduction and fluctuations compared to controls, with mean SD at -5 cm and -15 cm equal to 1.41% and 1.35%, respectively (**Fig. 3.11**, bottom graph). In this case, the high VWC fluctuations observed in both soil layers under OTCs could be attributed to the interplay between lower and higher evapotranspiration resulting from two contrasting chamber effects: collateral wind reduction may reduce water loss and increase VWC, whereas higher temperatures could increase plant water demand, hence reducing VWC.

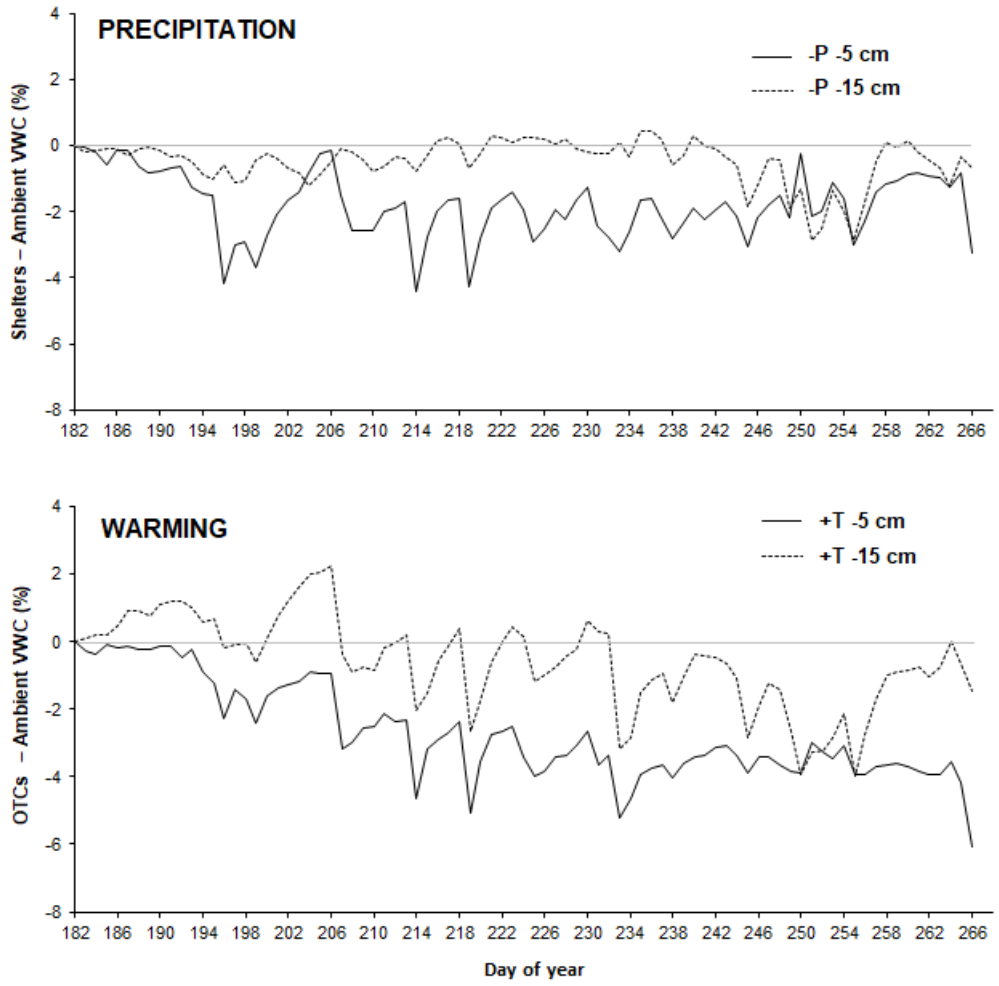


Fig. 3.11: Differences in soil volumetric water content (VWC, %) recorded at -5 cm and -15 cm depth during 2019 growing season. Top graph shows the mean daily shelter effect, -P [mean VWC in D and DW minus mean VWC in C and W (ambient rainfall)]. Bottom graph indicates the mean daily OTCs effect, +T [mean VWC in W and DW minus mean VWC in C and D (no OTCs)].

Differences in soil moisture under shelters could explain the higher daily average canopy temperatures and VPD, with lower VWC potentially causing an increase in temperature as a result of the reduction of the cooling action of water evapotranspiration when soils dry (Al-Kayssi et al., 1990). It should be noted, however, that obtaining a precise picture of soil water status is an extremely difficult task. This is mainly due to high spatial heterogeneity and temporal dynamics, which are, in turn, dependent on a number of factors, including soil type and structure, and the identity and performance of plants (Kreyling et al., 2017 and references therein). For instance, even within the same site the presence of a large proportion of stones in the measured volume of soil may reduce values irrespective of treatments, a bias which becomes even greater when measurements refer to a single depth, such as those presented above. Moreover, data on the topsoil layer – i.e. above 5 cm depth – is not gathered, so the effects of treatments on VWC in the first few cm of soil just below the surface might, in fact, be greater than those estimated on deeper soil layers.

This hypothesis was later confirmed in 2020 when checking soil moisture data collected for the 0-3.8 cm and 0-7.6 cm soil layer, which were measured with a portable instrument (TDR 100; Spectrum Technologies Inc., Chicago, IL, USA). Measurements were carried out on the same plots of the experiment presented in the current chapter on 5 different dates during the experimental period, with only 3 dates including both soil layers. During each sampling date, four records for each soil layer per plot were gathered and averaged to obtain the mean daily VWC for the relative plot.

On examination of the data, a greater difference between treatments could be observed in the 0-3.8 cm layer compared to values recorded in the 0-7.6 cm profile (**Fig. 3.12**). Considering all measurement dates, in this first layer the difference in VWC between D and C ranged from -4% to +1%, whereas for W it ranged from -11% to -4% and for DW from -10% to -1%. On the other hand, when considering differences in the 0-7.6 cm layer, differences between treatments and controls ranged from -2% to -4% (D), -8% to -4% (W) and -6% to -4% (DW). Soil moisture fluctuations were higher in the near-surface layer, as highlighted by measurements on 4 September (**Fig. 3.12**): on this date, VWC was highest in the near-surface layer, as a result of the intense rainfall events which had occurred in the preceding 7 days, with 124 mm of rain falling within the site. The VWC pattern on this date was the opposite of that observed on the other two measurement dates (26 August and 15 September) where the highest VWC was found at the 0-7.6 cm depth. These contrasting patterns highlight the intensity of drying and re-wetting cycles characterising the near-surface layer and means that this layer could undergo more severe water reduction when precipitation is reduced or a dry spell occurs.

This data, therefore, suggests that to fully assess soil moisture status and potential treatment effects, further measurements of the entire soil profile should be made, rather than limiting them

to a single depth – e.g. VWC in 0-5 cm layer instead of VWC at -5 cm. Furthermore, to overcome problematic issues related to the high spatial heterogeneity of soil texture in the field, recording data within a homogeneous matrix would be preferable to better assess treatment effects.

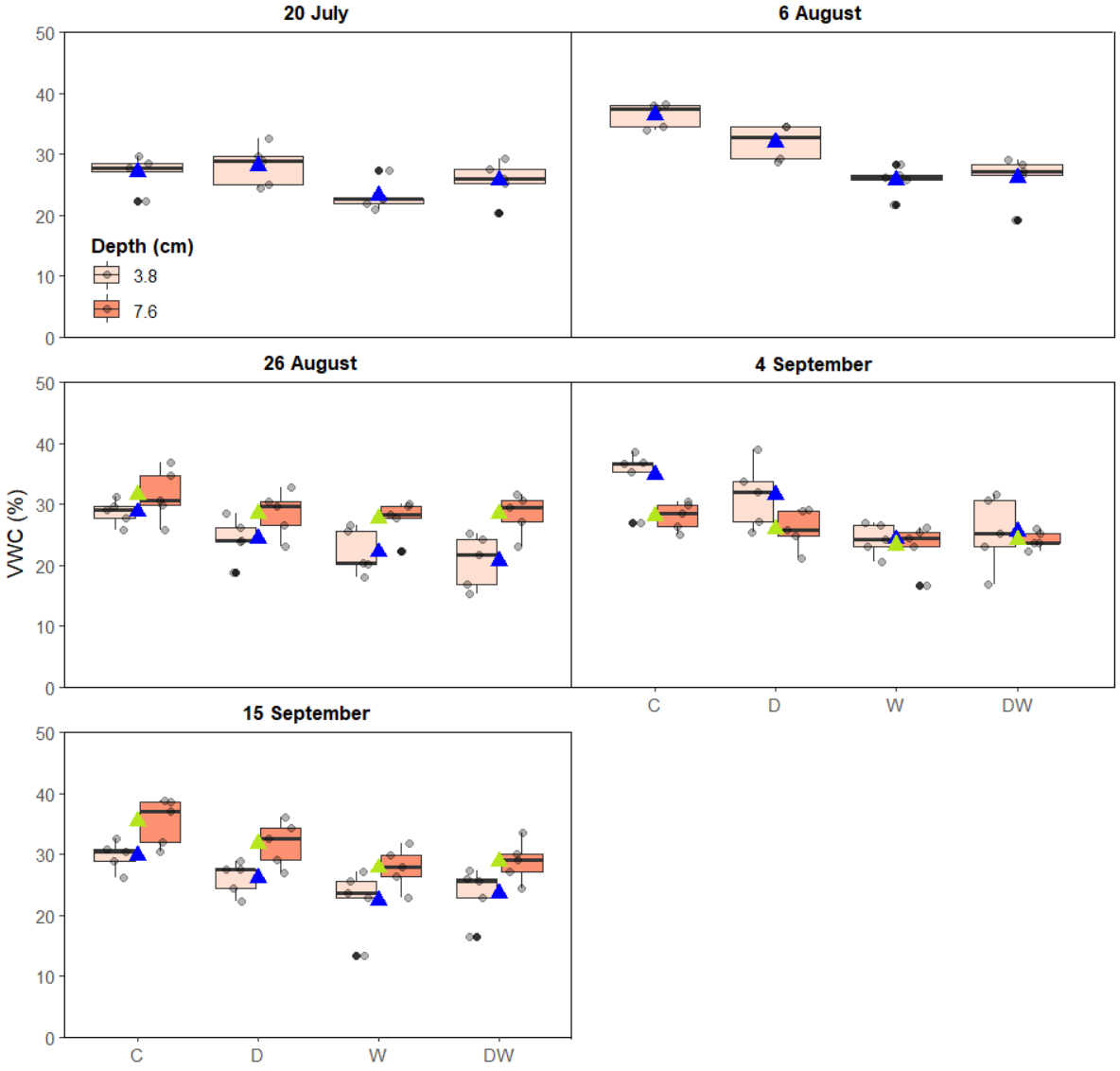


Fig. 3.12: Volumetric water content (VWC, %) measured by means of a portable instrument for the 0-3.8 cm and 0-7.6 cm soil layer; data recorded on 5 dates in 2020 in each of the 4 treatments [C – control; D – drought; W – warming; DW – drought + warming]. Values for layer 0-7.6 cm are missing for the first two dates. Triangles indicate the mean value for each soil layer (0-3.8 cm, blue; 0-7.6 cm, green). For each boxplot, dots represent single mean values measured within each plot for each treatment.

CHAPTER 4. STUDY I – SUMMER WARMING AND PRECIPITATION REDUCTION: EFFECTS ON NET PRIMARY PRODUCTION AND PLANT COVER OF AN ALPINE GRASSLAND

4.1 ABSTRACT

Alpine ecosystems are particularly sensitive to climate change. Although warming effects have been largely assessed, responses to reduced precipitation in the alpine zone still remain under-investigated. In the present study, a 2-year warming and precipitation reduction (c. -40%) experiment, using Open Top Chambers (OTCs) and shelters, was carried out in a *Carex curvula* grassland located in the Italian Alps to assess the effects of climate variables on ecosystem functions, both separately and in interaction with each other. Investigated responses included plant cover, net primary production (NPP) both above- and belowground (ANPP and BNPP) and root:shoot ratio. ANPP was estimated for vascular plants, plant functional types and the two most abundant species within the plant community. BNPP was assessed on the total 0-20 cm soil profile and separately for the near-surface layer (0-10 cm) and the deeper one (including 10-20 cm and 20-30 cm) to further consider potential root dynamics along the vertical profile. Pre-treatment values for plant cover, ANPP and root biomass for the year 2017 were included in analyses to account for the initial state of the community. Inter-annual variability was also assessed and effects of treatments on microclimate analysed.

Shelters effectively reduced cumulative rainfall (-41% in 2018 and -33% in 2019) and volumetric water content (VWC) at -5 cm depth, which was 3-4% less under drought conditions. In both years, OTCs increased air and soil temperature by +1 and +1.5 K, respectively, with slightly higher average temperatures when associated with shelters. Small but significant effects of reduced precipitation were detected on plant cover variation relative to pre-treatment values, with different responses exhibited by the most abundant *taxa*, mainly driven by the dominant forb in the grassland, which showed decreased cover under the shelters. On the other hand, treatments did not significantly affect ANPP, with the initial state of the community being a major driver explaining ANPP variations across plots; ANPP also exhibited a high inter-annual variability over the entire study period (2017-2019), with an overall decrease at all ANPP levels in the two years following 2017, a year characterised by an intense heat-wave and drought. With regard to BNPP, values for the 0-20 cm soil profile were significantly lower under shelters, with analysis across depths revealing a tendency towards lower BNPP for the near-surface layer in response to reduced rainfall, in contrast to the deeper layer, which showed similar values across treatments. A

marginally lower root:shoot ratio was found under shelters. However, overall NPP was not significantly affected by treatments.

Data from the study reveals that the investigated alpine grassland has a certain degree of resistance to short-term experimental warming and statistically "extreme" drought. Nevertheless, results from different NPP components highlight the need to consider both above- and belowground compartments when assessing climate change effects on ecosystem functions and different root responses along the vertical soil profile could also occur in the long term. Furthermore, inter-annual differences in ANPP suggest that naturally occurring extremes – i.e. drought and heat waves – could affect alpine grasslands.

4.2 INTRODUCTION

Warming and reduced precipitation patterns in the future will alter water availability, leading to drier conditions. This is expected to impact ecosystems in multiple ways: by altering species physiological performance (Signarbieux and Feller, 2012; Bollig and Feller, 2014; Niu et al., 2014); reducing microbial activity (Schimel, 2018); disrupting biotic interactions (Walter, 2018); and, no less importantly, affecting carbon and nutrient turnover through a reduction in plant productivity (Zhao and Running, 2010), an alteration in decomposition processes and overall carbon fluxes (van der Molen et al., 2011; Sippel et al., 2018). Net primary production (NPP), one of the central aspects governing of energy and matter, is particularly sensitive to changes in precipitation and water availability (Zhao and Running, 2010; Knapp et al., 2017b). Although the mechanisms by which water scarcity acts on plants performance are well known (Reddy et al., 2004; Lambers et al., 2008; Farooq et al., 2012), for various ecosystems the degree of resistance to altered precipitation patterns is still unclear, with a scarcity of precipitation experiments (Beier et al., 2012). In particular, effects of reduced rainfall on NPP in the upper alpine life zone – i.e. the area above the natural high-elevation treeline (Körner, 2003) – still need to be further investigated. Changes in ecosystem structure and functions are expected to be especially pronounced in areas characterised by strong seasonality, such as mountain regions, where annual growth is constrained by a short snow-free season and where current and predicted warming rates are among the highest globally (IPCC, 2013; Pepin et al., 2015).

Along with increasing temperatures, for various mountain areas there is increasing evidence coming from historical climate data pointing towards a trend of reduced summer rainfall having occurred in the last century (Basistha et al., 2009; Yang and Gong, 2010; Zhang et al., 2015; Brugnara and Maugeri, 2019). When coupled with decreased snow cover extent and duration (Dye, 2002), such a trend could lead to the likelihood of high-elevation systems experiencing water deficiency during the summer. This scenario is predicted to become more pronounced in

the future, particularly in some regions of the Alps (Gobiet et al., 2014), where altered precipitation patterns will be accompanied by greater inter-annual variability related to an increase in the magnitude and frequency of climate extremes such as heat waves and droughts (Calanca, 2007; IPCC, 2013; Orth et al., 2016).

Alpine environments are characterised by extreme abiotic features – strong seasonality, low temperatures, intense solar radiation, wind exposure, and strong diurnal temperature variations – which constrain aboveground plant morphology and lead to the presence of communities which are well-adapted to extreme conditions (Bliss, 1962; Bowman, 2001; Körner, 2003). In particular, abiotic factors such as low temperatures, snow-cover depth and duration and the consequent limited length of the growing season shape alpine plant communities and, hence, control primary production (Walker et al., 1994; Berdanier and Klein, 2011; Winkler et al., 2019). In addition, patterns of soil water availability drive the high degree of spatial heterogeneity observed in alpine tundra, further constraining plant biomass production during the growing period (Taylor and Seastedt, 1994; Fisk et al., 1998; Bowman and Fisk, 2001). In mid-latitude alpine regions, water supplies in summer are guaranteed by snowmelt input and rainfall, the latter being especially abundant at this latitude (Körner, 2003). A rainfall supply deficit, therefore, has only become an issue in many alpine regions recently, as a result of climate change. For these reasons, together with the difficulties of both forecasting future precipitation patterns and technically setting-up precipitation experiments, responses to reduced precipitation in the alpine zone still remain under-investigated (Wu et al., 2011; Beier et al., 2012; Knapp et al., 2017a). In the last decade, however, increasing attention has been paid to this topic (e.g. De Boeck et al., 2016; Zhang et al., 2017; De Boeck et al., 2018b; Liu et al., 2018).

With regard to increased temperatures, alpine tundra, and cold biomes in general, have been found to be highly sensitive to anthropogenic warming. The release of thermal constraints on vegetation has determined a notable trend towards increased productivity – a trend detected in both observational and experimental studies (Elmendorf et al., 2012a, b; Bjorkman et al., 2018; Berner et al., 2020). The positive effects of warming on plant growth, however, may be counteracted by altered precipitation patterns and soil drying (Wu et al., 2011; Yang et al., 2018), resulting in a change in the predicted responses to increased temperatures when water availability decreases (Zhao and Running, 2010). For instance, a study conducted on alpine species has demonstrated that the enhancing effect of warming on plant performance and growth was strongly limited when plants were not watered (Winkler et al., 2016). A meta-analysis assessing effects of experimental warming on plant growth across Arctic and alpine tundra has found a greater response in those sites characterised by higher soil moisture levels (Elmendorf et al., 2012b). Site-specific soil moisture has also been found to mediate aboveground net primary

production (ANPP) responses to warming across alpine ecosystems in the Tibetan Plateau (Yang et al., 2018).

Moreover, there is general agreement confirming the direction of the response of aboveground biomass to water limitation – i.e. ANPP reduction in response to drought – in manipulation studies using rain-off shelters in alpine environments, including subalpine grassland (Schmid et al., 2011; Gilgen and Buchmann, 2009; Prechsl et al., 2015; Schmid, 2017; Liu et al., 2018), even though the strength of the response is still not fully understood. The research conducted by Schimd et al. (2011) on three alpine grasslands exposed to different degrees of drought intensity (moderate and extreme) has illustrated a reduction of aboveground biomass already under moderate drought conditions (6 weeks of rainfall exclusion), with substantial differences detected across sites. On the other hand, reduced ANPP under the most extreme precipitation reduction level (only 1/12 of natural rainfall) has been reported on the Tibetan Plateau (Zhang et al., 2017), whereas significantly lower ANPP values were found after 18 days of full exclusion in alpine grassland monoliths, a reduction which was even greater in combination with heat waves (De Boeck et al., 2016). Idiosyncratic results such as these are probably due to the type of treatment applied and the diversity in plant communities and substrates (Huxman et al., 2004; Henry et al., 2018)

With regard to the type of treatment, results can differ depending on the press or pulse nature of the drought, with acute drought episodes (pulse) being more likely to exceed water stress thresholds than extended periods of reduced precipitation, such as press droughts (Hoover and Rogers, 2016; De Boeck et al., 2018a). Furthermore, precipitation experiments often apply a rainfall reduction based on an absolute quantity or a relative change in the amount of precipitation, but rarely based on site-specific historical records (Knapp et a., 2017a). In the light of this, manipulations testing site-specific extremes could provide additional information on the mechanisms behind ecosystem sensitivity towards climate change and would improve understanding of the ANPP-precipitation relationship within each site, allowing researchers to obtain a more robust generalization of results on a regional and global scale. The use of a common protocol, starting from the definition of drought and the type of treatments to be applied is also necessary to overcome the limits imposed by the variety of methodologies employed to analyse a single variable when synthesising results on a global scale (Knapp et al., 2017a; Henry et al., 2018). Such a manipulation protocol could also help to determine to what degree responses to drought are influenced by the identity and traits of the species present within the studied plant community.

As has been frequently observed, each species exhibits a different response to water shortage (Tardieu and Simonneau, 1998; Kreyling et al., 2008; Signarbieux and Feller, 2012; Niu et al., 2014). This variation in sensitivity is evidenced in high-elevation environments by the diverse

responses of plant functional types to drought. Grasses have been found to generally perform better − i.e. more drought-tolerant − than forbs (Winkler et al., 2016; De Boeck et al., 2016; Cremonese et al., 2017; Rosbakh et al., 2017) although contrasting results have also been observed (Signarbieux and Feller, 2012; Bollig and Feller, 2014), pointing to the need for a species-specific evaluation of the effects of drought on a community. This is also relevant since, as has been found in studies on warming and drought effects, different and complementary responses to altered abiotic conditions across species may be evened out at a community level, leading to an overall stability of the ecosystem in terms of biomass production (Klanderud, 2008; Kreyling et al., 2008).

However, in order to gain a comprehensive picture of primary production responses to reduced precipitation biomass partitioning and allocation to belowground organs also need to be considered, since the "hidden side" of biomass might compensate for the reduction observed in the standing matter. Roots, in fact, represent an important C stock, especially in alpine ecosystems, where over half of the total biomass is stored belowground (Körner and Renhardt, 1987; Grabherr, 1989; Fisk et al., 1998). The pivotal role roots play in water and nutrient uptake represents a fundamental aspect to take into consideration when assessing climate change effects, especially when investigating the impact of altered water availability.

Syntheses on root response globally to experimentally manipulated precipitation reveal a general tendency towards a decline in belowground net primary production (BNPP) for herbaceous plants under dryer conditions (Zhou et al., 2018; Zhang et al., 2018; Wang et al., 2019), whereas warming generally tends to enhance BNPP (Liu et al., 2018; Liu et al., 2021; Yang et al., 2020a). According to the optimal allocation theory, however, when a resource becomes scarce, plant species allocate major resources towards the organs allowing maximum uptake of the limiting resource (Bloom et al., 1985). A recent synthesis across biomes has confirmed this theory, revealing a greater allocation of belowground biomass when plants are exposed to stressful climate conditions and nutrient limitations (Qi et al., 2019). Plants could, therefore, shift biomass allocation towards their roots in such conditions, maintaining overall NPP and increasing root:shoot ratio (Wilcox et al., 2017). At present, there is little empirical evidence of belowground productivity responses to warming or water shortage in alpine ecosystems. When studies from lower elevation grassland sites are included, a variety of responses are reported, ranging from an increase in root production (Zhang et al., 2017; Schmid, 2017; Liu et al., 2018; Liu et al., 2021) to no significant variation (Gilgen and Buchmann, 2009; Prechsl et al., 2015; Liu et al., 2018; Liu et al., 2021) and, finally, to a reduction in belowground biomass (Fiala et al., 2009). In addition, root responses to altered precipitation patterns could differ along vertical soil profiles, with shifts towards the deeper ones (Zhang et al., 2019a). Although comparison is hindered by a variety of factors which could influence the final outcome – different treatment duration, the set of species

present and the methodology applied (Zhang et al., 2018) – the inconsistency in the results highlights the need for further research on root responses to drought in order to provide more information on potential carbon turnover in response to climate change.

The current study investigates the effects of increased temperatures and reduced summer precipitation manipulations on the productivity dynamics of an alpine grassland located at 2700 m a.s.l. in the Italian Central Alps. The research was carried out over a 3-year period (2017-2019), which includes pre-treatment data (2017) to account for the influence of the initial state of the community. After treatment effects on microclimate were analysed and inter-annual climate variation during the study period was accounted for, the data was examined with the following aims: (a) to assess whether temperature and reduced precipitation affect NPP; (b) to investigate whether effects can be detected in ANPP at different levels of organization (total vascular plants, plant functional types and species) together with estimated cover values of the most common *taxa*; and (c) to assess effects on root responses and biomass allocation patterns, including total BNPP and variation across the vertical soil profile. To account for covariation between manipulated microclimatic variables and ambient conditions (Hoover et al., 2018), differences in primary production across years were also considered. The study was carried out within the framework of two international networks: the International Drought Experiment, where sensitivity of terrestrial ecosystems to specific drought is tested globally by imposing site-specific extreme conditions and, secondly, the International Tundra Experiment, where the responses to warming of cold biomes are assessed.

4.3 MATERIALS AND METHODS

4.3.1 Study site and experiment design

The study site and experiment design are presented in Chapter 2 and Chapter 3, respectively. Materials and methods used for microclimate measurements, plant cover and biomass are described in the paragraphs below.

4.3.2 Microclimate

In 2018 and 2019 continuous measurements of temperature, relative humidity and soil moisture were recorded using 2 replicated devices for each treatment during the growing season. Canopy temperature (AT, °C) and relative humidity (RH, %) were monitored by placing sensors (EHT Temperature/RH Sensor, Decagon Devices, Inc.) 5 cm above the soil surface. Mean daily AT and RH were then used for computations of mean daily Vapour Pressure Deficit (VPD), a measure of the actual atmospheric air dryness. VPD was calculated following Jones (1992) – R package "plantecophys" (Duursma, 2015).

In addition, soil temperature (ST, °C) and moisture were recorded during the whole growing season. HOBO sensors (HOBO Pendant® Temperature/Light 64K Data Logger, Part # UA-002-64) were used to record soil temperatures at -5 cm belowground. Soil moisture was recorded by measuring the volumetric water content at -5 cm, -15 cm and -25 cm (VWC, %; WaterScout SM100, Spectrum Technologies, Inc.) in order to obtain a clear picture of water content along the vertical profile where roots were collected. Data for -5 cm and -15 cm is available in all treatments, while the -25 cm depth was only monitored for C and D plots. To quantify shelter effects and performance, together with a site-specific climate characterisation, the following additional climate data was gathered throughout the growing season, both under and outside the rain-out shelter: AT and RH at approx. 20 cm above the ground (Spectrum WD1650), total precipitation (P, mm, Rain Collector, Decagon Devices, Inc.), photosynthetic active photon flux density (PPFD, µmol m^{-2} s^{-1}) and solar radiation (SR, W m^{-2}) (Spectrum Technologies, Inc.). During the growing season microclimate data was logged hourly.

4.3.3 Plant cover and aboveground net primary production (ANPP)

To guarantee a continuous long-term data survey on the same plots, the visual percentage cover of both vascular and non-vascular species was recorded annually (including the pre-treatment year 2017). The non-destructive point intercept method (PIM) was also employed to indirectly estimate ANPP of vascular species within each of the monitored permanent plots (Jonasson, 1988). A 1 m^2 frame, with a 10 cm-mesh size grid (100 pins in total), was placed on top of each plot (**Fig. 4.1a**). The number of contacts for each species was recorded for each pin and then totalised to obtain the number of contacts per species per plot. Data was gathered in August, during the peak season (13.08.2017, 18.08.2018 and 27.08.2019) – the standard procedure for estimating ANPP in the alpine tundra (Bowman and Fisk, 2001).

To obtain an indirect estimate of ANPP production of vascular species, at the same time as the PIM, phytomass (hereafter "biomass") was harvested in both C and D plots from randomly selected 400 cm^2 quadrats (**Fig. 4.1b**) – 3 quadrats in 2017, 2 in 2018 and 2 in 2019 for each plot – after recording the number of pin contacts in 4 points using the same frame used in the permanent plots, with the exception of 2017, where no PIM was applied on harvested areas. The biomass samples gathered for each season were subsequently stored at -20°C until they were processed in the lab, where the detached, dead plant material from the previous year (litter), identified by its grey colour and major fragmentation, was separated from the current year's production. In 2018 and 2019, biomass was then sorted into species (**Fig. 4.1c, d**). All plant material was finally oven-dried at 65 °C for 48 hours, after which dry mass was recorded. The biomass value was then used to calibrate contacts and obtain an indirect ANPP estimate on the permanent plots by dividing the biomass value of every contacted species for the total number of

contacts for each of the harvested quadrats gathered in 2018 and 2019. Before biomass estimation for each species, an assessment was made to ascertain whether the biomass value related to contacts was not significantly different ($p>0.05$) between treatments – C and D – and years. Following this, for each quadrat an average biomass value of one contact for every species was obtained and then mediated across all quadrats where the species was present (**Table 4.1**). Finally, ANPP (g m^{-2} yr^{-1}) for 2017-2019 was estimated in all treatments by multiplying the total number of contacts for each species by the previously computed species-specific biomass value. Although the precision of such an estimation method has been questioned – i.e. high variability in biomass values of one contact and a variety of species never contacted – this method is, nevertheless, able to give a weighed estimate for each of the most abundant grassland species, which are those that are expected to contribute the most to ANPP variation in the short term.

Finally, ANPP for the main plant functional types (PFTs) present in the target grassland – sedges, represented only by *Carex curvula*, grasses and forbs – was also computed by grouping vascular plant species on the basis of common morphological and physiological traits which may determine differences in responses to climate change (Winkler et al., 2016; Liu et al., 2018) (**Table 4.1**).

4.3.4 Root biomass and belowground net primary production (BNPP)

In 2017, pre-treatment root biomass data (g m^{-2}) was gathered in 4 replicates per (pre)-treatment, whereas annual BNPP (g m^{-2} y^{-1}) was estimated for experimental seasons (2018 and 2019). Root biomass was gathered from soil cores (2 cm in diameter, 10 cm deep) extracted from 3 points in each plot at the end of the growing season (28.09.2017); for each point, three soil depths were sampled: 0-10 cm, 10-20 cm and 20-30 cm (**Fig. 4.2a-c**). BNPP for subsequent years was estimated through the ingrowth core method by refilling the holes with sieved, root-free soil which had been previously collected in the area surrounding the plots, corresponding to the same 3 depths – sampling on 19.09.2018 and 2019. Subsequently, in the lab, main roots of each sample were extracted from the soil and carefully washed with water to eliminate soil particles; almost all roots belonged to the fine root class size, with a diameter <2 mm (McCormack et al., 2015) (**Fig. 4.2c**). To recover the finest root fraction, which might have not been retrieved during the first stage of root processing, a 10-minute extraction of the finest roots was subsequently carried out for each soil sample and then added to the main biomass sample. Finally, all root material was oven-dried at 65 °C for 48 hours and weighed to the nearest 0.0001 g.

Table 4.1: Average biomass value (g) ± standard deviation for 1 point intercept contact for each vascular plant species used to calculate ANPP. Biomass values derive from the mean biomass of one contact in each harvested quadrat, which was then mediated across all quadrats where the species was present. N stands for sample size (no. of quadrats). The relative identification code (ID), plant functional type (PFT) and frequency (Freq) of each species in the 20 permanent plots are also reported. All the species present in the plots are listed in descending order of frequency, where NA indicates absent biomass value. Species names are standardized following Pignatti (1982).

ID	Species	PFT	Freq (%)	N	Biomass (g)
CC	*Carex curvula* All.	Sedge	100	39	0.282 ± 0.099
AP	*Alchemilla pentaphyllea* L.	Forb	100	37	0.496 ± 0.369
PA	*Poa alpina* L.	Grass	100	16	0.575 ± 0.526
LH	*Leontodon helveticus* Mérat	Forb	100	12	0.292 ± 0.304
PH	*Phyteuma hemisphaericum* L.	Forb	100	18	0.258 ± 0.226
SP	*Soldanella pusilla* Baumg.	Forb	100	21	0.209 ± 0.151
LA	*Leucanthemopsis alpina* (L.) Heyw.	Forb	100	8	0.118 ± 0.121
AR	*Agrostis rupestris* All.	Grass	100	10	0.146 ± 0.110
EM	*Euphrasia minima* Jacq. ex DC.	Forb	85	NA	NA
PG	*Primula glutinosa* Wulfen	Forb	65	4	0.687 ± 0.466
GS	*Gnaphalium supinum* L.	Forb	55	1	0.003
SH	*Salix herbacea* L.	Shrub	50	3	0.109 ± 0.081
LM	*Ligusticum mutellina* (L.) Crantz	Forb	35	6	0.535 ± 0.379
OD	*Oreochloa disticha* (Wulfen) Link	Grass	30	1	0.365
PV	*Polygonum viviparum* L.	Forb	30	1	0.012
PAU	*Potentilla aurea* L.	Forb	25	NA	NA
AV	*Avenula versicolor* (Vill.) Lainz	Grass	25	NA	NA
FH	*Festuca halleri* All.	Grass	15	1	0.025
HA	*Homogyne alpina* (L.) Cass	Forb	10	NA	NA
TA	*Taraxacum alpinum* (Hoppe) Hegetschw.(aggr)	Forb	10	NA	NA
SPR	*Sibbaldia procumbens* L.	Forb	10	NA	NA
AA	*Anthoxanthum alpinum* Love et Love	Grass	10	NA	NA
PC	*Phleum commutatum* Gaudin	Grass	5	1	0.195
HP	*Hieracium piliferum* Hoppe	Forb	5	NA	NA
PK	*Pedicularis kerneri* D. Torre non Huter	Forb	5	NA	NA
LAP	*Luzula alpinopilosa* (Chaix) Breistr.	Rush	5	NA	NA

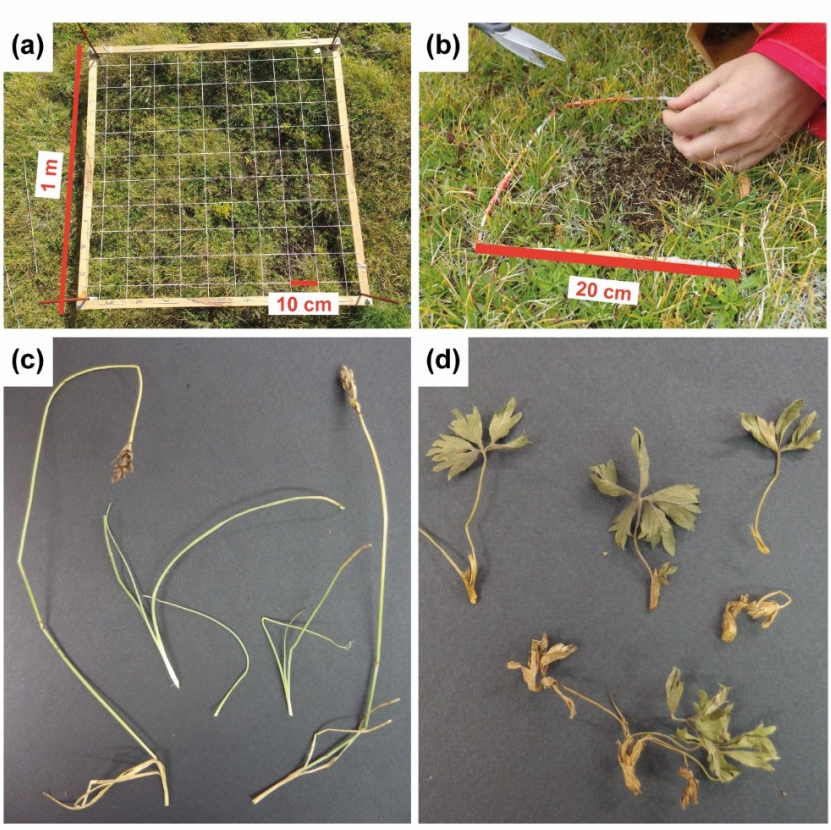


Fig. 4.1: **(a)** Frame placed on top of 1 m^2 permanent plot used for the point intercept method, with a 10 cm-mesh size grid; **(b)** Harvested quadrat (400 cm^2) for destructive estimate of ANPP, sorted by species – **(c)** *Carex curvula* and **(d)** *Alchemilla pentaphyllea.*

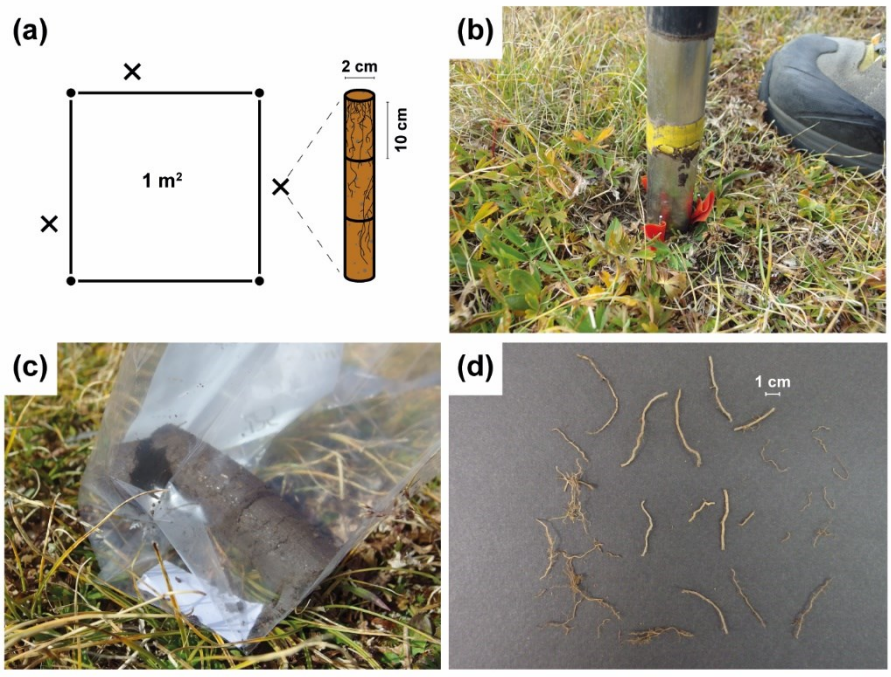


Fig. 4.2: **(a)** Root sampling scheme, consisting of soil core extraction from 3 points around each 1 m^2 permanent plot; from each point, three soil depths were sampled: 0-10 cm, 10-20 cm and 20-30 cm; **(b)** Ingrowth core extraction in the field; **(c)** Extracted ingrowth core sample; **(d)** Final root sample deriving from ingrowth core.

4.3.5 Net primary production (NPP) and root:shoot ratio

During the experimental period, NPP was estimated by summing ANPP and BNPP (4 replicates), with BNPP referring to the 0-20 cm profile. Root:shoot production ratio was estimated (BNPP/ANPP) on the same data.

4.3.6 Data analysis

To assess variation in microclimatic conditions resulting from experimental treatments (4-level factor: C, D, W and DW), partial linear redundancy analysis (RDA) was carried out on the following scaled variables (to zero-mean and unit-variance): precipitation, solar radiation, air temperature, relative humidity and vapour pressure deficit. Time was inserted as a 2-level factor (2018 and 2019) covariate. This partial canonical ordination, which could be regarded as a multivariate equivalent of partial linear regression, allows for the detection of the variation in microclimate which can be solely explained by experimental treatments, while holding the influence of time as a constant, or while partially excluding inter-annual variability.

Inter-annual variability in microclimate was subsequently explored by performing RDA on scaled microclimatic variables, which were measured in ambient conditions and constrained by the 3-level factor "year" (2017, 2018 and 2019).

Partial RDA was also used to assess if patterns in *taxa* cover change in 2018 and 2019, compared to the pre-treatment year (2017), could be explained by temperature (2-level factor: =T, no OTCs; +T, plots with OTCs), precipitation (2-level factor: =P, no shelters; -P, plots with shelters) or year (2-level factor: 2018 and 2019) while, at the same time, controlling for the effects of blocks (5-level factor). The effect of spatial variation in plant cover – i.e. the differences among blocks – was excluded by performing a multiple multivariate regression of the predictors of interest (year, temperature and precipitation) on the factor to be controlled (block), and then using the residuals of this analysis as explanatory variables.

In all above-mentioned ordinations, the significance of models, terms and canonical axes was tested by means of permutations; linear dependency among predictors was checked by computing variance inflation factors (Neter et al., 1996) with a cut-off value of 5. Model selection was performed by excluding non-significant terms and considering the stopping criterion based on adjusted R^2 of the global model proposed by Blanchet et al. (2008) – i.e. containing all the predictors – to prevent inflation of both type I error and explained variance.

With regard to changes in primary production, these were assessed by means of linear mixed effects models (LMMs) following the approach suggested by Zuur et al. (2009) and Crawley (2012), carrying out a top-down model selection. Starting from a saturated linear mixed model, which included all possible fixed effects, the resulting non-significant terms were removed sequentially to obtain a final parsimonious model retaining only significant fixed effects ($p>0.05$).

Model selection was performed using the ML method (maximum likelihood), while the final model was fitted using the REML method (restricted maximum likelihood) to obtain estimates of the effects of factors tested and their significance. Linear model assumptions were verified by visually checking the residual patterns and a variance structure was included to deal with heterogeneity of residuals whenever necessary. Akaike Information Criteria (AIC) was employed to test model goodness of fit and select the best random effect and variance structure – random effect and variance structure for each final model are reported in **Table 4.3** and **Table 4.4** in the Section 4.4. Pairwise comparison of significant terms was carried out by means of post-hoc Tukey contrast(s).

In detail, analysis of primary production was performed by testing the following response variables: total ANPP of vascular species, plant functional types (PFTs: forbs, grasses and sedges) and of the two dominant species (*Carex curvula* – CC, and *Alchemilla pentaphyllea* – AP); total BNPP; BNPP of each investigated soil layer; NPP; and root:shoot ratio. Since in both 2018 and 2019 the 20-30 cm profile lacked a sufficient number of replicates for DW treatment, NPP, total BNPP and root:shoot ratio were only analysed on the 0-20 cm profile. Root biomass sampling in 2017 revealed that this profile consisted of almost 90% of the total belowground biomass in the investigated 0-30 soil layer (see Chapter 2, Section 2.2.2).

To assess manipulation effects on response variables, the four treatments characterising the experimental design (see Chapter 3, Section 3.2) were coded into two categorical variables to test for temperature T (2-level: =T and +T), precipitation P (2-level: =P and -P) and their interaction. Together with T and P, fixed effects of models for ANPP of vascular species, total BNPP, NPP, and root:shoot ratio included the year (2-level factor: 2018 and 2019) and its interaction with treatments. In order to evaluate the initial state of the community prior to experiments, pre-treatment values were also included as a covariate (numerical variable). In addition, fixed effects of LMMs testing for ANPP of PFTs, together with that of species, also included PFTs (3-level factor: forbs, grasses and sedges) and species (2-level factor: CC and AP). Similarly, models testing for BNPP across depths also included depth as a fixed effect (2-level factor: surface and deep). For this last model, the level “surface” included the 0-10 profile values, while the level “deep” included root data from both the 10-20 and 20-30 profiles, after ANOVA model comparison using a 3- and a 2-level factor had revealed no significant differences between the two models (Crawley, 2012).

Finally, inter-annual variability in ANPP responses at all investigated levels – total vascular, PFTs and species – was tested for each response variable by setting the whole study period as a fixed effect (3-level factor: 2017, 2018 and 2019), with fixed effects also including PFTs and species for each of the relative response variables.

Statistical analysis was carried out in R (R Core Team, 2020). The following packages were employed: “vegan” (Oksanen et al., 2019) for RDA; “nlme” (Pinheiro et al., 2020) for LMMs; “lsmeans” (Lenth, 2016) and “multcomp” (Hothorn et al., 2008) for post-hoc comparisons and letter display; and “effects” (Fox and Weisberg, 2018) for regressions graphs.

4.4 RESULTS

4.4.1 Microclimate

Checking for cumulative precipitation under the shelters when compared to those recorded in ambient conditions during the entire experimental period, in 2018 rainfall amount was reduced by 41% (407 mm in C vs 239 mm in D), and in 2019 by 33% (347 mm in C vs 232 mm in D). Records form 2019 revealed there was a 17% reduction in PPFD under the shelters compared to ambient, with 447 ± standard deviation (SD) 149 µmol m^{-2} s^{-1} in D vs 370 ± SD 126 µmol m^{-2} s^{-1} in C – see Chapter 3, Section 3.3.2.

RDA on microclimate gathered in treatments indicated that the variation in microclimatic features was significantly explained by treatments (pseudo-$F_{1,395}$=6.25, p<0.001). In particular, the first ordination axis (RDA1) resulted significant (pseudo-$F_{1,395}$=14.54, p<0.001) while RDA2 resulted as only marginally significant (pseudo-$F_{1,395}$=3.68, p=0.060), explaining 3% and 1% of the variance, respectively.

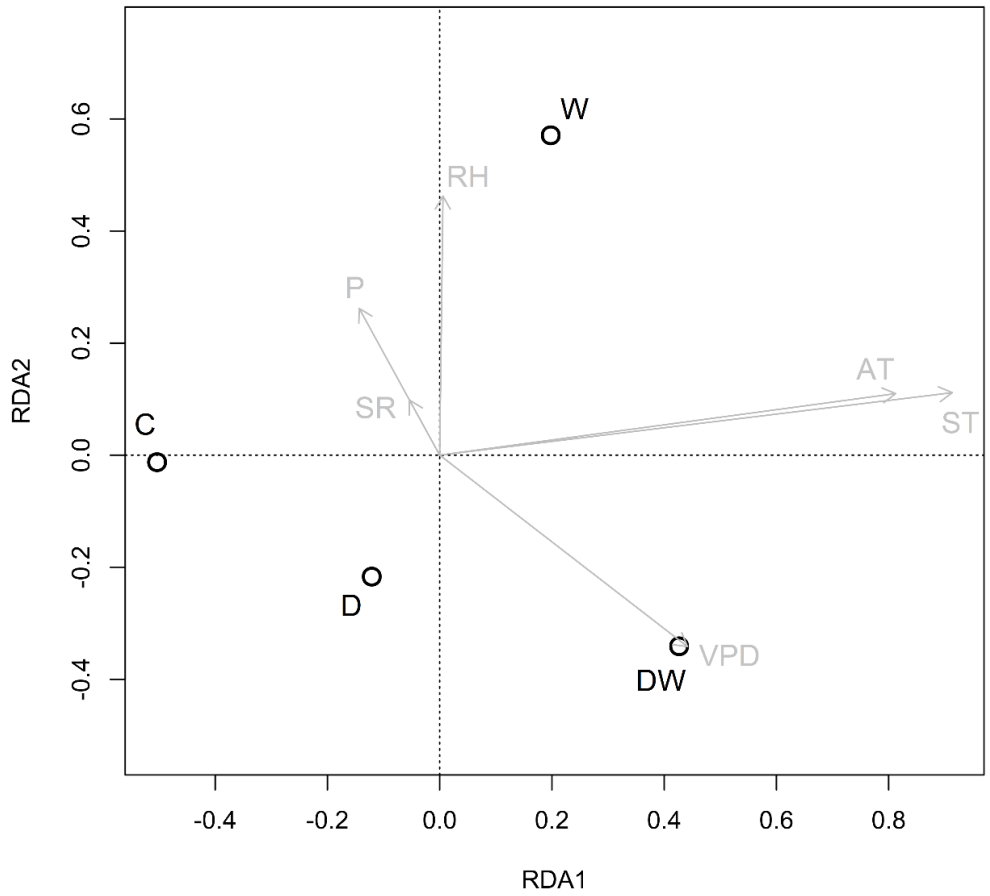


Fig. 4.3: Partial RDA correlation plot of microclimatic variables within treatments [C − control; D − drought; W − warming; DW − drought + warming], with 3% variance explained by RDA1 and 1% by RDA2. Data corresponds to the 50 days preceding the point intercept date. Microclimate variables: precipitation (P, mm); solar radiation (SR, W m^{-2}); air temperature (AT, °C), relative humidity (RH, %) and vapour pressure deficit (VPD, kPa) gathered at the canopy level, 5 cm above the soil surface; and soil temperature (ST, °C) measured at -5 cm.

In particular, the RDA correlation plot (**Fig. 4.3**) indicated that air and soil temperature (AT and ST) were positively related, whereas daily mean precipitation (P) was negatively related to vapour pressure deficit (VPD). Among treatments, plots under OTCs (W and DW) exhibited higher AT and ST compared to those with ambient temperature (C and D). In addition, the warming treatment (W) was characterised by higher relative humidity (RH), whereas areas under the shelters showed higher VPD, especially under the treatment which combined warming with precipitation reduction (DW). Average values of microclimate variables across treatments are reported in **Table 4.2**.

Table 4.2: Daily mean ± standard deviation (standard error, n=50) of microclimate variables recorded in treatments [C − control; D − drought; W − warming; DW − drought + warming] for the year 2018 and 2019; codification of treatments for temperature (T) and precipitation (P) is also reported below treatment acronyms. Mean values are calculated from the 50 days preceding the point intercept date (day of PI excluded). Microclimate variables: precipitation (P); solar radiation (SR) at 20 cm; air temperature (AT), relative humidity (RH) and vapour pressure deficit (VPD) at 5 cm (canopy) from the soil surface; and soil temperature (ST) at -5 cm.

YEAR	**2018**				**2019**			
Snowmelt date	5 June				21 June			
Treatment period	15 June - 25 September				01 July - 24 September			
Point intercept date	12 August				27 August			
TREATMENT	**C** =T =P	**D** =T -P	**W** +T =P	**DW** +T -P	**C** =T =P	**D** =T -P	**W** +T =P	**DW** +T -P
P [mm]	4.91 ± 8.09 (1.14)	3.34 ± 6.15 (0.87)	-	-	4.43 ± 6.67 (0.94)	2.99 ± 4.99 (0.71)	-	-
SR [W m^{-2}]	227.03 ± 77.78 (11.00)	225.10 ± 73.11 (10.34)	-	-	211.77 ± 75.30 (10.65)	200.97 ± 67.57 (9.56)	-	-
AT [°C]	10.76 ± 2.46 (0.35)	11.17 ± 2.51 (0.35)	12.27 ± 2.21 (0.31)	12.35 ± 2.98 (0.42)	10.51 ± 2.47 (0.35)	11.06 ± 2.56 (0.36)	12.15 ± 2.41 (0.34)	12.73 ± 2.95 (0.42)
RH [%]	77.95 ± 7.28 (1.03)	76.98 ± 7.37 (1.04)	79.68 ± 7.26 (1.03)	76.53 ± 7.04 (1.00)	77.82 ± 5.99 (0.85)	77.75 ± 6.50 (0.92)	79.87 ± 5.80 (0.82)	77.28 ± 6.25 (0.88)
VPD [kPa]	0.29 ± 0.09 (0.01)	0.31 ± 0.09 (0.01)	0.30 ± 0.11 (0.02)	0.34 ± 0.11 (0.02)	0.29 ± 0.09 (0.01)	0.30 ± 0.11 (0.01)	0.29 ± 0.11 (0.02)	0.35 ± 0.13 (0.02)
ST [°C]	10.58 ± 1.78 (0.25)	11.08 ± 1.74 (0.25)	11.95 ± 1.67 (0.24)	11.69 ± 1.73 (0.24)	10.64 ± 1.27 (0.18)	11.59 ± 1.39 (0.20)	11.73 ± 1.28 (0.18)	12.21 ± 1.34 (0.19)

Concerning the RDA on microclimatic features across the 3-year study period, which includes the pre-treatment year (2017), only the first axis significantly explained the variation observed in the data (pseudo-$F_{1,147}$=23.03, p<0.001), with 12% of variance explained by RDA1 as against 1% explained by RDA2 (**Fig. 4.4**). The factor "year", therefore, was found to significantly influence microclimate (pseudo-$F_{2,147}$=11.65, p<0.001). In particular, conditions in 2017 were drier compared to the following two years (2018 and 2019), as shown by higher VPD values and number of days without precipitation (DWP) which, in turn, were negatively related to RH. In contrast, the second year of treatments (2019) was the wettest, resulting in higher RH and lower VPD.

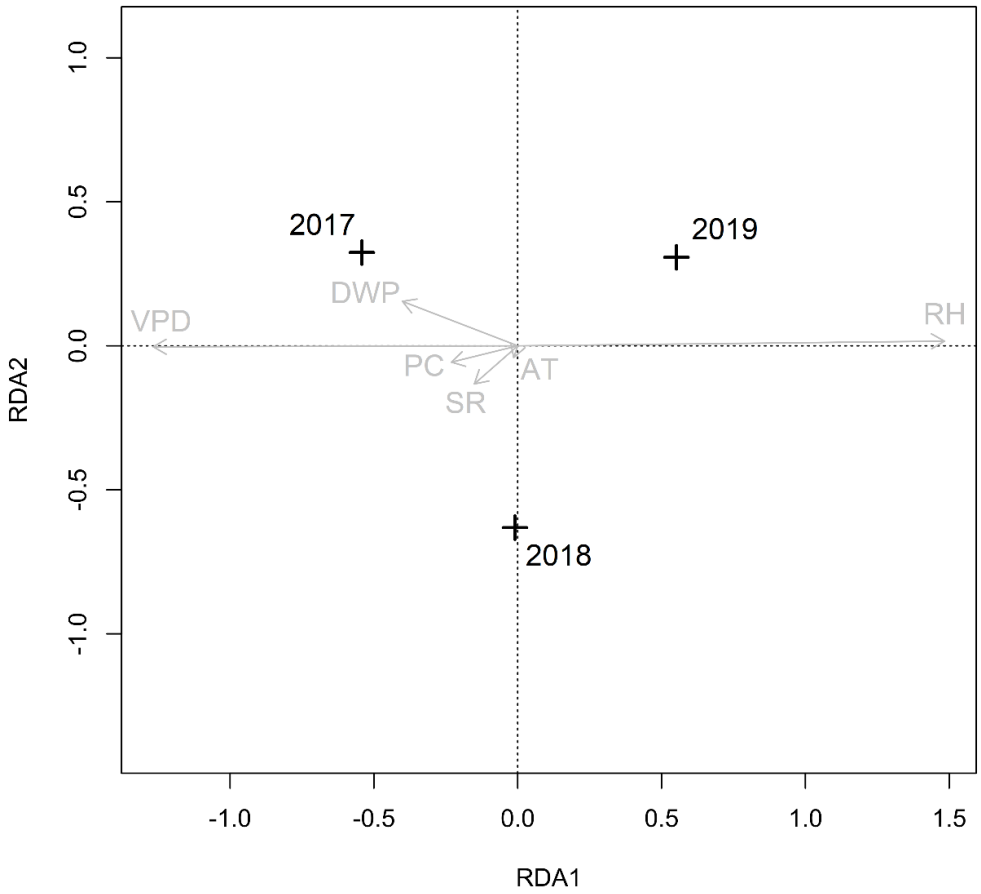


Fig. 4.4: RDA correlation plot of the microclimatic variables across the 3-year study period (2017-2019), with 12% of variance explained by RDA1 and 1% by RDA2. For each year, data corresponds to the 50 days preceding the point intercept date. Microclimate variables: cumulative precipitation (PC, mm); number of days without precipitation (DWP); solar radiation (SR, W m^{-2}); air temperature (AT, °C), relative humidity (RH, %) and vapour pressure deficit (VPD, kPa) gathered at 20 cm above the soil surface; and soil temperature (ST, °C) measured at -5 cm.

With regard to soil moisture across treatments (**Fig. 4.5a**), daily values of VWC recorded at -5 cm in D and DW were on average 3.2% and 4.1% lower than those recorded in C, respectively; mean VWC was also 3.5% lower in W compared to controls. At greater depths (-15 cm and -25 cm), the VWC was similar between control and treatments. Overall, the deeper the sampled soil, the lower the VWC, with -15 cm and -25 cm showing on average 3% and 9.6% less VWC compared to the most superficial layer (-5 cm), respectively. Finally, in the near surface (-5 cm) shelters exerted a greater influence on VWC compared to the next deepest soil layer (-15 cm), with mean standard deviation at -5 cm and -15 cm equal to 0.98% and 0.69%, respectively (**Fig. 4.5b**).

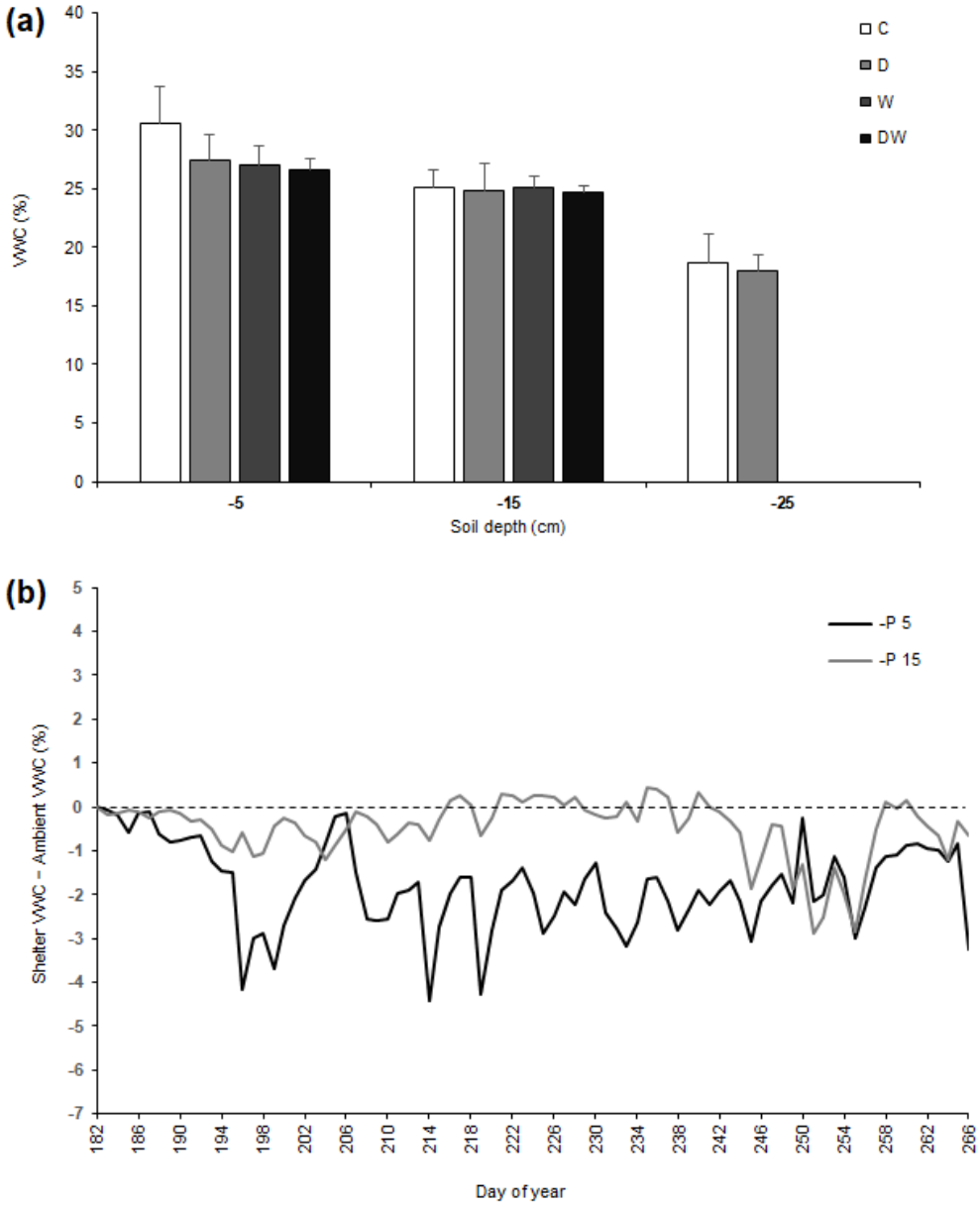


Fig. 4.5: Soil volumetric water content (VWC, %) recorded in 2019: **(a)** Comparison of mean daily VWC across treatments [C − control; D − drought; W − warming; DW − drought + warming] at three depths (-5 cm, -15 cm and -25 cm); for -25 cm only C and D data is available; data corresponding to the period between the beginning of treatments and the point intercept date; error bars indicate standard deviation; **(b)** Mean daily shelter effect (-P) [i.e. the difference between mean VWC in D and DW (rainfall under shelters) minus mean VWC in C and W (ambient rainfall)] recorded at -5 cm and -15 cm depth.

4.4.2 Plant cover

Partial RDA on the cover variation across treatments and years (2018 and 2019) indicated that changes in plant cover, compared to the pre-treatment year (i.e. cover variation), while keeping the spatial differences in cover constant (i.e. partial canonical ordination including block as a covariate), were significantly affected by inter-annual variability (pseudo-$F_{1,33}$=4.33, p=0.004) and experimentally reduced rainfall (pseudo-$F_{1,33}$=3.22, p=0.021), whereas they were not influenced by experimental warming. In particular, the factors year and precipitation were respectively associated with the first (pseudo-$F_{1,33}$=4.36, p=0.027) and second (pseudo-$F_{1,33}$=3.19, p=0.033) ordination axes (**Fig. 4.6**), which explained 9% (RDA1) and 6% (RDA2) of the variance in species cover changes, respectively. Moreover, cover variations differed across *taxa*, with changes being mainly driven by the two most abundant species, *Carex curvula* (CC) and *Alchemilla pentaphyllea* (AP). The former exhibited a +2% average increase in 2018, but decreased by -5% in 2019; the latter species showed increased cover values (+3%) in ambient conditions (=P) while these decreased (-3%) under shelters (-P). The remaining *taxa*, which are characterised by lower cover values compared to the dominant species, CC and AP, exhibited low cover variation across both treatments and years.

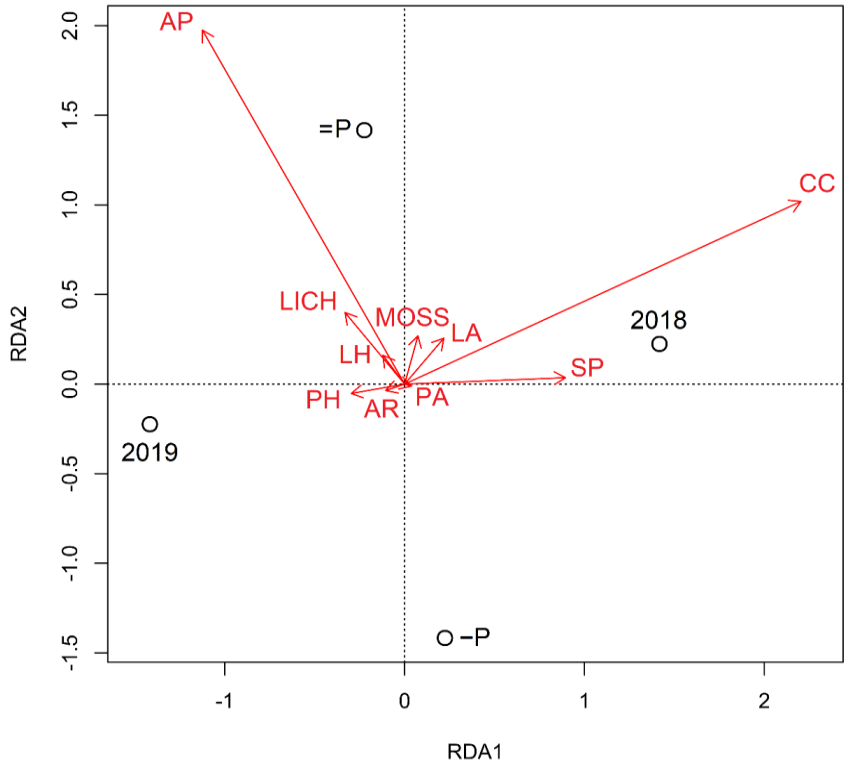


Fig. 4.6: Partial RDA correlation plot of cover variation of mosses, lichens and 8 most frequent species across treatments [=P, ambient (C and W); -P, precipitation reduction (D and DW)] in the two treatment years (2018 and 2019), with 9% of variance explained by RDA1 and 6% by RDA2. Data corresponds to the difference between cover values for 2018 and 2019 with those for the 2017 pre-treatment year. *Taxa*: *Alchemilla pentaphyllea* (AP); *Agrostis rupestris* (AR); *Carex curvula* (CC); *Leucanthemopsis alpina* (LA); *Leontodon helveticus* (LH); Lichens (LICH); mosses (MOSS); *Poa alpina* (PA); *Phyteuma hemisphaericum* (PH); *Soldanella pusilla* (SP).

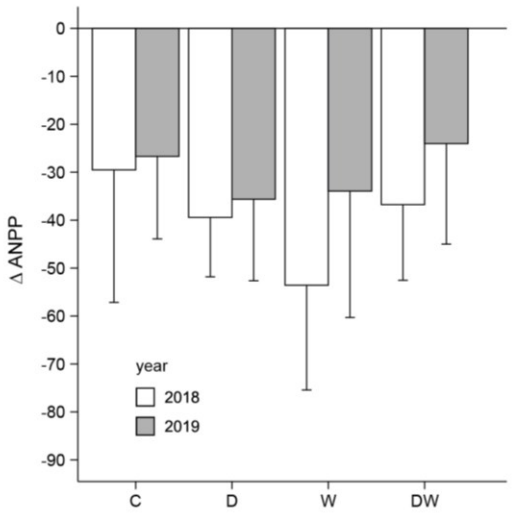
4.4.3 ANPP

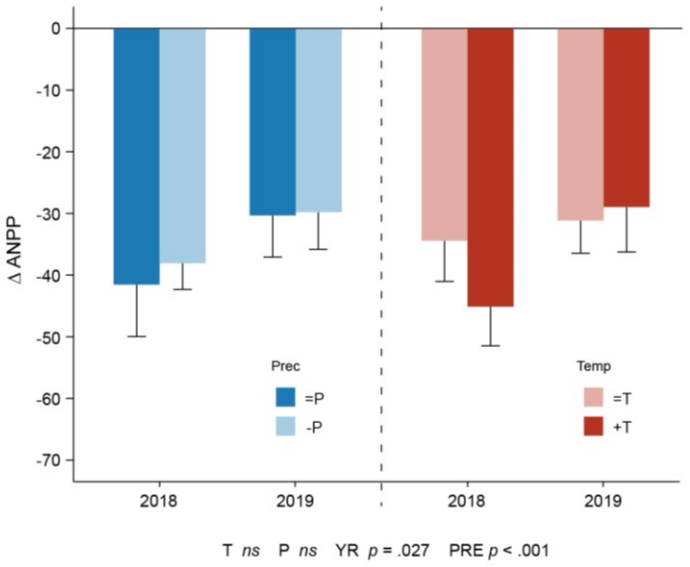
No effects of increased temperature, extreme precipitation reduction and inter-annual variability on aboveground productivity was found for ANPP ($p>0.05$) at any of the considered levels – total vascular (**Fig. 4.7a**), PFTs (**Fig. 4.7b**) and species (**Fig. 4.7c**) – and were, therefore, excluded during the model selection process. On the other hand, most of the variation at all levels of analysis was explained by pre-treatment values, with ANPP estimated for 2018 and 2019 positively correlated with values recorded in 2017 (**Fig. 4.8a-c**, left-hand graphs). ANOVA output on LMMs for ANPP are shown in **Table 4.3**, where only significant terms are reported.

Table 4.3: Results of LMMs for above- and belowground net primary production (ANPP and BNPP) and root:shoot ratio in response to treatments. Analyses of ANPP were carried out at different levels of organization – total vascular plants, plant functional types and species. Only significant terms and interactions retained in the final model are reported: YR, treatment years (2018 and 2019); PRE, pre-treatment values (2017 ANPP values); PFT, plant functional types (forbs, grasses and sedges); SP, species (*Carex curvula* and *Alchemilla pentaphyllea*); DPT, depths (superficial and deep); and P, precipitation [ambient (no shelters) and reduced (plots with shelters)]. Random and variance structures included in the final model are also reported; in particular, depending on the model, variance structure was homogenised for: pre, pre-treatment values (2017 ANPP values); t, temperature [ambient (no OTCs) and warmed (plots with OTCs)]; and p, precipitation (ambient and reduced). LMMs for net primary production (NPP) are not reported, since fixed effects resulted as non-significant during the model selection process ($p>0.05$).

Y	Fixed effects	Random	Variance	df (N,D)	F-value	p-value
ANPP						
Vascular	YR	block	-	1,33	5.321	0.027
	PRE			1,33	29.106	<0.001
Plant functional types	PFT	block	pre	1,109	18.748	<0.001
	YR			1,109	12.399	0.001
	PRE			1,109	111.907	<0.001
	PTF × YR			1,109	5.929	0.004
Species	SP	block/plot	pre ; sp	1,56	88.369	<0.001
	YR			1,56	21.218	<0.001
	PRE			1,56	121.400	<0.001
	SP × YR			1,56	19.313	<0.001
BNPP						
Total (0-20 cm)	P	block	t ; p	1,25	7.090	0.013
Depths	DPT	plot	dpt	1,67	79.613	<0.001
	P			1,14	4.202	0.060
	DPT × P			1,67	5.815	0.019
Root : shoot	P	plot	t	1,14	5.149	0.040

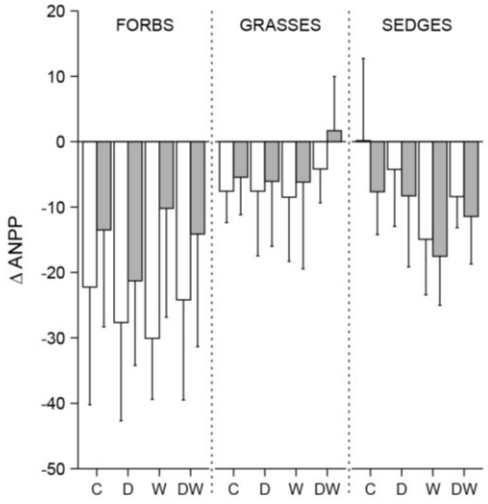
(a) Total vascular

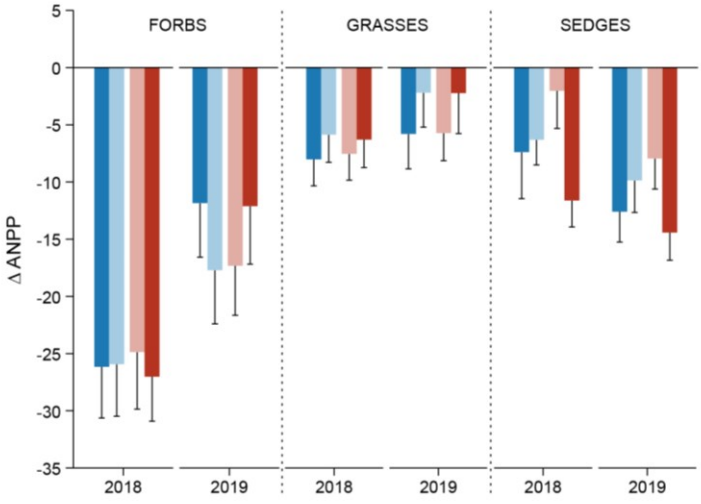




T *ns* P *ns* YR $p = .027$ PRE $p < .001$

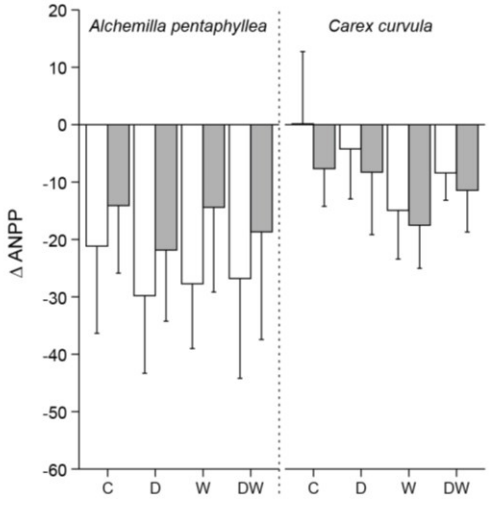
(b) Plant functional types

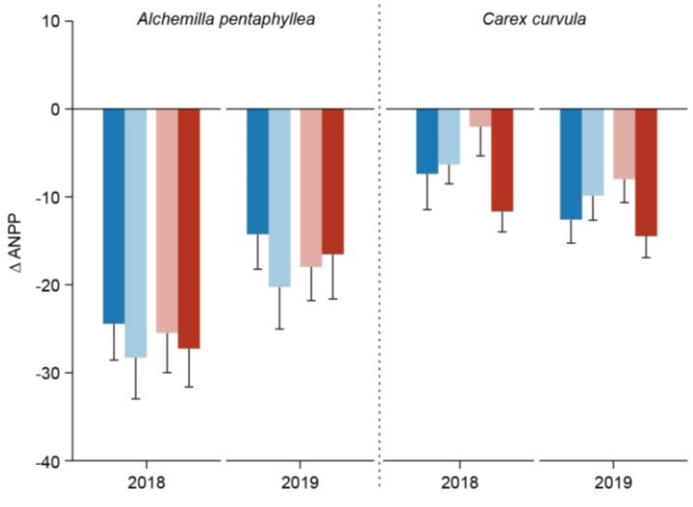




T *ns* P *ns* PFT $p < .001$ YR $p = .001$ PRE $p < .001$ PFT × YR $p = .004$

(c) Species



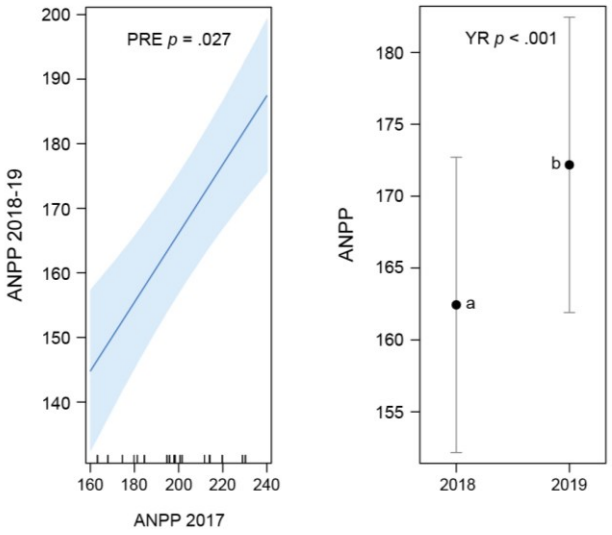


T *ns* P *ns* SP $p < .001$ YR $p < .001$ PRE $p < .001$ SP × YR $p < .001$

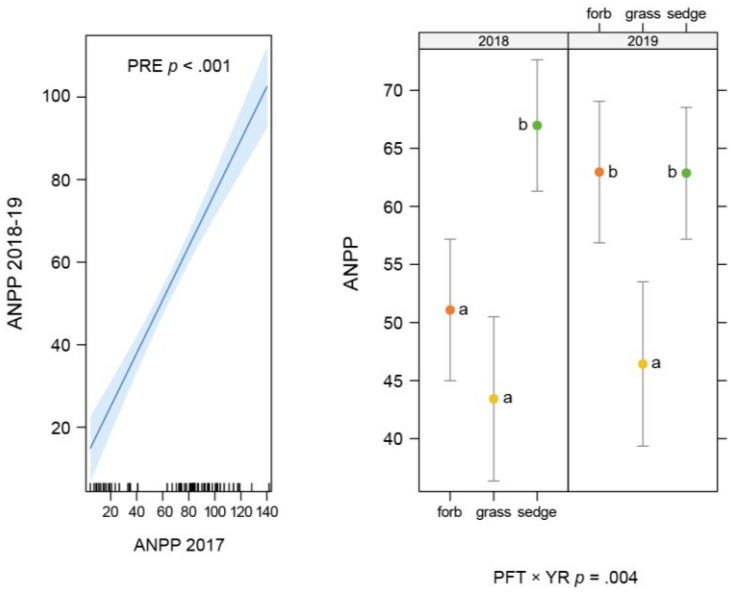
Fig. 4.7 (p. 70): Aboveground net primary production (ANPP, g m^{-2} y^{-1}) of **(a)** vascular plants, **(b)** plant functional types (PFT, forbs, grasses and sedges) and **(c)** the two dominant species (SP, *Carex curvula* and *Alchemilla pentaphyllea*) estimated for the two treatment years (YR, 2018 and 2019). ANPP is reported as ΔANPP, the difference between 2018 and 2019 with the 2017 pre-treatment year (PRE). Left-hand graphs indicate average value ± standard deviation measured in all treatments [C − control; D − drought; W − warming; DW − drought + warming]. Right-hand graphs compare average value ± standard error (n=10) estimated for different conditions of precipitation [P: =P, ambient precipitation (C and W plots); -P, precipitation reduction (D and DW plots)] and temperature [T: =T, ambient temperature (C and D plots); +T, warming (W and DW plots)]; the p-value of each fixed effect is reported, with "ns" indicating non-significant terms (p>0.05) excluded during the model selection process.

At a more detailed level, when considering total ANPP of vascular plants, 2019 production was 6% higher than the previous year [estimated values, 172 ± 95% confidence interval (CI) 10 g m^{-2} y^{-1} in 2019 vs 162 ± CI 10 g m^{-2} y^{-1} in 2018] (**Fig. 4.8a**, right-hand graph). Turning to ANPP of the three main PFTs, sedges were the most abundant group, followed by forbs and grasses (**Fig. 4.7b**, **Fig. 4.8b**). PFTs were also found to respond differently across the two years, with sedges and grasses exhibiting similar values in 2018 and 2019, whereas forbs showed a significant increase in the second year compared to the previous year (**Fig 4.8b**, right-hand graph). As far as analysis on the most abundant species is concerned, results reflected those of PFTs (**Fig. 4.8c, right panel**). Estimated ANPP of CC was significantly higher than AP and a general increase in production was recorded in 2019 compared to 2018. Species responded differently across the two study years, exhibiting contrasting temporal trends: while values for AP were significantly higher in 2019 compared to 2018, those for CC were slightly lower during the second treatment year, although this turned out not to be significant, as revealed by the post-hoc test.

(a) Total vascular



(b) Plant functional types



(c) Species

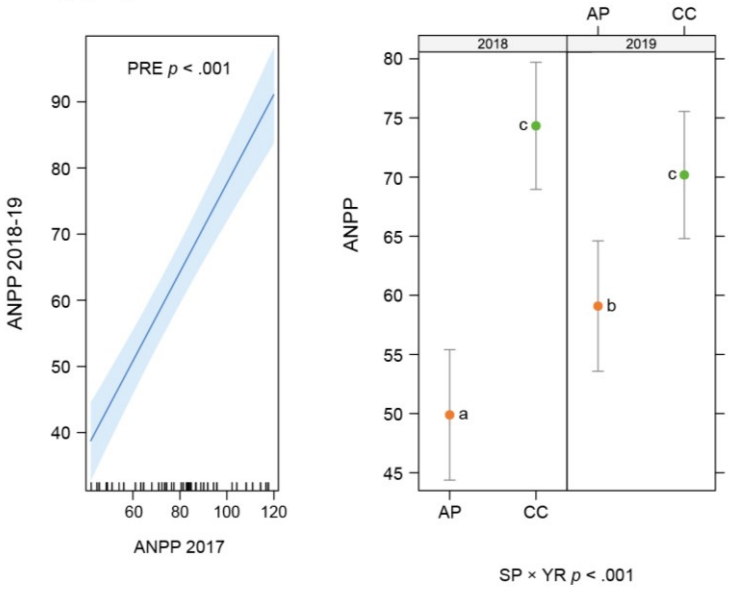


Fig. 4.8 (p. 72): LMMs results on aboveground net primary production (ANPP, g m^{-2} y^{-1}) of **(a)** vascular plants, **(b)** plant functional types (PFT, forbs, grasses and sedges) and **(c)** the two dominant species (SP, *Carex curvula* and *Alchemilla pentaphyllea*) estimated for the two treatment years (YR, 2018 and 2019). Left-hand graphs report the relation between ANPP estimated in 2018 and 2019 with those for the pre-treatment year (PRE: 2017). Right-hand graphs indicate the temporal trend of ANPP (YR: years) and the interaction between years and groups (PFT: plant functional types; SP: species); bars represent the 95% confidence interval. The p-value of each fixed effect is reported.

Since the sampling year was a significant factor in explaining differences across data when only ANPP values for 2018 and 2019 were considered, further analysis was performed to test responses over the whole study period (2017-2019), including the pre-treatment year (**Table 4.4**). This analysis highlighted an overall decrease in the two years following 2017 at all ANPP levels (**Fig. 4.9a-c**). In 2017, total ANPP of vascular plants was equal to 202 ± 10 g m^{-2} y^{-1}, whereas in 2018 and 2019 estimated values were 20% and 15% lower, respectively (**Fig. 4.9a**). At the PFT level, all groups exhibited lower ANPP values in 2018 and 2019 compared to the pre-treatment year (**Fig. 4.9b**): in 2018, ANPP of forbs was by 27% significantly lower (68 ± CI 7 g m^{-2} y^{-1}) compared to the 2017 (95 ± CI 9 g m^{-2} y^{-1}), while it increased in 2019 (80 ± CI 8 g m^{-2} y^{-1}); ANPP of grasses was by 36% lower in 2018 (11 ± CI 3 g m^{-2} y^{-1}) compared to 2017 (17 ± CI 4 g m^{-2} y^{-1}) followed by a slight, non-significant, increase in 2019 (14 ± CI 3 g m^{-2} y^{-1}); sedges exhibited an overall non-significant decrease (80 ± CI 6 g m^{-2} y^{-1} in 2018 and 76 ± CI 5 g m^{-2} y^{-1} in 2019) compared to 2017 (81 ± CI 6 g m^{-2} y^{-1}). The interaction at a species level was also maintained when 2017 was included in the analysis (**Fig. 4.9c**), with an evident decrease in 2018 of both AP (-38%) and CC in 2018 (-8%,) compared to 2017 – AP: 70 ± CI 12 g m^{-2} y^{-1} in 2017 vs 43 ± CI 10 g m^{-2} y^{-1} in 2018; CC: 88 ± CI 6 g m^{-2} y^{-1} in 2017 vs 81 ± CI 6 g m^{-2} y^{-1} in 2018. This was followed by a tendency towards an increase of AP in the following year (52 ± CI 9 g m^{-2} y^{-1} in 2019) and a continuous slight decrease in CC values (76 ± CI 6 g m^{-2} y^{-1}).

Table 4.4: Results of LMMs for aboveground net primary production (ANPP) of all vascular plants, plant functional types and species across the 3-year study period (2017-2019). Only significant fixed effects and interactions retained in the final model are reported: YR, years (2017-19); PFT, plant functional types (forbs, grasses and sedges); and SP, species (*Carex curvula* and *Alchemilla pentaphyllea*). Random effects and variance structures included in the final model are also reported.

Y	Fixed effects	Random	Variance	df (N,D)	F-value	p-value
Vascular	YR	plot	year	2,38	35.066	<0.001
Plant functional types	PFT			2,152	205.021	<0.001
	YR	plot	pft	2,152	10.425	<0.001
	PFT × YR			4,152	2.512	0.044
Species	SP			1,95	10.200	0.002
	YR	plot	year ; sp	2,95	7.051	0.001
	SP × YR			2,95	3.991	0.022

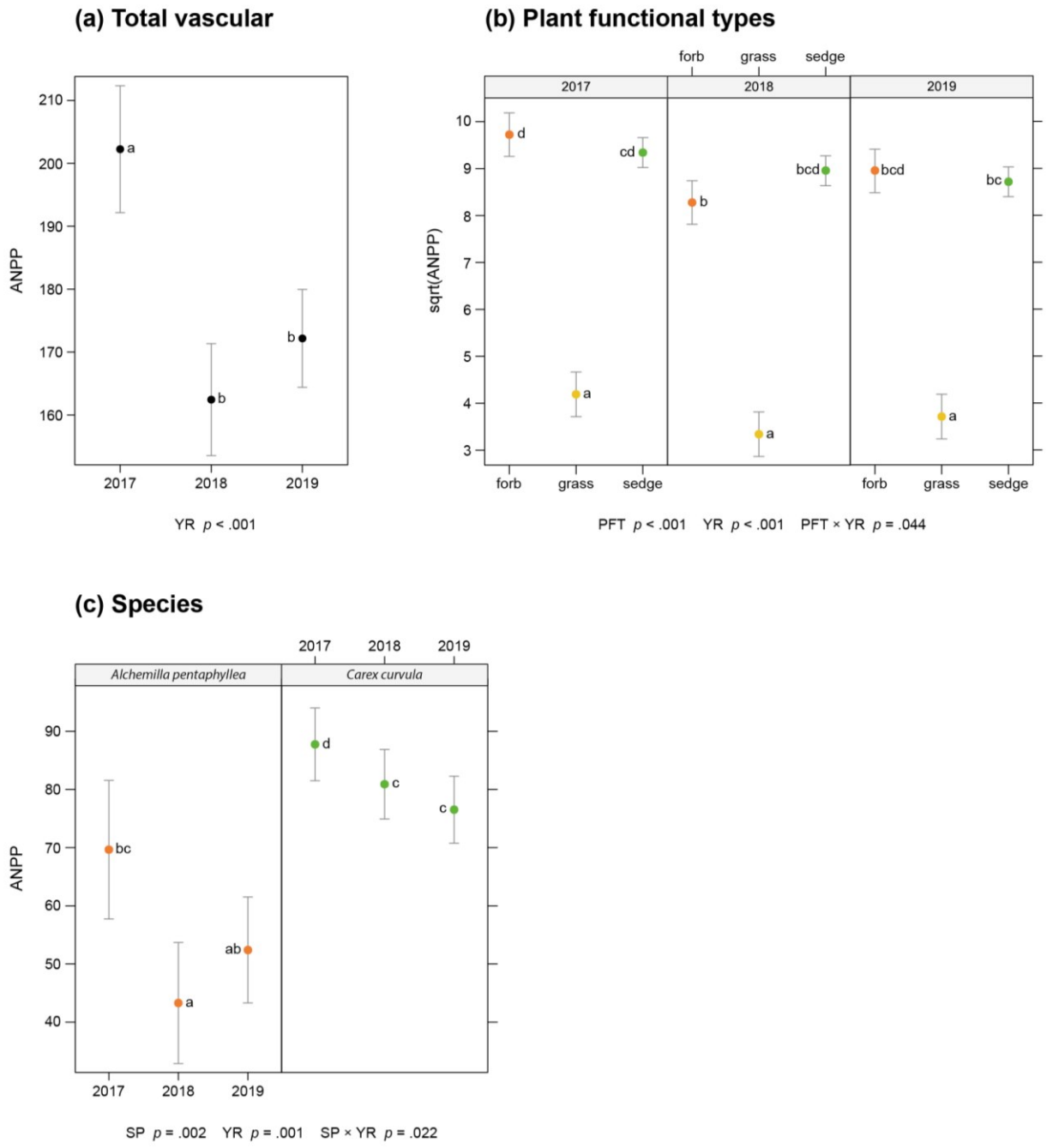


Fig. 4.9: Output of LMMs results on aboveground net primary production (ANPP, g m^{-2} y^{-1}) for **(a)** vascular plants, **(b)** plant functional types (PFT: forbs, grasses and sedges) and **(c)** the two dominant species (SP: *Carex curvula* and *Alchemilla pentaphyllea*) estimated for the 3-year study period (YR: 2017- 2019). The 95% confidence interval is reported and the p-value of each fixed effect is specified.

4.4.4 BNPP

ANOVA output for BNPP is shown in **Table 4.3**, where only significant terms are reported. Precipitation reduction significantly affected total BNPP (0-20 cm), with mean values 19% lower under shelters (estimated value, 253 ± CI 50 g m^{-2} y^{-1}) when compared to ambient precipitation conditions (311 ± CI 58 g m^{-2} y^{-1}) (**Fig. 4.10a**). In contrast, warming alone or in interaction with drought was not significant in explaining the variation observed (**Fig. 4.11a**). Pre-treatment values and years were also excluded during the model selection process as they were found not to significantly influence total BNPP.

Analysis of different depths – surface (0-10 cm) and deep (10-20 cm and 20-30 cm) – revealed a heterogeneous distribution of roots along the investigated soil profile, with most BNPP present in the near-surface layer (approx. 62%) (**Fig. 4.11b**). Furthermore, the distribution of roots in the soil profile was not consistent between plots under ambient precipitation conditions (=P) and those under shelters (-P) (interaction term in **Table 4.3**, **Fig. 4.10b**). In detail, a tendency towards lower BNPP (-22%) was observed in the 0-10 profile under shelters compared to that in ambient conditions, whereas in the deep profile BNPP values under the shelters were slightly higher (+14%) than controls (**Fig. 4.10b**). Post-hoc comparison within the same depth, however, revealed that differences across the P treatment were not significant (**Fig. 4.10b**). Even within depths, pre-treatment values and years were non-significant factors when explaining BNPP variations.

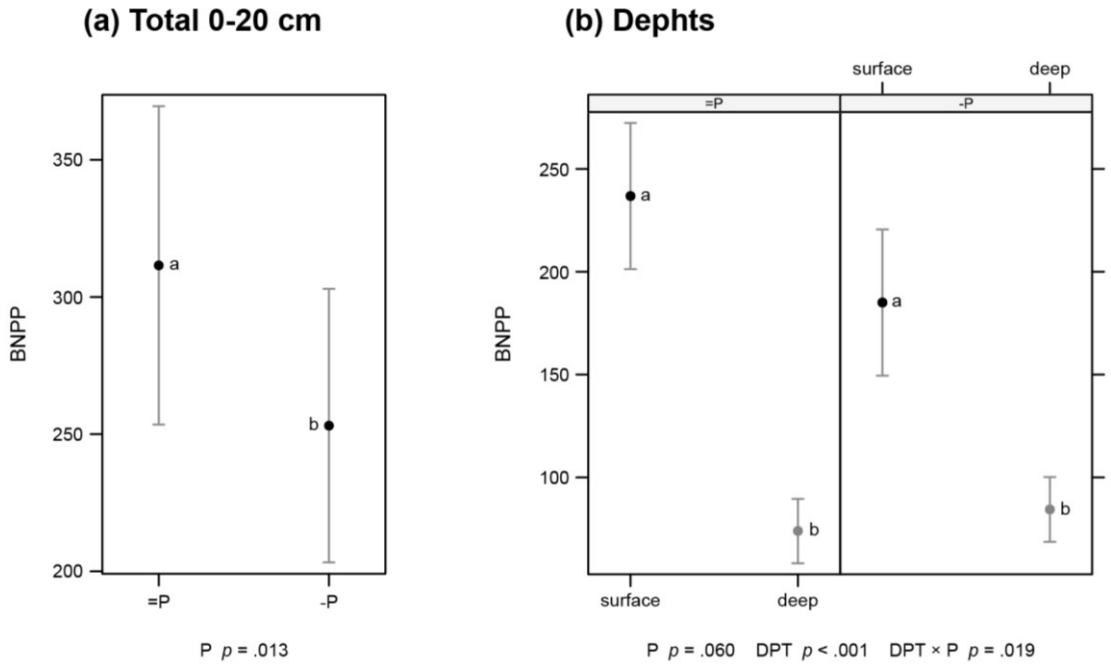


Fig. 4.10: Output of LMMs results on belowground net primary production (BNPP, g m-2 y-1) in response to different precipitation [P: =P, ambient precipitation (C and W plots); -P, precipitation reduction (D and DW plots)] of **(a)** the whole 0-20 cm soil profile and **(b)** across the two investigated depths [DPT: superficial (0-10 cm soil profile); deep (10-20 cm and 20-30 cm soil profiles) estimated in the two treatment years (2018 and 2019). The 95% confidence interval is reported and the p-value of each fixed effect is specified.

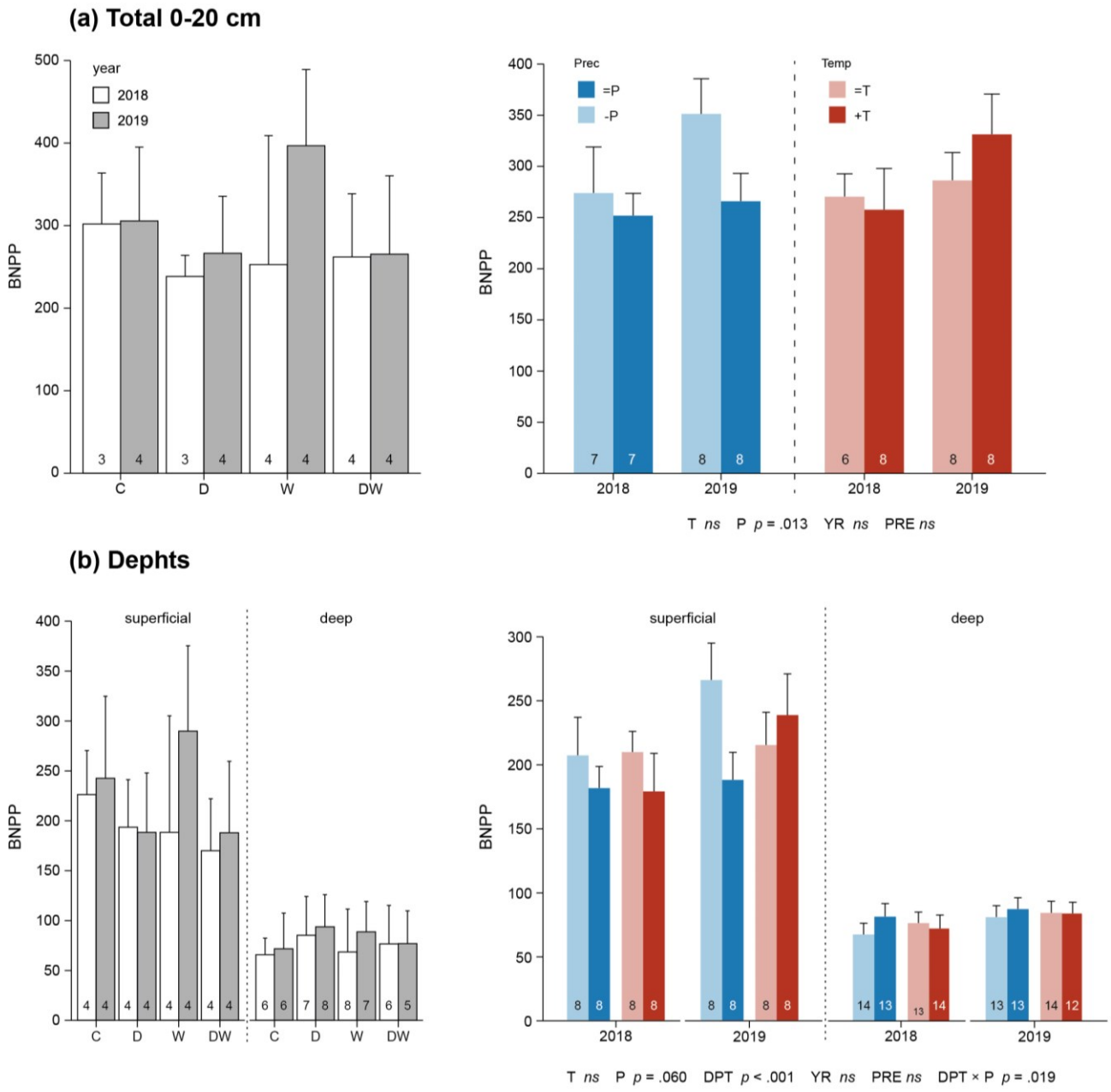


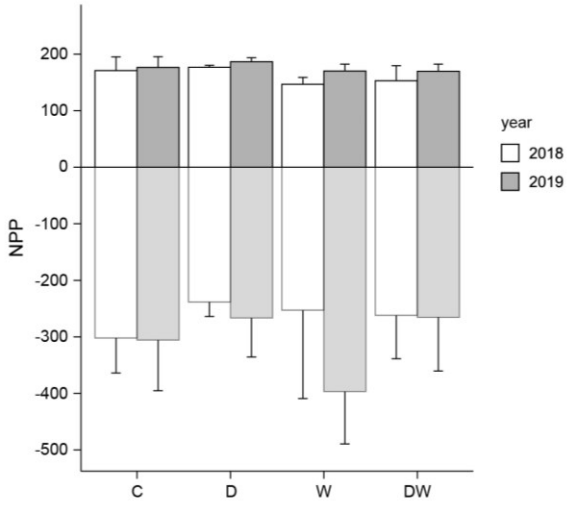
Fig. 4.11: Belowground net primary production (BNPP, g m^{-2} y^{-1}) estimated for the two treatment years (YR: 2018 and 2019) **(a)** for the whole 0-20 cm soil profile and **(b)** across the two investigated depths [DPT: superficial (0-10 cm soil profile); deep (10-20 cm and 20-30 cm soil profiles). Left-hand graphs indicate average value ±standard deviation measured in all treatments [C − control; D − drought; W − warming; DW − drought + warming]. Right-hand graphs compare average value ± standard error estimated for different conditions of precipitation [P: =P, ambient precipitation (C and W plots); -P, precipitation reduction (D and DW plots)] and temperature [T: =T, ambient temperature (C and D plots); +T, warming (W and DW plots)]; the p-value of each fixed effect is reported, with "ns" indicating non-significant terms (p>0.05) excluded during the model selection process. The sample size is reported at the base of the graphs.

4.4.5 NPP and root:shoot ratio

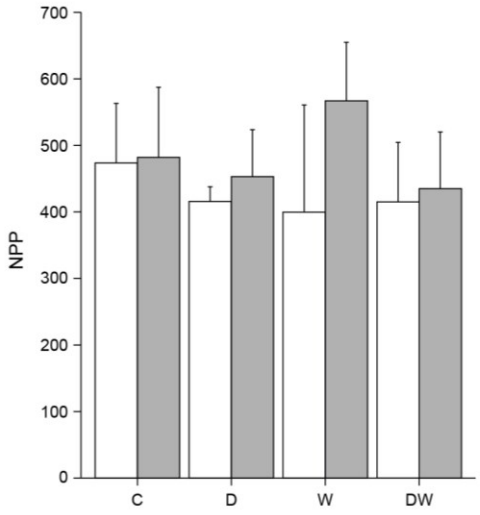
Analysis performed by means of model selection showed that neither increased temperature, reduced precipitation nor their interaction significantly affected overall NPP values in the target grassland ($p>0.05$) (**Fig. 4.12a, b**). Root:shoot ratio values (**Fig. 4.12c**), on the other hand, were found to be 19% lower under precipitation reduction treatments compared to those in ambient conditions [1.44 ± CI 0.22 (n=2) g m^{-2} y^{-1} in -P vs 1.79 ± CI 0.22 g m^{-2} y^{-1} in =P] (**Table 4.3**). During the selection process, years were excluded as they did not significantly influence NPP or biomass partitioning either on their own or in interaction with treatments.

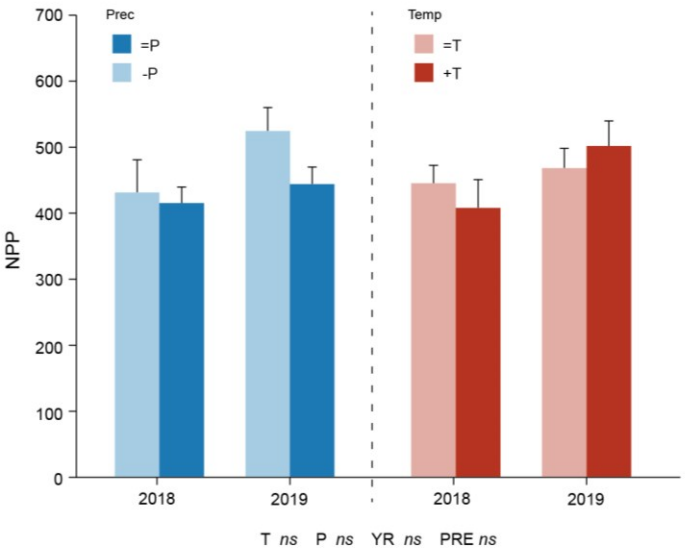
Fig. 4.12 (p. 78): **(a)** Above- and belowground net primary production (g m^{-2} y^{-1}), **(b)** net primary production (NPP, g m^{-2} y^{-1}), and **(c)** root:shoot ratio, estimated in the two treatment years (YR, 2018 and 2019). Left-hand graphs indicate average value and standard deviation measured in all treatments [C − control; D − drought; W − warming; DW − drought + warming]. Right-hand graphs compare average value and standard error (n=8, except for =P and -P in 2018, n=7) estimated for different conditions of precipitation [P: =P, ambient precipitation (C and W plots); -P, precipitation reduction (D and DW plots)] and temperature [T: =T, ambient temperature (C and D plots); +T, warming (W and DW plots)]; the p-value of each fixed effect is reported, with "ns" indicating non-significant terms ($p>0.05$) excluded during the model selection process.

(a) Aboveground and belowground net primary production

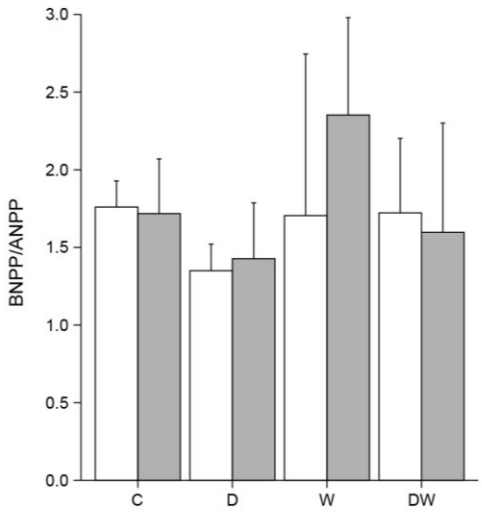


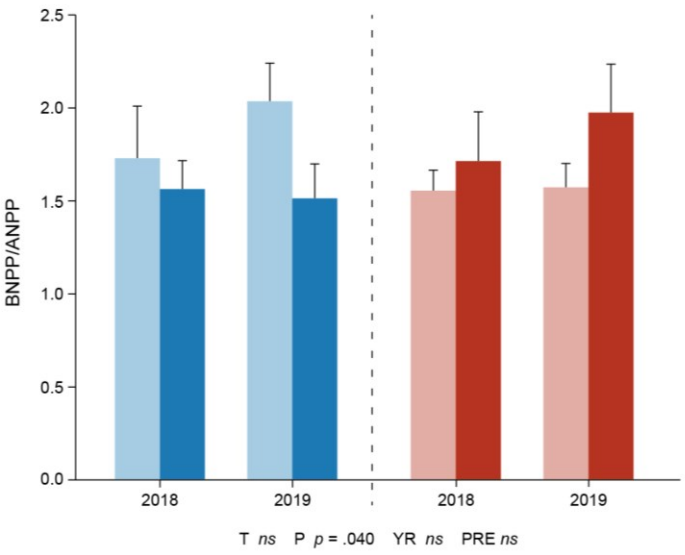
(b) Net primary production





(c) Root:shoot ratio





4.5 DISCUSSION

Although it is clear that microclimate features were effectively modified by manipulations, short-term results from the present study reveal that the investigated alpine grassland is, to a certain extent, resistant in terms of NPP to experimentally increased temperature and site-specific precipitation reduction. However, when plant cover across the main species within the community was analysed, there was evidence of precipitation reduction effects and a change in cover values over time for the two dominant species, *Carex curvula* and *Alchemilla pentaphyllea*. In addition, with regard to primary production, diverse responses were detected above- and belowground, with non-significant ANPP responses to treatments, together with lower root production in response to -P during the first 2 years of manipulation.

It is generally acknowledged that NPP responses could dampen the effects of climate treatments on above- or belowground ecosystem compartments. For instance, in a meta-analysis on responses to experimental changes in temperature and precipitation, Wu et al. (2011) highlighted the fact that warming and precipitation reduction may counteract each other, resulting in no significant effect of treatments. This was also found to be the case in alpine grasslands in the Tibetan Plateau, where artificial warming and drought caused a decrease in ANPP, while increasing BNPP, resulting in no significant change when assessing effects on NPP (Liu et al., 2018). This pattern was also observed in a *Carex curvula* grassland in the Swiss Alps (Schmid, 2017).

Analysis from the present study, in contrast, revealed lower BNPP under shelters, but this was not sufficient to affect the NPP of the entire community. Overall, effects of treatments were detected when results of different levels of investigation were taken into account. Moreover, ANPP significantly varied over the time period under consideration (2017-19) with the initial state of the community being a major driver of variation across the plots.

4.5.1 Effects of treatments on microclimate

During the two treatment years, microclimate data revealed that treatments effectively altered temperature and precipitation. In 2018 there was a 41% reduction in cumulative rainfall under shelters − an amount corresponding to the statistical extreme reduction calculated on the basis of a site-specific historical record. In 2019 there was only a 33% reduction – this lower figure could be explained by the nature of climatic events during that year: under heavy rainfall roof panels on shelters may have been less efficient, causing raindrops to bounce in the space between each panel, eventually reaching the area under the shelter; strong winds during rainfall events could also be a factor because of rain entering the area under the shelter from the open sides. Unfortunately, no data is currently available to test this last hypothesis, as the site's

weather station was not functioning correctly. Aside from these considerations, it is also worth noting that the weather station measuring ambient precipitation is situated about 1 km from the study site, leading to the possibility of bias in data comparison between precipitation treatments.

As far as temperature is concerned, OTCs effectively increased canopy air and soil temperature (approx. 1 and 1.5 K), with slightly higher average temperatures in chambers located under the shelter (**Fig. 4.3**). Since similar air temperatures at 20 cm were measured both in ambient precipitation conditions and under the shelters (see Chapter 3, Section 3.3.2), temperatures may have been influenced by the lower VWC in D and DW plots. Soil water content can, in fact, directly influence soil properties – soil thermal conductivity and heat capacity – therefore, impacting soil temperature (Al-Kayssi et al., 1990) which, in turn, tends to increase air temperature at the canopy level.

With respect to soil moisture, VWC measured in 2019 showed lower moisture values under shelters, especially in the layer nearer the surface (-5 cm), while differences between treatments decreased at -15 cm and -25 cm, revealing more stable moisture conditions at these depths (**Fig. 4.5a**). In addition, fluctuations in shelter effect on VWC was slightly greater in the near-surface layer, which could have been the result of a more intense drying-re-wetting cycle (**Fig. 4.5b**). The warming treatment plots also exhibited lower VWC compared to controls (**Fig. 4.5a**), which is in line with results on the impacts of OTCs on microclimate reported in 20 studies in alpine and polar sites, with soil drying being an artefact effect of chambers (Bokhorst et al., 2013).

It should be noted, however, that soil moisture reduction under the shelters was not nearly as intense as precipitation reduction, with VWC approx. -3.5% compared to ambient values. In a study evaluating precipitation effects on productivity using shelters similar to the one in the present study, Henry et al. (2018) also report reduced effects treatments on VWC. These outcomes are not surprising, since soil moisture can be reconstituted by a number of factors that are difficult to control in rainfall exclusion experiments. As reported by Schmid (2017), these factors include: (a) surface and sub-surface runoff during strong rainfall events; (b) wind-blown rain reaching the area under the shelter (as mentioned above); and (c) fog and dew deposits. Stable belowground humidity levels may also help prevent excessive drying of the upper soil layers through upward water flow. Having said this, it is generally acknowledged that obtaining a precise picture of soil water status is an extremely difficult task. This is mainly due to high spatial heterogeneity and temporal dynamics, which are, in turn, dependent on a number of factors, including soil type and structure, and the identity and performance of plants (Kreyling et al., 2017 and references therein). For instance, even within the same site the presence of a large proportion of stones in the measured volume of soil may reduce values irrespective of treatments. It is, therefore, necessary to take further measurements of the whole vertical profile

under investigation, rather than limiting measurements to a single depth and recording data within a homogeneous matrix to better assess treatment effects on soil moisture status.

4.5.2 Increased temperature: no effects of two years of artificial warming

With regard to warming, the fact that there was no evidence of an enhanced response to increased temperatures seems to be in contradiction with the general thrust of research indicating that cooler ecosystems are more sensitive to warmer conditions; the release of the main abiotic constraint driving productivity in these environments has, in fact, been found to produce a general increase in ANPP (Elmendorf et al., 2012b; Bjorkman et al., 2018; Berner et al., 2020). Along with this general pattern, it has also been observed that: (a) the magnitude of change in response to warming is site-specific and varies across plant functional types (Elmendorf et al., 2012a, b); (b) species-specific responses to increased temperature have been detected in other cold ecosystems (Jónsdóttir et al., 2005; Klanderud, 2008; Hoffmann et al., 2010); and (c) inherent variability in investigated species' traits could mask treatment effects (Hollister et al., 2005). The absence of a warming effect on above-ground production (**Fig. 4.7a**) could, therefore, depend on which species are present in the investigated grasslands, as supported by findings from other studies on ANPP. For instance, sedge production was found to be reduced in response to 4 years of experimental temperature increase in alpine grasslands of the Tibetan Plateau (Liu et al., 2018). Furthermore, inconsistent responses across sedges species belonging to the genus *Carex* within an alpine heathland were reported by Klanderud (2008), highlighting large variability in responses for this group reported in the literature and leading the author to speculate that soil moisture was the driver of such diversity. Erschbamer (2001), also investigating the genus *Carex*, reported a greater decrease in the number of shoots of *C. curvula* in OTCs after two years compared to controls, although differences were not statistically significant. *Carex curvula* showed the same tendency in the present study, with a decreasing trend (**Fig. 4.7c**), although this resulted as non-significant when differences were statistically assessed. With regard to forbs in alpine ecosystems, on the other hand, Winkler et al. (2016) found these to be generally stimulated by warming when water was not a limiting resource. Forbs in the present community actually showed greater ANPP values in OTCs compared to ambient temperature conditions in 2019 and lower values under shelters (**Fig. 4.7b**). However, when analysing *Alchemilla pentaphyllea*, the dominant forb within the community, such a tendency was less evident (**Fig. 4.7c**). As far as grasses are concerned, the species *Poa alpina*, the second most diffuse and abundant graminoid within the community under study, was found to have both a weak (bulbiferous form) or no response (viviparous form) to artificial warming (Erschbamer et al., 2007). In a similar way, grasses in this study did not seem to respond to warming over the two years, with constant ANPP values (**Fig. 4.7b**). Although the large variability in the data does not

allow robust conclusions to be reached, there is, nevertheless, a preliminary indication of inconsistent responses to warming by the community's main species – overall, the sedge-dominated community could be generally less responsive to warmer temperatures in the short-term compared to other alpine ecosystems when aboveground production is analysed.

Turning to belowground responses, the even greater variability in BNPP data, especially for the warming treatment, did not allow any effect of increased temperatures on root production to be detected, although a trend towards greater BNPP in W could be detected for the year 2019 (**Fig. 4.11a**). These differences were not related to the initial condition of plots, since no relation between BNPP with the initial standing biomass present during the pre-treatment year revealed after analysis, as opposed to that found for ANPP. This was probably due to the fact that in 2017 root biomass represented the sum of both new and old, standing roots which are characterised by slow turnover rates (Grabherr, 1989) – in this year root biomass was, in fact, on average almost 3 times more than root production for a single year (see Chapter 2, Section 2.2.2). No significant responses – just increasing trends – were also reported for alpine ecosystems subjected to 3-4 years of experimental warming (Liu et al., 2018; Liu et al., 2021). The already existing adaptation of alpine plants to large diurnal-nocturnal thermal fluctuations, with a difference of up to 13 K, was suggested by Liu et al. (2020) as a possible explanation of the stability of BNPP to the +1.5 K experimentally increased temperature.

Taking the results as a whole, there is reason to believe that a 2-year period of increased temperature may not be enough to produce any clearly detectable effect on biomass production. A lag between start of treatment and ecosystem response may be underway (Chapin et al., 1995). This is backed up by the meta-analysis by Wu et al. (2011) which found that increase in biomass responses to warming were only apparent in long-term climate change experiments (>5 years).

4.5.3 Precipitation reduction: differences between above- and belowground responses

Aboveground responses: plant cover

Analysis on plant cover revealed small but significant effects of reduced precipitation on cover variation relative to values prior to climate manipulations, with *taxa* exhibiting diverse responses (**Fig. 4.6**). In particular, the effect of precipitation reduction was mainly driven by *Alchemilla pentaphyllea*, the most abundant forb within the grassland, which exhibited reduced cover values under the shelters (-3%) compared to increased values in ambient conditions (+3%). Other *taxa*, such as the forb *Leontodon helveticus* or cryptogames (mosses and lichens) also showed reduced values under reduced rainfall conditions, while *Carex curvula* cover only slightly decreased. In contrast, the most abundant grasses either slightly increased their cover under drought – as was the case of *Agrostis rupestris* – or showed no variation, like *Poa alpina*. Cover results were partially supported by trends observed in the ANPP data: for instance, grasses exhibited a greater

(non-significant) average ANPP value under shelters, partially in line with the cover increase observed for *Agrostis rupestris*. On the other hand, *Alchemilla pentaphyllea* showed a tendency towards lower ANPP in reduced rainfall plots, thus confirming visual cover estimation output.

The variety of responses highlighted by the RDA could be the result of the direct effects of drought on species' physiological functioning and/or an indirect consequence of modified plant-plant interactions. It is well established that species exhibit different tolerance to drought (Tardieu and Simonneau, 1998; Signarbieux and Feller, 2012; Bollig and Feller, 2014; Niu et al., 2014), which depends on a number of traits allowing certain species to better cope with water scarcity. These include greater rooting depth (Zeiter et a., 2016; Stampfli et al., 2018), smaller leaf area (De Boeck et al., 2016) and isohydric stomatal behaviour (Tardieu and Simonneau, 1998). For alpine species, such species-specific difference was observed in early studies conducted at the end of the last century. For instance, Oberbauer and Billings (1981) observed a great variability in leaf conductance and rooting depths along natural gradients of soil moisture so that, when faced with drought, some species were more affected than others – e.g. early leaf senescence recorded in some species, while others continued to flower despite drier conditions. The variability in rooting depth across species was identified as one of the main traits determining the variety of water-use patterns across species (Oberbauer and Billings, 1981). Another early study by Peterson and Billings (1982) which analysed the responses of 11 alpine species to drought – simulated as a reduction in water frequency – showed that almost half of the *taxa* were not significantly affected by reduced water availability, while 7 species showed reduced growth. In this case, responses were associated with the species' original habitat, with the most sensitive being those living in habitats receiving meltwater from permanent or long-lasting snowbanks and thus not experiencing water shortage.

Species within the present research community are, therefore, likely to be characterised by particular traits enabling some species to cope with drier conditions better than others. For instance, according to Landolt ecological indicator values (Landolt et al., 2010), *Alchemilla pentaphyllea* is a typically chionophile species, which thrives in snow-dominated patches and areas with high soil moisture levels; this species has traits which allow it to cope with a short growing season – high relative growth rate and clonal reproduction (Körner, 2003; Choler, 2005) – but, on the other hand, the ecological niche to which it is adapted may also have selected a set of traits making *A. pentaphyllea* particularly vulnerable to lower water availability. Regarding *Leontodon helveticus*, the larger leaf area characterising leaves of this species compared to that of grasses, for instance, may induce greater water loss though transpiration (De Boeck et al., 2016), hence representing a disadvantage in drier conditions. Moreover, the high levels of frost tolerance reported for both *Carex curvula* and *Poa alpina* (Körner, 2003) could represent an

adaptive strategy to freezing dehydration which could guarantee a certain resistance of these species to reduced water availability.

Another factor which needs to be taken into consideration is the influence of abiotic stress on the balance of biotic interactions – i.e. competition and facilitation (Kreyling et al., 2008; Walter, 2018). For instance, Grant et al. (2014) found that facilitation mechanisms increased under drought in temperate grasslands, with some species being released from growth competition in relation to reduced water availability. In contrast, among plant functional types, Stampfli et al. (2018) found that competition drove the decline in forbs after drought as a result of the higher performance of grasses. In the present research, cover changes of some species may, therefore be induced by alterations in the cover of neighbours: the increase of the grass *Agrostis rupestris* under shelters may be a consequence of reduced cover of the most abundant species so that competition for light was released. In *Carex curvula* grasslands, *A. rupestris* was, in fact, among the species most able to fully and rapidly recolonize free patches, where all species and the top soil layer had been artificially removed (Erschbamer et al., 2003).

Unfortunately, the short-term data available does not allow any hard and fast conclusions to be reached. The set of traits underlying the different degree of sensitivity to drought of the species within the investigated community needs to be identified, together with more detailed measurements on resource acquisition strategies. This should be accompanied by focused experiments investigating neighbour effects in order to disentangle the role that biotic interactions have in driving ecosystem responses to climate change.

Aboveground responses: ANPP

Despite the convergence of cover and production data on the direction of change at species level, the lack of significant effects of precipitation reduction on ANPP highlights the fact that the degree to which altered climate variables acted on the investigated community was not sufficient to produce a significant change in aboveground production as estimated using the point intercept method. In fact, in the present study no treatment effect was detected on ANPP during the first two treatment years (**Fig. 4.7a-c**), in line with other studies assessing precipitation reduction effects on herbaceous ecosystems (Kreyling et al., 2008; Jentsch et al., 2011; Carroll et al., 2021), but in contrast with drought experiments in alpine grasslands, which reported a general tendency towards a decrease in ANPP under decreased rainfall scenarios (Schmid et al., 2011; Gilgen and Buchmann 2009; Schmid, 2017; Zhang et al., 2017; Liu et al., 2018). However, there is a lack of consistency in the literature on the magnitude of ANPP responses for alpine ecosystems. The absence of a precipitation reduction effect on ANPP in the studied grassland is, for instance, in accordance with outcomes reported in Zhang et al. (2017), who found that in along an artificial precipitation gradient, alpine grasslands exhibited a significant reduction of plant growth only

under the most extreme reduction scenario (-92% of annual precipitation). In a *C. curvula* alpine grassland located in the Swiss Alps, instead, the ANPP reduction observed by Schmid et al. (2011) and Schmid (2017) was the result of full-rainfall exclusion which, according to the author, effectively reduced precipitation by 50%. Results from the present study, therefore, indicate once more that a variety of complex mechanisms are at work in determining responses of a single investigated variable. These mechanisms include the type of experimental drought applied, possible interactions of drought with other abiotic variables (e.g. temperature), the duration of the experiment, the mean annual precipitation (MAP) characterising the site and, finally, the biotic interactions present within the investigated ecosystem.

With regard to the interplay with other variables, the present study tested the effects of combined drought and increased temperature, with no significant ANPP response. This is likely to be the result of the extent to which passive OTCs act on both temperature and air dryness, which is not equivalent to naturally occurring drier, warmer years – naturally occurring droughts are usually accompanied by heat waves, which not only determine a temperature increase but also higher evaporation loss and higher VPD (De Boeck et al., 2010; De Boeck and Verbeeck, 2011). For instance, De Boeck et al. (2010) reported a difference in average maximum temperatures during heat waves equal to +8.0 K, accompanied by an increase in the daily number of hours of sunshine (>50% in summer) and a two-fold increase in VPD (>1.2 kPa). During drought and heat waves, plant responses to high temperatures and water shortage are particularly affected by VPD, which drives mechanisms of stomatal closure, hence influencing photosynthetic activity, rates of transpiration and soil water loss (see recent review by Grossiord et al., 2020). By further depleting soil moisture resources, a combination of these two climate extremes is, therefore, likely to determine larger productivity responses than those observed in single factor drought experiments (De Boeck et al., 2016; Hoover et al., 2018). The grassland analysed in the present study was not subject to such conditions in the DW treatment, since average daily maximum temperatures in OTCs were only 3.6 K higher than ambient temperature across the two seasons, and VPD values (average 0.3 kPa), although slightly higher in treatments, were well below those experienced during natural droughts. Rainfall frequency was also high in both treatment years: in 2018, 60% of the 50-day period preceding the point intercept date were rainy days, with 62% during the same period in 2019; in both experimental growing seasons, there were on average 1.8 consecutive days without precipitation – see Chapter 3, Section 3.3.2 for graphs on precipitation distribution during the growing season. It has to be noted, however, that as the present study was not set-up to simulate heat waves, these results merely underline that, under a slightly warmer scenario, effects of reduced precipitation amounts would not be intensified in the short term.

As far as the type of experimental treatment is concerned, the high rainfall frequency was probably a determining factor in defining the intensity of the drought treatment, since the passive

precipitation reduction imposed is known to be strongly tied to climate conditions naturally occurring in the surrounding environment (Hoover et al., 2018). Even in experiments using full-exclusion shelters, a study quantifying the influence of weather conditions on the eco-physiological responses of *Plantago lanceolata* demonstrated how responses were highly dependent on ambient temperature and VPD, which modulated drought-treatment responses (Kreyling et al., 2017). Furthermore, the duration of the drought period could influence ANPP responses to altered precipitation (Sala et al., 2012). In the *Carex curvula* grassland studied by Schmid (2017), the impacts on ANPP, although already significant after 6 weeks, further increased under the most extreme drought scenario (12 weeks). In the current study, the shelters used at the Gavia site acted only on rainfall amount without any influence on the number of days without rain. The type of precipitation applied corresponds to a chronic, press reduction which, by inducing slow reduction of soil water content, have a lower probability of exceeding water stress thresholds (Hoover and Rogers, 2016; De Boeck et al., 2018a). It is, therefore, to be expected that effects on ANPP of drought simulated through the type of shelters employed within this study are minimal in both very dry or very wet years (Henry et al., 2018), although some studies in the literature evidence negative effects on ANPP in those years naturally experiencing well below average precipitation (Bloor et al., 2010) or when the system actually undergoes a decrease in soil water content values, reaching moisture thresholds that trigger significant ecosystem responses (Hoover et al., 2014).

Moreover, a 2-year period of drought, while not significantly affecting plant responses in the short term, may have a cumulative effect over multiple years of drought, which can only be detected by long-term-experiments. Recurrent climate extremes can, in fact, weaken the resistance of a community or of a species to further perturbation (Walter et al., 2011; Dreesen et al., 2014). Such considerations underline the need for: (a) longer time series available to assess the full extent of precipitation reduction on ANPP; and (b) simulations of more severe drought to identify benchmarks for change in ecosystem structure and functions, and improve the mechanistic understanding of ecosystem processes and performance under extreme events (Beier et al., 2012; Estiarte et al., 2016; Knapp et al., 2017b).

In view of the above, there might be other explanations for the observed results on ANPP when compared to other drought manipulation studies. The high levels of MAP (>1000 mm) recorded at the site may buffer the effects of precipitation reduction on ANPP in contrast to what can be observed in sites with lower MAP. Such a hypothesis is supported not only by outcomes deriving from studies comparing drought responses of different grassland sites along an increasing MAP gradient (Gilgen and Buchmann, 2009, Knapp et al., 2015a), but is also confirmed by a recent meta-analysis on primary productivity responses to extreme drought of grassland and forest ecosystems (Stuart-Haëntjens et al., 2018). In particular, the authors of the meta-analysis

found that MAP was the main predictor of ecosystem resistance to drought – i.e. higher MAP relates to greater resistance – as a result of drier ecosystems "operating closer to water limitations" (Stuart-Haëntjens et al., 2018). Furthermore, in snow-dominated ecosystems, such as the one under investigation, winter precipitation recharges water reservoirs through snowmelt input, contributing to high levels of soil moisture during the first part of the growing period, where plants are most active (Taylor and Seastedt, 1994; Williams et al., 2009). Considering that alpine communities mainly consist of perennial herbaceous species with fast development rates (Körner, 2003), it is likely that a c. 40% reduction of summer rainfall, extended to the entire growing season, is not sufficient to cause water stress in the target plant community in the short term, given the amount of water available from snowmelt.

The lower biomass of the studied plant community compared to other grasslands could also explain ANPP stability to precipitation reduction. Experimentally constructed communities with low biomass, for instance, were found to be more resistant to drought in terms of ANPP than those characterised by higher biomass values, which is probably due to lower soil water resource depletion resulting from both lower evapotranspiration and less competition for soil water (Wang et al., 2007). With 202 ± 23 g m^{-2} (data from the pre-treatment year), the grassland in the present study had lower biomass values than those of lower-elevation grasslands analysed in drought experiments. For instance, the subalpine grasslands investigated by Gilgen and Buchmann (2009) and by Prechsl et al. (2015) had a maximum ANPP value twice as high as the aboveground biomass of the alpine grassland under study.

A further hypothesis to explain the initial productivity resistance of the ecosystem to reduced rainfall could be found in the characteristics of alpine grassland species – longevity, clonality and slow growth (de Witte et al., 2012) – where responses to drought are difficult to record over a short time period (Beier et al., 2012). The dominant grassland sedge, *Carex curvula*, in particular, exhibits these traits (de Witte et al., 2012; Grabherr, 1989; Steinger et al., 1996), which are considered to be the main factor explaining ecosystem persistence in the face of climate change (Windmaißer and Reisch, 2013). The species is also characterised by a continuous presence of green leaves, together with a basal meristem which is able to continuously produce new tissue, enabling *C. curvula* to compensate, in the short-term, for lower rates of production due to unfavourable conditions during the growing season (Erschbamer and Winkler, 1995) – in the drier conditions reproduced in this study, it might, therefore, take some time before ANPP effects are detected.

In conclusion, as explained in the previous section, biotic interactions could play a decisive role, with an overall stabilizing effect on ANPP. High-elevation plant communities subject to high levels of abiotic stress generally exhibit positive interactions rather than competition when neighbours are present, with biomass, growth and reproduction greater than those observed in

lowland species (Callaway et al., 2002). This could be especially true for alpine grasslands such the present one, where the dense mat produced by *Carex curvula* might prevent not only the dominant species itself, but also co-occurring *taxa* occupying different ecological niches, experiencing excessive water loss (Körner, 2003). However, verification of this hypothesis with regard to the community under study would require specific neighbourhood removal experiments in the future.

Belowground responses: BNPP

Although manipulations did not affect ANPP, significantly lower root values were recorded under the precipitation reduction treatment (**Fig. 4.10a**). Analysis across depths revealed a lower BNPP in the near-surface layer in response to reduced rainfall, while deeper layers showed similar values across precipitation treatments (**Fig. 4.10b**). These results were partially due to lower values effectively being observed under the shelters together with higher average values recorded in the warming treatment (**Fig. 4.11b**). By comparing BNPP responses under shelters (-P) with those recorded in ambient conditions – including both warming and controls – the difference was sufficient to produce significantly lower values under reduced precipitation treatments. This reading was confirmed by the fact that no significant effect of treatments on BNPP responses ($p>0.05$) was evident when analysing treatments as a 4-level categorical factor. When looking at the general picture, however, it appears that reduced rainfall amount effectively led to lower BNPP, which was sufficiently low to result in marginally lower root:shoot ratios under the shelters (**Fig. 4.12c**), potentially indicating a different sensitivity to manipulations between the below- and aboveground compartments. This last result is in accordance with a previous finding which reported major drought impacts on the root biomass of grassland species compared to that aboveground (de Vries et al., 2016; Carroll et al., 2021). In another study, a meta-analysis of fine root responses to altered precipitation, major root responses to drought were found in short-term experiments (<3 years), after which adjustments of fine roots take place, which level out BNPP responses in the long-term (Zhang et al., 2019a).

At first glance, BNPP responses to precipitation reduction seem to contradict the general theory stating that a major allocation to the belowground portion of plants occurs during periods of water scarcity (Bloom et al., 1985; Qi et al., 2019). However, when investigating within a single ecosystem, other intrinsic features could explain the diversity in BNPP responses to hydrological limitation. These include: vegetative functional composition, root depth distribution and species-specific root traits, the timing of the event and the duration of the experiment (Wilcox et al., 2017; Zhou et al., 2018; Wang et al., 2019; Zhang et al., 2019a). Studies have confirmed the diversity of root responses of grassland ecosystems to drought, with increase in root production (Zhang et al., 2017; Liu et al., 2018; Liu et al., 2021), non-significant responses (Gilgen and

Buchmann, 2009; Prechsl et al., 2015) and even a decrease (Fiala et al., 2009; Zhang et al., 2018; Wang et al., 2019) all being reported. Along with these varied results, inconsistent BNPP responses to altered precipitation have also been found among different grassland types within a single biome (Wilcox et al., 2015) and among sites applying the same experimental protocol (Henry et al., 2018). In this last case, Henry et al. (2018), who assessed NPP responses to drought using the same experimental approach on drought that was applied in this study, found a site-specific root production response, with 2 out of 13 sites exhibiting lower values after a single year of manipulation. It could be that, in a similar way to ANPP, belowground responses are probably influenced by the vegetation type under investigation (Wang et al., 2019) together with specific species traits within the community (Zhou et al., 2018). Roots of herbaceous plants, for example, are highly responsive to rainfall reduction, a sensitivity which could depend on their root distribution, given that a large fraction of belowground material stands within the upper layer, which is more subject to drying (Wang et al., 2020). Furthermore, under drought conditions grassland species have been found to rely on water resources available in the top-soil layer (Prechsl et al., 2015). These attributes, taken in concomitance with increased root mortality under drier conditions (Zhou et al., 2018) could determine overall reduced root production.

The hypothesis described above could explain the observed results in the present study, considering that the majority of root biomass and root production (approx. 70%, see Chapter 2, Section 2.2.2) is located in the 0-10 cm soil layer (**Fig. 4.10b, Fig. 4.11b**), reflecting a typical feature of *Carex curvula* grasslands (Grabherr, 1989). The near-surface soil profile within the studied grassland was characterised by higher VWC compared to deeper soil profiles, but also showed greater variability in mean daily values probably due to higher rates of wetting and drying following the seasonal precipitation pattern. This last factor might have been sufficient to alter root production in the upper soil layer as a result of modified belowground traits.

Furthermore, findings from the present study are likely to be dependent on species-specific belowground traits, which might also explain results which contrast with other studies conducted in mountain and alpine grasslands (Liu et al., 2018; Stampfli et al., 2018; Liu et al., 2021). The ability to shed fine lateral roots in response to lower soil moisture, for instance, was suggested as a possible aspect explaining the BNPP reduction observed in the grass *Deschampsia cespitosa*, as opposed to the shrub *Calluna vulgaris* which did not respond in the same way (Arndal et al., 2014). However, in a pot study conducted on a temperate grassland, lower specific root length – a resource acquisitive trait – and increased root tissue density – a resource-conservative strategy – were found to be the main features explaining a decrease in root biomass (de Vries et al., 2016); differences across species were also identified, with slow growing species more affected by drought than fast growing ones. Obviously, it is clear that currently available data for the *Carex curvula* grassland under study does not permit an accurate assessment of whether morphological

root adjustments took place – root production was not defined at a species level and root traits were not measured. Moreover, the use of the in-growth method for BNPP estimation did not allow an assessment of the effects on root turnover, especially where fine roots are concerned, found to be particularly vulnerable to precipitation reduction (Zhang et al., 2018). In tandem with a trait-based approach, these aspects will have to be addressed in the future in order to shed light on the complex mechanisms underpinning observed responses. Bearing in mind that short-term results cannot lead to any robust conclusions, such differences highlight the need for further investigation of root distribution dynamics. A trait-based base approach might also enable major clarity in understanding root responses across the vertical profile.

It was interesting to observe that there was a different BNPP response occurring between the superficial and deep-soil layer, with a tendency towards lower values in the 0-10 cm layer and a tendency toward an increase being detected below this level. In herbaceous ecosystems, shifts in root distribution in response to altered water availability have been assessed in some other studies, with contradictory results and a variety of suggested causes. For instance, Liu et al. (2018) reported an increase of BNPP in deeper soil layers within an alpine grassland in response to a 4-year drought manipulation, but resulting from a shift in community composition towards more deep-rooted species. On the other hand, in temperate grasslands, Prechsl et al. (2015) found an increase in root biomass in the 0-5 cm soil profile under drought conditions and no shift towards deeper layers, with species relying mostly on the top-layer water resource. In addition, Wu et al. (2018) found that two alpine grassland species exhibited different responses to drought, with a shift in root production towards deeper soil layers in one species, whereas there was no significant change in the other. In a 6-year study on a semi-arid steppe, Zhang et al. (2019a) observed that the quantity of roots in diverse layers consistently followed precipitation patterns, with larger amounts of belowground material in the near-surface layer occurring with increased precipitation and larger quantities in the deeper layers (10-30 cm) with decreased rainfall. It should be noted that such responses may be more evident in dry ecosystems where plant communities are already situated at the limit of the hydric resource. In the present study, it could be the case that increasingly variable soil moisture availability in the near-surface layer may lead to shifts to deeper layers in the longer term. It is equally possible that shifts have already occurred within the 0-10 cm profile, remaining undetected due to the vertical scales imposed in the investigation. Finally, in deeper layers, the lower fraction of roots may make it difficult to detect responses in the short term. Again, further research is needed, at a more detailed scale of analysis, to understand whether root distribution changes are occurring within the alpine ecosystem in question.

4.5.4 Responses across years: importance of inter-annual variability

A striking difference in aboveground production of vascular plants was observed across different years, with lower values in 2018 and 2019 compared to 2017, the year preceding the start of manipulations – approx. 20% and 15% less, respectively (**Fig. 4.9a**). In 2018, the same trend was observed in both the main PFTs (forbs, grasses and sedges; **Fig. 4.9b**) and species (*C. curvula* and *A. pentaphyllea*; **Fig. 4.9c**), whereas in 2019 responses differed at both PFT and species level: while forbs and grasses increased, sedges still exhibited a declining trend, with the two main species showing the same patterns as their respective functional groups. These results were partially confirmed by cover percentages, with higher values being observed for grasses and the majority of forbs in 2019 whereas lower cover percentages were recorded for *C. curvula* (**Fig. 4.6**). An investigation of the main drivers of such a change could not be carried out on account of the short time series available and the direction of this change cannot, as yet, be detected; ANPP values – particularly high in 2017 or particularly low in 2018 and 2019 – could have simply been within the range of natural variability. It was interesting, however, to note how ANPP could vary from one year to the next and, on the basis of general climate trends over the last few years, various hypotheses can be put forward as an explanation of such an outcome.

The first, most obvious reason could be the greater amount of rainfall available for plants in 2017 compared to the following years over the entire growing season, in accordance with the model of the temporal ANPP-annual precipitation relationship for herbaceous systems (Knapp et al., 2017b). However, data available for a longer time series (5 years), gathered in an experimental snowbed site within the same valley, revealed that 2015 and 2017 were particularly hot and dry growing seasons compared to other years, negatively affecting cover of vascular species (data not published). During these two years, intense heat waves and drought occurred during the summer over a large part of Europe (Orth et al., 2016; Hartfield et al., 2018). These events could have had a negative effect on plant performance in alpine ecosystems, as has already been reported by studies analysing impacts on ecosystems of similar climate episodes (Jolly et al., 2005; Bragazza, 2008; Griffin and Hoffmann, 2012). With regard to the 2015 heat wave, Cremonese et al. (2017) observed a reduced greening peak maximum and advanced senescence in a subalpine grassland in the western Italian Alps as a consequence of both high temperatures and dry conditions. In the present study, the RDA correlation plot of microclimatic variables across the 3 years (**Fig. 4.4**) shows that 2017 was effectively drier than the two following seasons, with higher VPD and more days without rain. It could, therefore, be possible that the high difference in ANPP values between 2017 and the two following years under study may, to some degree, be a consequence of the extreme events reported for 2017.

Following an environmental stress, ecosystems are known to manifest legacy or carry-over effects, exhibiting responses different to those expected on the basis of current-year climate

conditions (Frank et al., 2016; Sala et al., 2012). Legacy effects have, in fact, been observed in temperate grasslands in the years following full-exclusion drought manipulations (Stampfli et al., 2018). The negative cover patterns displayed by forbs in the present study is in line with recent findings by De Boeck et al. (2018b), which observed lower green cover and biomass values for the investigated alpine grassland in the 2 years following the heat and drought experimental manipulation. In this study, lower recovery rates for forbs were recorded, as a result of their greater leaf surface, horizontal leaf orientation and larger specific leaf area, distinguishing this group from the more long-lived and conservative graminoids (among which *C. curvula*), which were less affected by hot droughts (De Boeck et al., 2016; Cremonese et al., 2017). However, the constant declining trend observed for *C. curvula* in the current research could also point to the fact that other important drivers may be acting concurrently, one of which could be the difference in growing season length which might explain dominant sedge ANPP response. In a study quantifying the exact amount of solar radiation invested in annual CO_2 assimilation, Körner (1982) found that *C. curvula* was highly sensitive to the sum of sunshine hours received. When considering the period between snowmelt and the growing season peak – i.e. point intercept and biomass harvesting date – 2019 was characterised by a short growing season of 62 days compared to 84 days in 2017 and 68 days in 2018. Such a difference, resulting in diverse amounts of received solar radiation, might explain the higher values observed in 2017 within the grassland and the lack of an increase exhibited by the dominant sedge in the following two years. Unfortunately, these hypotheses cannot be supported by the available data, which lacks a sufficiently long time series and species-specific physiological measurements. The different response across years and species does, however, highlight the importance of considering inter-annual variability when analysing ANPP responses, especially in the current scenario, with climate reports indicating increasingly frequent natural climate extremes (Hartfield et al., 2018; Blunden and Arndt, 2020).

4.5.5 The importance of the initial state of the community

Analysis revealed the importance of the initial (pre-treatment) abundance in explaining variations in ANPP during the two subsequent treatment years: highest and lowest ANPP values were recorded in those areas that were initially characterised by abundant and low biomass, respectively; this relation was revealed by analysis at all levels of organisation – total vascular, PFTs and species (**Fig. 4.8**, left-hand graphs). Belowground production, on the other hand, was not found to be dependent on the root biomass present before the beginning of treatments, due to the fact that biomass represented the sum of both new and old roots characterised by slow turnover rates (Grabherr, 1989). Bias in the interpretation of results was, thus, avoided by

including pre-treatment data as a covariate in the analysis, thereby disentangling the role of spatial variability from that of the treatments applied.

4.6 CONCLUSION

Results obtained from the first two years of the experiment showed that neither warming nor a chronic (press) precipitation reduction were accompanied by significant changes in the overall NPP of the target ecosystem. Results on plant cover and on different components of NPP, however, highlighted that negligible impacts on key functions at the community level do not necessarily imply that there have been no responses at lower organization levels to changing environmental conditions. In fact, the major *taxa* exhibited different responses to precipitation reduction when plant cover variation was analysed and belowground production was reduced by lower rainfall to a sufficient degree to determine marginally lower root:shoot ratios under shelters. The data, therefore, revealed the importance of considering both above- and belowground compartments when assessing climate change effects on ecosystem functions and the fact that responses are strongly dependent on the species present within the community. A future trait-based assessment at a species level is, therefore, necessary to fully identify the set of traits driving both above- and belowground responses and, thus, obtain a more detailed picture of the mechanisms underlying the observed resistance to drought. Alternative approaches in addition to the in-growth core method, such as the use of mini-rhizotrons, could be employed to allow non-destructive, time-efficient root measurements and enable root distribution dynamics along the soil vertical profile to be accurately measured, alongside fine-root turnover rates (Iversen et al., 2012 and references therein). In addition, longer time series are needed to verify whether short- and long-term responses differ (Chapin et al., 1995; De Boeck et al., 2019) and whether recurrent drought – or naturally occurring climate extremes – could weaken the resistance of plant species and the community itself to further perturbations (Dreesen et al., 2014).

Climate extremes have become increasingly frequent over the last few years. Evidence from other studies and from the inter-annual differences in ANPP found in the present study suggest that naturally occurring extremes (drought and heat waves) could not only have an effect on the current year, but also impact ecosystem responses in the years following the event. Furthermore, other aspects of drought, apart from precipitation reduction, may be equally important in defining to what degree the system is actually resistant to drought events. These include the association of drought with other extremes, such as heat waves, and the timing and duration of its occurrence. Finally, there is a need for deeper, more nuanced insights into the ecological thresholds triggering important changes in ecosystem functions, which could then enable a better

understanding of the relationship between primary production and precipitation dynamics (Reichstein et al., 2013; Estiarte et al., 2016; Knapp et al., 2017b).

4.7 CONTRIBUTIONS

Alessandro Petraglia and Michele Carbognani initiated the project, designed and supervised the experiment. Roberto Vacchelli, Andrea Comelli, Luca Barchi and Cristian Cavozzi also worked on the design of the shelters and the set-up of the experiment. T'ai G. W. Forte, AP and MC planned the research and led the field sampling. Giorgio Chiari, Stefano Meroni, Giorgia Andreoli, Thai Linh Bacchilega, Marco Boschetti, Paola Chiudinelli, Matteo Gaudenzi, Matteo Maria Maniscalco, Mattia Malerba, Letizia Pagliani, Silvia Quaresmini, Niccolò Maria Sarti and Maria Timco also participated in the field sampling and lab work. TGWF and MC performed the statistical analyses. TGWF wrote the manuscript, which was subsequently critically revised by AP and MC.

CHAPTER 5. STUDY II – STABILITY OF LITTER DECOMPOSITION UNDER CLIMATE CHANGE IN SNOW-DOMINATED ECOSYSTEMS: EVIDENCE FROM THE ALPINE TUNDRA

5.1 ABSTRACT

It is generally assumed that litter decomposition processes in high-elevation ecosystems will be altered by climate change. Despite this, short-term decomposition may be unaffected by altered summer climate conditions in systems characterised by a strong seasonality, where important quantitative and qualitative alterations of litter occur during the snow-covered season.

In the present study, effects of warming (+T) and precipitation reduction (-P) on decomposition during the summer were tested in an alpine grassland, both singularly and in interaction, by means of a manipulation experiment using standard litter – green and rooibos teabags – which was incubated both on the soil surface and belowground. Litter decay was evaluated over a whole one-year period (2018-2019), and considering the snow cover period (SCP) and the snow-free period (SFP) separately, in order to disentangle the potential effect of seasons from that of treatments. To obtain a clearer picture of the actual variation in plant material decomposability across seasons and litter types, and to link standard litter decomposition with that of local litter, native litter was also incubated in ambient conditions, both on the surface – leaves from 14 species and wood from *Vaccinium gaultherioides* – and belowground (roots). Field incubation started in fall 2018, with litter samples collected after 9 months (SCP) and 1 year; decomposition for SFP was subsequently indirectly estimated. Moreover, to disentangle the role of micro-environmental conditions from that of litter alteration on summer decomposition, a further set of teabags was incubated belowground for 3 months during the 2019 SFP. Mass loss proportion (ML) and the rate of mass loss (RML, % day^{-1}) were calculated to estimate decomposition.

Results showed that +T and/or -P during the summer did not significantly affect either ML_{year} or ML_{SFP} of standard litter. On the other hand, ML significantly differed between litter types (green > rooibos) and incubation depths, with the influence of the latter being not consistent between the two litter types – i.e. increasing ML_{year} and ML_{SFP} with depth for green tea, whereas the opposite pattern was found for ML_{year} of rooibos tea. For native litter, most of the litter types showed higher ML and RML during the SCP compared to the SFP, with the following ML hierarchy: forbs > sedges > grasses > shrubs > mosses. Leaf litter ML and RML was greater than that of roots. ML of green tea was similar to that of the dominant sedge *Carex curvula*, whereas ML of rooibos tea resembled that recorded for shrubs, in particular *Salix herbacea*. Finally, the degree of litter alteration had a greater effect on standard litter decomposition compared to micro-environmental conditions, with fresh litter exhibiting higher RML than partially decomposed litter.

Findings showed that, although in high-elevation ecosystems environmental conditions during the summer would seem to be more favourable for decomposition, the action of decomposers is limited by the state of alteration characterising end-of-winter litter. In all likelihood, decomposition processes within alpine ecosystems characterised by strong seasonality will, therefore, not be altered in the short term by changed summer climate conditions. When investigating decomposition dynamics, the microscale environment at which such processes take place needs to be considered – aboveground vs belowground – and the simultaneous use of local leaf litter and teabags could improve the interpretation of results deriving from standard litter.

5.2 INTRODUCTION

Decomposition is one of the main processes regulating carbon stocks and nutrient cycling in ecosystems (Swift et al., 1979), consisting in the physical, chemical and biological breakdown of organic matter into CO_2 and inorganic nutrients. Physical mechanisms include leaching – i.e. the initial removal of soluble material – and fragmentation by soil fauna and abiotic agents; chemical mechanisms involve the oxidation and condensation of organic matter; and biological mechanisms consist in degradation mainly driven by the catabolic activity of fungi and bacteria (Aerts, 1997; Gavazov, 2010; Berg and McClaugherty, 2013). The rate at which these processes occur are mainly determined by the abiotic environment, the quantity and quality of the substrate, and the type of soil community (Swift et al., 1979) which, in turn, are not only influenced by climate change but also contribute to the amount of greenhouse gases and humus released into the atmosphere and the soil, respectively (Berg and McClaugherty, 2013).

Over the last decades there has been increasing concern regarding the positive feedback between warming and the carbon cycle in tundra ecosystems (Coûteaux et al., 1995; Schuur et al., 2008; Gavazov, 2010) on account of the important carbon reservoir represented by tundra soils – they stock the highest carbon concentrations among terrestrial biomes (Coûteaux et al., 1995; Yang et al., 2008) – and also the high sensitivity of decomposition compared to that of net primary production recorded in cold regions, which could lead to a rapid decrease of soil organic carbon content under global warming, thus releasing further amounts of CO_2 into the atmosphere (Kirschbaum, 1995). It is for these reasons that understanding the implications of changing climate conditions with respect to the carbon cycle in cold biomes represents a fundamental task in ecological research (Coûteaux et al., 1995; Gavazov, 2010).

Climate change is expected to affect carbon and nutrient turnover in tundra ecosystems (Aerts, 2006; Cornelissen et al., 2007; Gavazov, 2010). This can occur directly, by influencing the activity of decomposers (Allison et al., 2013; Melillo et al., 2017) or indirectly, by altering litter quality and quantity through both short-term and long-term changes; the former is achieved

through modifications in tissue chemical composition (Shaw and Harte, 2001) while the latter changes involve shifts in the composition of plant communities and soil biota (Cornelissen et al., 2007; Aerts et al., 2012).

At present, however, there is no general consensus on the effects of increased temperatures on litter decomposition and C pools in general (Lu et al., 2013). For instance, various studies in tundra ecosystems have found an enhancing effect of warming on decomposition rates along natural gradients and in manipulation experiments (Hobbie, 1996; Aerts et al., 2006; Althuizen et al., 2018; Liu et al., 2021). Other studies, however, have found that litter quality has an overriding effect on litter decomposition dynamics when compared with climate conditions (Bokhorst et al., 2007; Aerts et al., 2012; Carbognani et al., 2014a). Furthermore, no effects (Bokhorst et al., 2007) or decreasing decomposition rates (Aerts, 2006; Lu et al., 2013) under warmer conditions have been revealed in studies where temperatures were artificially increased by means of Open Top Chambers (OTCs), which could have been the result of soil drying as a collateral effect of OTCs (Blok et al., 2016; Christiansen et al., 2017; Brigham et al., 2018).

Low levels of soil moisture are known to act on litter decay rates by reducing the activity of decomposers and altering their abundance and composition (Coûteaux et al., 1995; Bloor and Bardgett, 2012; Christiansen et al., 2017). In a meta-analysis carried out on 34 studies simulating increased temperatures in cold biomes, Aerts (2006) found that low soil moisture levels represented the main limiting factor on increased decomposition rates under warmer conditions. Such an outcome confirmed the findings of a previous study carried out in a sub-arctic heath in Sweden by Robinson et al. (1995), which found increasing decomposition rates under warming when simultaneous watering took place. Moreover, studies conducted in temperate and Mediterranean ecosystems confirmed lower decomposition rates in response to reduced precipitation (Sanaullah et al., 2012b; Santoja et al., 2015). In the light of the available results, therefore, it can be postulated that even in cold ecosystems, including those at high elevation, the enhancing action of increased temperatures on decomposition might be hampered in the future by alterations in precipitation patterns during the summer (Gavazov, 2010) – a climate scenario expected to affect various alpine regions by the end of the century (Gobiet et al., 2014).

Few field studies have investigated the effects of drier summers on decomposition in the alpine tundra (Benot et al., 2014; Hagedorn and Joos, 2014; Bernard et al., 2019), with inconsistent results probably due to the use of local litter and the experimental framework employed, in which the effects of drier conditions cannot be disentangled from other variables. While drought was found to significantly decrease leaf litter mass loss in three managed grasslands in Switzerland (Hagedorn and Joos, 2014), the remaining studies, which were performed in high-elevation herbaceous ecosystems, found only weak or no effects of drought on decomposition rates. For instance, in a subalpine grassland in the French Alps, mass loss of only

one species out of the 3 investigated was found to be affected by extreme weather after one year of incubation (Benot et al., 2014). This reduction was, however, only marginally significant and the experimental manipulation was not factorial – overnight warming coupled with 80% precipitation reduction – with effects that could not, therefore, be disentangled. The same difficulty applies to the findings of Bernard et al. (2019), where no significant effect of anticipated snowmelt combined with drought was detected on the decomposition kinetics of native litter in subalpine environments. The results of these studies, taken together with the evidence of non-significant influences of climate manipulations on decomposition observed in other studies in tundra ecosystems (Aerts, 2006; Aerts et al., 2012; Carbognani et al., 2014a) raise the further question of whether, and by how much, ecosystem processes in cold environments are resistant to changing environmental conditions and, if so, what characteristics drive such a response. It could be hypothesised that the long snow-covered season following litter fall could act as a buffer, dampening potential effects of altered summer climate conditions on litter decomposition, hence resulting in no significant change in litter mass loss (Carbognani et al., 2014a).

Seasonally snow-covered ecosystems, such as those in the alpine tundra, are regarded as being particularly sensitive to the global climate crisis (Seddon et al., 2016), undergoing higher warming rates compared to average trends recorded globally (e.g. IPCC, 2013; Pepin et al., 2015). In these systems, plant communities are dominated by slow growing, low statured, perennial plant species whose life cycles are strongly influenced by a variety of abiotic factors – low average temperatures, strong thermal seasonal variation, high irradiance, nutrient scarcity, and hydrological dynamics – all of which are driven by the interaction of precipitation, wind exposure and topography (Körner, 2003; Winkler et al., 2019). Snowpack, in particular, decouples plants and soil from the atmosphere during the winter, provides water and nutrients at the beginning of the growing season, and limits the time available for growth to the relatively short, warm snow-free period (Taylor and Seastedt, 1994; Fisk et al., 1998; Litaor et al., 2008). Nevertheless, while the production of biomass is mainly limited by and confined to the snow-free season, litter decomposition takes place all year round in most alpine areas, with an important amount of organic material decay occurring under the snowpack (Schinner, 1983; O'Lear and Seastedt 1994; Hobbie and Chapin, 1996; Saccone et al., 2013*;* Chen et al., 2020). Substantial mass loss during the period preceding the growing season is the consequence of both leaching (Bokhorst et al., 2010) and the predominant action of microbial communities, which manage to thrive even under temperatures approaching 0 °C (Brooks et al., 1996). During this period litter undergoes a series of significant changes, starting with its physical structure and chemical composition, which determine the degree to which the nutritional requirements of decomposers are satisfied and, therefore, define the overall quality of the litter (Robinson, 2002; Berg and McClaugherty, 2013). In fact, litter quality – one of the main drivers of decomposition (Cornwell et al., 2008; Zhang et

al., 2008) – is not a static feature in the ecosystem, exclusively dependent on the initial proprieties of the diverse plant tissues constituting the vegetation from which it originates; litter quality represents a dynamic ecosystem feature, changing over time as a result of mass loss and nutrient release (Berg and McClaugherty, 2013). After a long period under the winter snowpack, litter decomposability may have been reduced and could, therefore, be generally less affected by abiotic factors during the growing season.

Given the important contribution, both quantitative and qualitative, of biological activity during the winter, seasonality has already been identified as a key element in analysing climate change effects on litter decomposition (Hobbie and Chapin, 1996; Blok et al., 2016). In tundra ecosystems, year-round processes, which are not restricted to the short, relatively warm summer period, could be generally less sensitive to warmer, drier snow-free periods. As far as can be ascertained, the extent to which the snow cover period contributes to the resistance of litter decomposition to changes in summer climate is not yet fully understood in alpine ecosystems. Studies addressing the effects of altered summer conditions often do not consider winter mass loss, reporting either annual mass loss (Benot et al., 2014; Carbognani et al., 2014a) or carrying out incubations at the beginning of the growing season, hence simulating unrealistic conditions (Hagedorn and Joos, 2014). Specifically, with regard to drought experiments in the alpine context, the interpretation of outcomes is further limited not only by the small number of studies available and the experimental set-up, with manipulated factors not being singularly assessed (Benot et al., 2014; Bernard et al., 2019), but also by the use of local litter and/or different litterbag mesh size used across experiments.

During the last decade, a protocol has been developed using standard litter (Keuskamp et al., 2013), which has been implemented in recent studies. This has permitted better identification of the major drivers of decomposition across terrestrial biomes on both a regional and global scale (Djukic et al., 2018; Didion et al., 2016; Petraglia et al., 2019; Fanin et al., 2020; Sarneel et al., 2020). Standard litter, represented by green and rooibos tea, is a cost effective, easy-to-use method for estimating decomposition dynamics under future climate scenarios, allowing for easy replication and comparison across studies (Keuskamp et al., 2013). These two types of tea could be identified as substrates representing the two extremes of litter quality found in natural conditions: on one hand, green tea represents high quality litter, characterised by a high soluble fraction and a low C:N ratio, features which make it similar to native litter made up of herbaceous plants (forbs and grasses); on the other, rooibos tea represents low quality litter, with a high lignin content and a high C:N ratio making it recalcitrant to decomposition in a similar way to litter consisting of woody plant species. The similarity with local litter is indirectly estimated when comparing mass loss percentages reported by Djukic et al. (2018) with those reported in other studies using native litter: for instance, green tea mass loss values of 62% after a 3-month

incubation period are similar to annual decomposition values reported for forbs (80-90% in Carbognani et al., 2014a; ~60% in Benot et al., 2014; both studies used larger mesh-size litterbags). Rooibos tea, with a 22% mass loss is, on the other hand, more similar to mass loss values reported for shrubs (35-40% in Carbognani et al., 2014a; 20-25% in Baptist et al., 2010). Among stand graminoids, reported mass loss values resembled those of green tea in some studies (e.g. 70-90% in Carbognani et al., 2014a) while in others values were more similar to the mass loss of rooibos tea (e.g. approx. 40% in Benot et al., 2014 and in Baptist et al., 2010). Differences in incubation periods and litterbag mesh size, together with inconsistencies across plant functional types, suggest that making generalizations about decomposition dynamics of native litter based on data obtained using standard litter could be misleading. Similarities between the two types of litter (standard and indigenous) have never been tested in alpine environments through simultaneous incubation within the same site. Such a comparison is necessary to link potential decomposition rates estimated using teabags with real decomposition rates gathered using local litter (Didion et al., 2016) and also to assess the degree to which standard litter is able to encompass the variability in decomposability of the litter originally present within a site. In addition, the simultaneous use of indigenous litter would lead to a better understanding of how decomposition processes might be affected by long-term shifts in community composition.

The goal of the present study was to investigate the effects summer temperature and precipitation reduction – on short-term litter decomposition in a typical high-elevation ecosystem (*Carex curvula* alpine grassland) characterised by an 8-month long period of snow cover. Evaluation of litter decay was carried out for different periods: annually, over the whole of a single year; and with a separate estimation of litter decay during winter (snow cover period, SCP) and summer (snow-free period, SFP) in order to disentangle potential seasonal effects from those of climate treatments. Standard litter was also employed, with teabags being incubated on both the soil surface and belowground to account for the different micro-environmental conditions experienced by indigenous litter – i.e. leaves and roots. Simultaneously, native litter – i.e. foliage, wood and roots – was also incubated in control plots, with no treatments, to obtain a clearer picture of the actual variation in decomposability of diverse litter types within alpine grasslands during the SCP and SFP, and to link potential ML and RML of standard litter with the actual values of local litter. Finally, to establish the role of microclimate conditions and the degree of litter alteration on decomposition rate during the SFP, mass loss rates of standard litter were compared to those of a further set of teabags. These represented fresh, unaltered litter, that had been incubated at a depth of -8 cm at the beginning of the summer and retrieved in the autumn. Results have been presented in order to verify the following hypothesis:

- Mass loss of standard litter is mainly influenced by the type of litter (litter quality), with no effect of a 3-month increased summer temperature and reduced precipitation on short-term litter decay in the target alpine ecosystem (HP1).
- The incubation period preceding the summer, largely dominated by snow, contributes to a large fraction of litter decay and reduces the initial differences among litter types (HP2).
- The alteration of litter during the SCP has a stronger influence over short-term responses of litter decay than altered summer climate conditions (HP3).

5.3 MATERIALS AND METHODS

5.3.1 Study site and experiment set-up

The study site and the experiment set-up are presented in Chapter 2 and Chapter 3, respectively. Materials and methods used for microclimate measurements and litter incubation are presented in the paragraphs below.

5.3.2 Microclimate

Microclimate data was logged every hour during the snow-free growing season using two replicated devices for each treatment. Air temperature (T_{air}) was registered using sensors placed 5 cm above the soil surface (EHT Temperature/RH Sensor, Decagon Devices, Inc.). HOBO sensors (HOBO Pendant® Temperature/Light 64K Data Logger, Part # UA-002-64) were used to record soil temperatures at the same depth as teabags and litterbags employed in the decomposition experiment: on the soil surface (0 cm), at -5 cm, -8 cm and -15 cm belowground. Soil temperature was logged at all depths during the entire 1-year incubation period, except for measurements at -8 cm, which were only taken during the snow-free growing season. Soil moisture was also recorded by measuring the volumetric water content at both -5 cm and -15 cm (VWC, %; WaterScout SM100, Spectrum Technologies, Inc.). Precipitation data was also gathered throughout the growing season, both under and outside the rain-out shelter (Rain Collector, Decagon Devices, Inc.).

5.3.3 Litter experiments

The litter experiments were conducted from fall 2018 to fall 2019 (25/09/2018 – 26/09/2019). Fine-mesh litterbags of 0.2-0.25 mm were used to assess climate effects on decomposition, in order to exclude litter decay resulting from soil macro- and mesofauna activity, and also to avoid loss of litter fragments (Xie, 2020). The aims of the present study were addressed by dividing the

experiment into two parts: (a) Experiment I, addressing HP1 and HP2 – i.e. the influence of litter quality vs climate change during the snow-free period (hereafter SFP, summer) and the influence of seasonality by comparing the snow cover period (hereafter SCP, winter) and SFP; and (b) Experiment II, addressing HP3 – i.e. evaluating the influence of seasonal change in litter quality vs that of microclimate on litter decomposition processes.

(a) Experiment I

In the present study, standard litter was employed to enable cross-site comparison together with study replicability. Specifically, green tea (Lipton, EAN 8722700055525) and rooibos tea (Lipton, EAN 8714100770542) were used as a proxy for high and low quality litter, respectively (see Keuskamp et al., 2013 for details). Teabags, made of non-woven PET fabric (0.2 mm mesh size) contain approx. 2 g of material. Before burial, each bag was identified with a code number and its air-dried mass was recorded.

Standard litter was placed in the study site at the end of the growing season, on 25 September 2018, and incubated at three different depths: on the surface (0 cm), at -5 m and -15 cm. To disentangle the potential effects of the SCP – no climate manipulations – from those of the SFP when treatments were applied, two sets of teabags of the same size were prepared to account for two retrieval dates: a first set to be retrieved immediately after snowmelt and a second set to be collected at the end of the growing season, after one year of incubation. Although snow is present for the majority of the SCP, it is important to note that this period also includes days before snow onset and days where snowmelt is ongoing. Each of the two sets of teabags consisted of one bag for each type and each depth, which were then incubated in each plot (**Fig. 5.1**). A total of 240 tea bags (120 green and 120 rooibos) were, therefore, deployed: 20 plots × 2 sets of tea bags × 2 tea types × 3 depths. The first set of teabags was retrieved after 273 days of incubation (25 June 2019), whereas the second set was collected after 366 days (26 September 2019).

Litterbags using native material – leaves, wood and roots – were also incubated together with teabags in control areas (no treatment) and collected at the same time as the two retrieval dates specified above (**Fig. 5.1**). This was done in order to gain an overview of the variation in different litter type decomposability present within the grassland and link potential mass loss and decomposability derived from teabags with the values of native species present in the target ecosystem.

Plant material was collected in the field at the beginning of September 2018, which represented different litter types: leaves for 14 of the most common species and encompassing 5 plant functional types (PFTs) – sedges, grasses, forbs, shrubs and mosses; wood material from *Vaccinium gaultherioides*; and roots gathered from -5 cm and -15 cm of depth, respectively (**Fig. 5.1, Table 5.1**). Given the difficulty of preparing litterbags made of leaf material composed of

completely senescent leaves before the beginning of the snow-covered season, green leaves harvested at the very end of the season and near to the senescent phase were used instead. Once collected, plant material was air-dried for approx. one week, until subsample mass was stable for two consecutive days. A total of 169 litterbags were prepared, containing approx. 1 g of dry mass; organza material was used for each bag, with a mesh size of 0.25 mm, similar to that of the teabags.

As for foliage and wood material, two litterbags were prepared for each litter type and placed in control plots on the soil surface (5 replicates) so as to have one set of bags for each retrieval date. Only 4 litterbags made of foliage from *Leontodon helveticus* were incubated during the winter as there was insufficient material. Root litter, used to assess belowground decomposition, was placed at -5 cm and -15 cm.

Once retrieved, all the material – standard and native litter – was first cleaned to remove soil particles and new roots, then oven-dried for 48 hours at 65 °C and finally weighed.

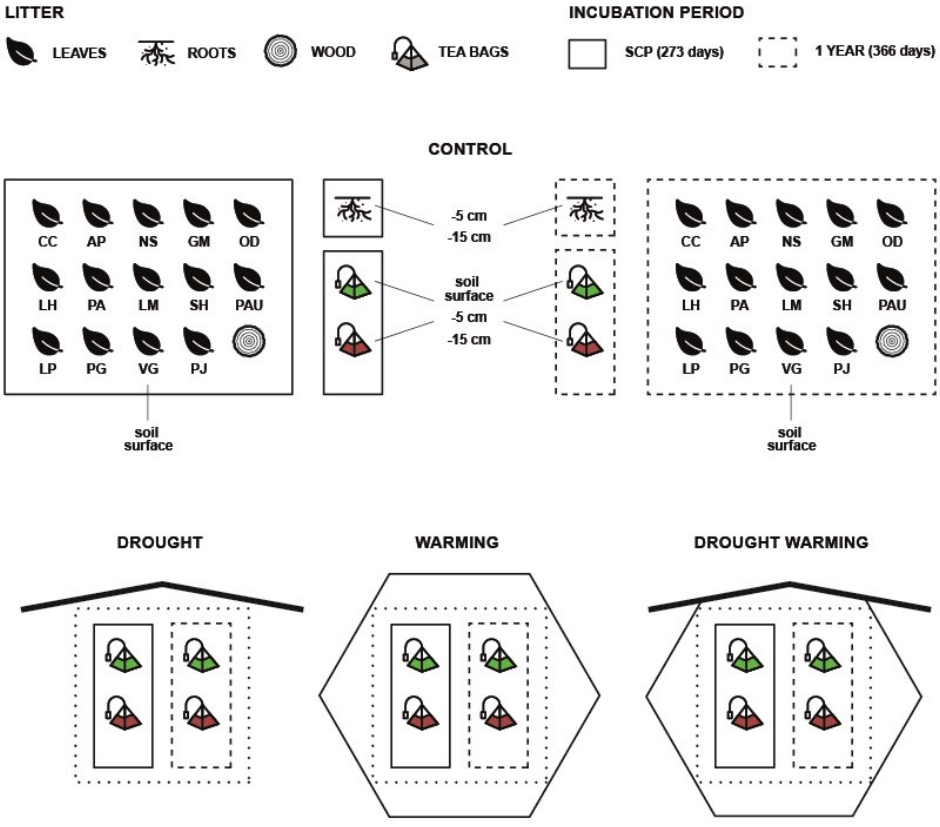


Fig. 5.1: Incubation scheme for standard litter (teabags) and native litter within treatment plots [control, drought, warming; drought + warming]. The type of litter (green and rooibos tea for standard litter; leaves, wood and roots for native litter), the incubation depth (soil surface, -5 cm and -15 cm) and the incubation period (snow cover period, SCP; one year, YEAR) are indicated. Native litter was exclusively incubated in control plots; letters under the "leaf" symbol indicate species identity (see "ID" in Table 1).

(b) Experiment II

A set of teabags was buried at a depth of -8 cm on 5 July 2019 and incubated for approx. 90 days, following the protocol of Keuskamp et al. (2013). Two pairs of teabags, each consisting of one green and one rooibos bag, were placed in control plots. A total of 20 bags were, therefore, employed for the summer decomposition experiment (**Table 5.1**) and collected on 26 September, the same retrieval date as that for the litterbags in Experiment I. This set of bags represented fresh, unaltered standard litter which had not undergone incubation during the SCP and was, therefore, used to identify the role of micro-environmental conditions (hereafter called "microclimate") and the degree of litter alteration on the decomposition rate during the SFP.

Table 5.1 (p. 105): List of litter types used in the study, the relative plant functional type (PFT), the abbreviations used in Fig. 5.1 (ID) and relative burial depths. Mean mass loss (proportion) ± standard deviation is reported, together with the total number of samples [in brackets] effectively used for analysis after discarding damaged material. Values are provided for each incubation period (SCP, snow cover period; YEAR, 12 months; SFP, snow-free period) and treatment (C − control; D − drought; W − warming; DW − drought + warming); temperature (T) and precipitation (P) codification for treatments is also reported below treatment acronyms.

				SCP	YEAR				SFP			
Litter type	**PFT**	**ID**	**Depth (cm)**	**C** =T =P	**C** =T =P	**D** =T -P	**W** +T =P	**DW** +T -P	**C** =T =P	**D** =T -P	**W** +T =P	**DW** +T -P
Experiment I												
Standard litter (tea bags)												
Green	-	G_0	0	0.44 ± 0.01 [4]	0.48 ± 0.02 [5]	0.46 ± 0.02 [5]	0.48 ± 0.03 [5]	0.48 ± 0.02 [5]	0.04 ± 0.02 [5]	0.03 ± 0.02 [5]	0.05 ± 0.03 [5]	0.04 ± 0.02 [5]
Green	-	G_5 G_{SCP} fresh and G_{SFP} alter	-5	0.44 ± 0.01 [5]	0.53 ± 0.02 [5]	0.53 ± 0.01 [5]	0.53 ± 0.01 [5]	0.52 ± 0.02 [5]	0.08 ± 0.03 [5]	0.09 ± 0.01 [5]	0.09 ± 0.01 [5]	0.07 ± 0.02 [5]
Green	-	G_{15} G_{SCP} fresh and G_{SFP} alter	-15	0.45 ± 0.04 [5]	0.54 ± 0.01 [5]	0.54 ± 0.07 [5]	0.56 ± 0.04 [5]	0.58 ± 0.07 [5]	0.09 ± 0.01 [5]	0.09 ± 0.07 [5]	0.11 ± 0.04 [5]	0.13 ± 0.07 [5]
Rooibos	-	R_0	0	0.22 ± 0.01 [5]	0.25 ± 0.02 [4]	0.21 ± 0.01 [5]	0.23 ± 0.01 [5]	0.23 ± 0.00 [5]	0.03 ± 0.02 [4]	-0.00 ± 0.01 [5]	0.01 ± 0.01 [5]	0.02 ± 0.00 [5]
Rooibos	-	R_5 R_{SCP} fresh and R_{SFP} alter	-5	0.18 ± 0.02 [5]	0.21 ± 0.02 [5]	0.20 ± 0.01 [5]	0.21 ± 0.01 [5]	0.21 ± 0.02 [5]	0.04 ± 0.02 [5]	0.03 ± 0.01 [5]	0.03 ± 0.01 [5]	0.03 ± 0.02 [5]
Rooibos	-	R_{15} R_{SCP} fresh and R_{SFP} alter	-15	0.17 ± 0.02 [4]	0.20 ± 0.01 [5]	0.20 ± 0.02 [5]	0.21 ± 0.02 [5]	0.19 ± 0.02 [5]	0.03 ± 0.01 [5]	0.03 ± 0.02 [5]	0.04 ± 0.02 [5]	0.02 ± 0.02 [5]
Native litter												
Carex curvula	Sedge	CC	0	0.43 ± 0.04 [5]	0.53 ± 0.05 [5]	-	-	-	0.10 ± 0.05 [5]	-	-	-
Nardus stricta	Grass	NS	0	0.32 ± 0.01 [5]	0.40 ± 0.01 [5]	-	-	-	0.09 ± 0.01 [5]	-	-	-
Oreochloa disticha	Grass	OD	0	0.35 ± 0.03 [5]	0.44 ± 0.01 [4]	-	-	-	0.10 ± 0.01 [4]	-	-	-
Poa alpina	Grass	PA	0	0.56 ± 0.01 [5]	0.63 ± 0.02 [5]	-	-	-	0.07 ± 0.02 [5]	-	-	-
Alchemilla pentaphyllea	Forb	AP	0	0.54 ± 0.04 [5]	0.60 ± 0.02 [4]	-	-	-	0.06 ± 0.02 [4]	-	-	-
Geum montanum	Forb	GM	0	0.50 ± 0.08 [5]	0.56 ± 0.03 [5]	-	-	-	0.05 ± 0.03 [5]	-	-	-
Leontodon helveticus	Forb	LH	0	0.54 ± 0.02 [4]	0.62 ± 0.04 [5]	-	-	-	0.08 ± 0.04 [5]	-	-	-
Ligusticum mutellina	Forb	LM	0	0.39 ± 0.02 [5]	0.46 ± 0.02 [5]	-	-	-	0.07 ± 0.02 [5]	-	-	-
Potentilla aurea	Forb	PAU	0	0.55 ± 0.05 [5]	0.62 ± 0.02 [5]	-	-	-	0.07 ± 0.02 [5]	-	-	-
Primula glutinosa	Forb	PG	0	0.60 ± 0.03 [5]	0.67 ± 0.03 [5]	-	-	-	0.06 ± 0.03 [5]	-	-	-
Loiseleuria procumbens	Shrub	LP	0	0.27 ± 0.04 [5]	0.30 ± 0.01 [5]	-	-	-	0.03 ± 0.01 [5]	-	-	-
Salix herbacea	Shrub	SH	0	0.19 ± 0.01 [5]	0.25 ± 0.03 [5]	-	-	-	0.06 ± 0.03 [5]	-	-	-
Vaccinium gaultherioides	Shrub	VG	0	0.27 ± 0.01 [5]	0.33 ± 0.03 [5]	-	-	-	0.06 ± 0.03 [5]	-	-	-
Polytrichum juniperinum	Moss	PJ	0	0.05 ± 0.01 [5]	0.10 ± 0.02 [5]	-	-	-	0.05 ± 0.02 [5]	-	-	-
Wood (*V. gaultherioides*)	-	Wd	0	0.11 ± 0.05 [5]	0.19 ± 0.04 [4]	-	-	-	0.07 ± 0.04 [4]	-	-	-
Roots	-	Rts_5	-5	0.08 ± 0.01 [5]	0.11 ± 0.01 [5]	-	-	-	0.04 ± 0.01 [5]	-	-	-
Roots	-	Rts_{15}	-15	0.07 ± 0.01 [5]	0.10 ± 0.02 [5]	-	-	-	0.02 ± 0.02 [4]	-	-	-
Experiment II												
Standard litter (tea bags)												
Green	-	G_{SFP} fresh	-8	-	-	-	-	-	0.60 ± 0.04 [10]	-	-	-
Rooibos	-	R_{SFP} fresh	-8	-	-	-	-	-	0.21 ± 0.02 [10]	-	-	-

5.3.4 Estimates of litter decomposition: mass loss proportion and rate of mass loss

The decomposition periods considered within the present study refer to the SCP, the SFP and one single year. For each period and at each depth, early-stage decomposition for each litter type was assessed, estimating the proportion of mass loss (ML) and the rate of mass loss (RML).

ML was calculated as $ML = (M_0 – M_f)/M_0$ where M_0 and M_f represent the initial and final oven-dried litter mass, respectively. In order to estimate the initial oven-dried mass (M_0), the air-dried value was multiplied by the ratio between air- and oven-dried mass (65 °C for 48 hours), which was calculated using an independent sample subset: for standard litter, 10 teabags for each type; and for native litter, 3 samples for each litter type.

The average daily percentage of mass loss (rate of mass loss – RML, % day^{-1}) was computed by dividing each ML value by the number of incubation days for each period: (a) ML of litter from Experiment I divided by 273 days, 93 days and 366 days for RML_{SCP}, RML_{SFP} and RML_{year}, respectively; and (b) ML of litter for Experiment II divided by 83 days for RML_{SFP}.

For litter from Experiment I, mass loss and RML for the snow cover period (ML_{SCP} and RML_{SCP}) and for the single year (ML_{year} and RML_{year}) were directly estimated from samples collected on the two retrieval dates; in particular, standard litter values from the SCP are also referred to as "RML_{SCP} fresh", since they represent winter decomposition of fresh, unaltered litter. For each litter type and depth, decomposition for the growing season (ML_{SFP} and RML_{SFP}) was indirectly computed by subtracting the mean ML_{SCP} value from each ML_{year} value (**Table 5.1**). In this case, standard litter values are also referred to as "RML_{SFP} alter", since they represent summer decomposition of altered litter which has already been subjected to winter decay.

With regard to standard litter from Experiment II, RML_{SFP} values were directly estimated and are referred to as "RML_{SFP} fresh" in the last set of the analysis.

5.3.5 Data analysis

Linear mixed-effect models (LMMs) were employed to assess decomposition of both standard and native litter. A step-by-step model selection approach was followed, excluding all non-significant terms ($p>0.05$); residual patterns were visually checked to verify linear model assumptions, and goodness of fit and selection of the best random and variance structure for each model was done by means of the Akaike Information Criteria (AIC) (Zuur et al., 2009).

To test HP1 – i.e. identify whether increased summer temperatures and reduced precipitation affected standard litter decay after one year and during the SFP – analyses were performed considering climate treatments as two 2-level factor categorical variables: (a) temperature, with =T (ambient temperature; areas without OTCs, including sheltered plots) and +T (temperature increase; all plots with OTCs, including those with shelters); and (b) precipitation, with =P (ambient precipitation, including unsheltered plots with OTCs) and -P (reduced precipitation; all

sheltered plots, including those with OTCs). Together with temperature and precipitation, litter type (2-level factor; green and rooibos tea) and incubation depth (3-level factor; surface, -5 cm and -15 cm) and three-way interactions were considered as fixed effects, whereas "column" was set as the random factor, indicating the vertical profile in which three teabags for each type were incubated at three different depths. To deal with the heterogeneity of residuals among groups, a combination of variance structures for "litter type" and "incubation depth" was also inserted in the models. Pairwise comparison of significant terms was computed by means of post-hoc Tukey constrast ($p>0.05$).

To test HP2 – i.e. disentangle the SCP and SFP contribution on mass loss and rate of decomposition for native and standard litter – LMMs were performed including the incubation period (2-level factor; SCP and SFP) and litter type (23-level factor; native litter and standard litter at three depths) as fixed effects and blocks as the random factor. The variance structure for "litter type" was incorporated in the ML model, while a combination of variance structures for "litter type" and "incubation period" was applied to the RML model.

Finally, to test HP3 – i.e. assess the influence of litter alteration and microclimate conditions on rate of decomposition – RML was related to the incubation depth (numeric variable) and a 6-level factor response variable (Comb) combining the type of litter (green and rooibos tea), the incubation period (SCP and SFP) and the degree of litter alteration (altered and fresh litter). The vertical profile of incubation, "column", was set as the random factor and a combination of variance structures for "litter type" and "incubation depth" was applied.

Analyses were carried out in R (R Core Team, 2020) using the following packages: "nlme" (Pinheiro et al., 2020), "effects" (Fox and Weisberg, 2018), "visreg" (Breheny and Burchett, 2017), "lsmeans" (Lenth, 2016) and "multcomp" (Hothorn et al., 2008).

5.4 RESULTS

5.4.1 Microclimate

Snowfall estimated from soil temperature data occurred on 27 October 2018, thirty-one days after standard and native litter had been incubated within the site, with snowmelt occurring around 21 June 2019, four days before the first litterbag retrieval (**Fig. 5.2a**). There was, therefore, persistent snowpack, within the site for 238 days, representing 87% of the period designated as "SCP" for litter.

Mean soil temperatures experienced by litter during the SCP were around 0 °C, with no substantial difference between plots assigned to different summer treatments; highest and lowest values were recorded on the soil surface, with temperatures ranging from a minimum of -7.07 °C (22 October 2018) to a maximum of 35.11 °C (25 June 2019) (**Table 5.2, Fig. 5.2a**). Mean

belowground values were higher compared to those on the soil surface, with +0.37 K ± standard error (SE) 0.04 (n=4) and +0.67 K ± SE 0.03 (n=4) at -5 cm and -15 cm, respectively (**Table 5.2**).

During the experimental period (SFP), cumulative precipitation was reduced by 33% under the shelters (**Table 5.2**). More than half of the SFP was characterised by rainy days (56% with precipitation >0 mm), with an average of 3 days ± standard deviation (SD) 1.6 of consecutive rainfall (**Fig. 5.2b**). The 13-day long period without rainfall shown by the data for precipitation actually corresponds to a cold spell which occurred during the second week of September (6-12 September 2019), which was characterised by snowfall (which the rainfall gauge failed to correctly record), a sharp fall in mean daily soil surface temperatures and a lack of thermal differences across treatments (**Fig. 5.2a, b**).

Despite this cold spell, differences in mean daily soil temperatures between control and warming treatments during the entire SFP were evident, with the largest difference exhibited on the soil surface (average difference of +3.20 K and +3.71 K for W and DW, respectively) and a progressive reduction taking place as the soil became deeper (+1.18 K in W and +1.65 K in DW at -5 cm; +0.62 K in W and +0.90 K in DW at -15 cm) (**Table 5.2**). Mean temperatures recorded in D were also higher compared to controls (+1.13 K, +0.98 K and +0.60 K at 0 cm, -5 cm and -15 cm).

With regard to soil moisture, trends were consistent with precipitation events within the site (**Fig. 5.2b**). All treatments showed reduced VWC compared to control plots, with the major difference across treatments occurring at the near-surface soil layer, with D plots exhibiting 2.91% lower VWC compared to controls, followed by W (-3.81%) and DW (-4.49%) (**Table 5.2, Fig. 5.2b**).

Table 5.2: Mean microclimate values ± standard deviation recorded during the snow-covered period (SCP) and snow-free period (SFP) in the study site treatments [C − control; D − drought; W − warming; DW − drought + warming]; temperature (T) and precipitation (P) codification for treatments is also reported below treatment acronyms. It should be noted that no manipulation was carried out during the SCP. Data available for the following: cumulative precipitation; soil temperature at soil surface; belowground soil temperature at -5 cm, -8 cm and -15 cm; and corrected volumetric water content at -5 cm and -15 cm. Values: $mean_{daily}$, daily (24 h) average value; $mean_{max}$, average daily maximum; and $mean_{min}$, average daily minimum. Records refer to the period when instruments were installed at the site: T soil surface, at -5 cm and at -15 cm (26 Sept 2018 - 25 June 2019 for SCP; 26 June - 24 Sept 2019), precipitation (07 July - 24 Sept 2019), VWC (01 July - 23 Sept 2019) and T soil -8 cm (06 July - 24 Sept 2019).

PERIOD	**SCP**				**SFP**			
TREATMENT	**C** =T =P	**D** =T -P	**W** +T =P	**DW** +T -P	**C** =T =P	**D** =T -P	**W** +T =P	**DW** +T -P
Precipitation [mm] [a]	-	-	-	-	347	232	347	232
T soil surface [°C]								
$mean_{daily}$	0.02 ± 2.16	0.24 ± 1.89	0.09 ± 1.79	0.22 ± 1.67	10.41 ± 3.53	11.54 ± 3.59	13.61 ± 4.28	14.13 ± 4.09
$mean_{max}$	1.68 ± 6.24	1.89 ± 6.02	1.44 ± 5.21	1.52 ± 5.02	22.94 ± 8.28	24.03 ± 7.88	31.32 ± 9.78	30.46 ± 8.21
$mean_{min}$	-0.82 ± 1.16	-0.58 ± 0.99	-0.63 ± 0.96	-0.48 ± 0.92	3.26 ± 1.96	3.96 ± 2.05	3.64 ± 2.26	4.57 ± 2.27
T soil - 5 cm [°C]								
$mean_{daily}$	0.37 ± 1.82	0.59 ± 1.71	0.51 ± 1.74	0.56 ± 1.72	9.66 ± 2.34	10.64 ± 2.27	10.84 ± 2.21	11.31 ± 2.20
$mean_{max}$	0.67 ± 2.50	0.91 ± 2.46	0.79 ± 2.43	0.86 ± 2.45	13.22 ± 3.40	14.04 ± 3.22	14.10 ± 3.09	15.03 ± 3.00
$mean_{min}$	0.14 ± 1.39	0.35 ± 1.23	0.30 ± 1.29	0.34 ± 1.24	6.75 ± 1.91	7.85 ± 1.94	8.19 ± 1.90	8.33 ± 1.96
T soil - 8 cm [°C]								
$mean_{daily}$	-	-	-	-	10.13 ± 2.14	10.51 ± 1.93	10.45 ± 1.93	10.75 ± 1.79
$mean_{max}$	-	-	-	-	11.89 ± 2.53	12.36 ± 2.27	12.23 ± 2.29	12.52 ± 2.05
$mean_{min}$	-	-	-	-	8.53 ± 1.93	8.85 ± 1.80	8.84 ± 1.71	9.19 ± 1.68
T soil - 15 cm [°C]								
$mean_{daily}$	0.74 ± 1.84	0.78 ± 1.79	0.86 ± 1.82	0.88 ± 1.81	9.28 ± 1.95	9.87 ± 1.95	9.90 ± 1.88	10.17 ± 1.98
$mean_{max}$	0.84 ± 2.01	0.89 ± 1.97	0.95 ± 2.01	0.98 ± 2.00	10.49 ± 2.09	11.07 ± 2.02	11.06 ± 1.94	11.42 ± 2.08
$mean_{min}$	0.65 ± 1.70	0.70 ± 1.63	0.77 ± 1.66	0.79 ± 1.64	8.16 ± 1.87	8.82 ± 1.90	8.87 ± 1.83	9.08 ± 1.92
VWC [%]								
$mean_{daily}$ -5 cm	-	-	-	-	31.82 ± 3.28	28.91 ± 2.90	28.01 ± 2.17	27.33 ± 1.66
$mean_{daily}$ -15 cm	-	-	-	-	26.12 ± 2.44	25.71 ± 2.43	25.58 ± 1.43	25.04 ± 0.79

a Values of precipitation in C recorded in snowbed site, approx 1 km from the study site

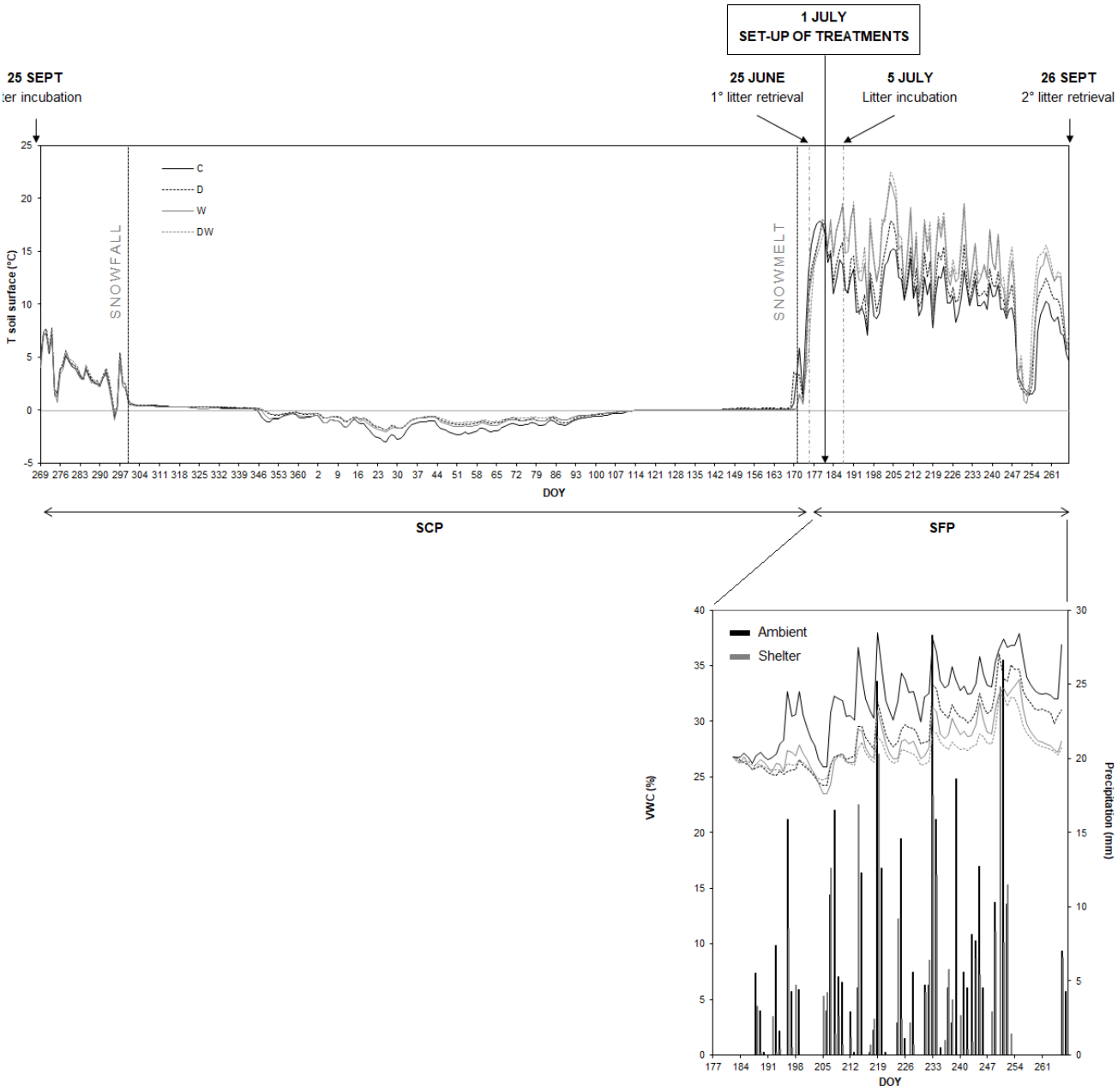


Fig. 5.2: The graph above illustrates mean daily temperatures (°C) recorded on the soil surface from the 26 September 2018 (day of the year, DOY 269) to the 24 September 2019 (DOY 266) for each treatment [C − control; D − drought; W − warming; DW − drought + warming]. It should be noted that no manipulation was carried out during the winter. Mean snowfall date [27 October 2018 (DOY 300)] and mean snowmelt date [21 June 2019 (DOY 172)] are indicated, together with the summer treatments set-up date. Litter incubation and retrieval dates are also reported: 25 September 2018 (DOY 267) refers to incubation of standard and native litter (Experiment I); 25 June 2019 (DOY 176) refers to retrieval of the first set of litterbags (decomposition during the snow-covered period, Experiment I); 5 July 2019 (DOY 186) refers to incubation of standard litter during the summer (Experiment II); and 26 September 2019 (DOY 269) refers to retrieval of both the second set of litterbags (annual decomposition, Experiment I) and standard litter incubated during the summer (Experiment II). The graph below represents mean daily volumetric water content (VWC, %) recorded at -5 cm in each treatment during the SFP, with VWC reported as the variation in soil moisture relative to the initial mean value recorded on the first day of measurements; daily precipitation under ambient conditions (black bars) and under shelters (grey bars) is also indicated.

5.4.2 Effect of treatments on mass loss of standard litter after one year and during the SFP

After one year, mass loss of standard litter had not been influenced by increased temperature and reduced precipitation, nor by their interaction (**Table 5.3a**, **Fig. 5.3**). On the other hand, significant differences were found between high- and low-quality litter and between different incubation depths (**Table 5.3a, Fig. 5.4a**). In particular, the influence of incubation depth was not consistent between the two litter types (significant interaction term in **Table 5.3a**), with high-quality litter showing increasing mass loss along the depth profile, whereas low quality litter displayed the opposite pattern (**Table 5.4, Fig. 5.4a**).

Climate manipulations were also found not to have influenced mass loss during the snow-free period (SFP) (data not shown) but, again, there were significant differences between litter types and depths (**Table 5.3b, Table 5.4, Fig. 5.4b**). In addition, the increase in mass loss with depth was greater in high-quality litter than it was in low-quality litter (significant interaction term in **Table 5.3b, Table 5.4**). During the SFP, despite mass loss of high-quality litter still being higher compared to low-quality litter, the difference between the two types was smaller compared to that observed after one year (**Fig. 5.4b**).

Table 5.3: Results of LMMs for mass loss after one year (YEAR) and during the snow-free period (SFP). All variables tested as fixed effects are reported, but results are shown only for significant terms; non-significant terms excluded during the model selection procedure are reported as "ns". Only the significant two-way interaction is shown; three-way interactions tested among variables were not significant and, therefore, not reported.

	df (N,D)	F-value	p-value
(a) YEAR			
Temperature	ns	ns	ns
Precipitation	ns	ns	ns
Type	1,38	1393.667	< 0.001
Depth	2,75	35.337	< 0.001
Type × Depth	2,75	50.43	< 0.001
(b) SFP			
Temperature	ns	ns	ns
Precipitation	ns	ns	ns
Type	1,38	18.981	< 0.001
Depth	2,75	23.253	< 0.001
Type × Depth	2,75	7.251	0.001

Table 5.4: Results of Tukey comparison of least square means ± standard error of mass loss values for the two types of standard litter (green and rooibos tea) incubated at three depths (0, -5 and -15 cm) during 3 periods: snow-covered period (SCP), snow-free period (SFP) and one year (YEAR). Means on the same row sharing the same letter are not significantly different by Tukey-adjusted mean separations (p>0.05).

Period	Green tea			Rooibos tea		
	0 cm	-5 cm	-15 cm	0 cm	-5 cm	-15 cm
SCP	0.436 ± 0.006 (a)	0.444 ± 0.007 (a)	0.452 ± 0.015 (a)	0.218 ± 0.005 (b)	0.177 ± 0.007 (c)	0.172 ± 0.017 (bc)
SFP	0.041 ± 0.005 (a)	0.081 ± 0.005 (b)	0.103 ± 0.010 (b)	0.013 ± 0.004 (c)	0.032 ± 0.003 (a)	0.028 ± 0.006 (ac)
YEAR	0.478 ± 0.005 (a)	0.525 ± 0.005 (b)	0.556 ± 0.010 (b)	0.231 ± 0.004 (c)	0.209 ± 0.003 (d)	0.200 ± 0.006 (d)

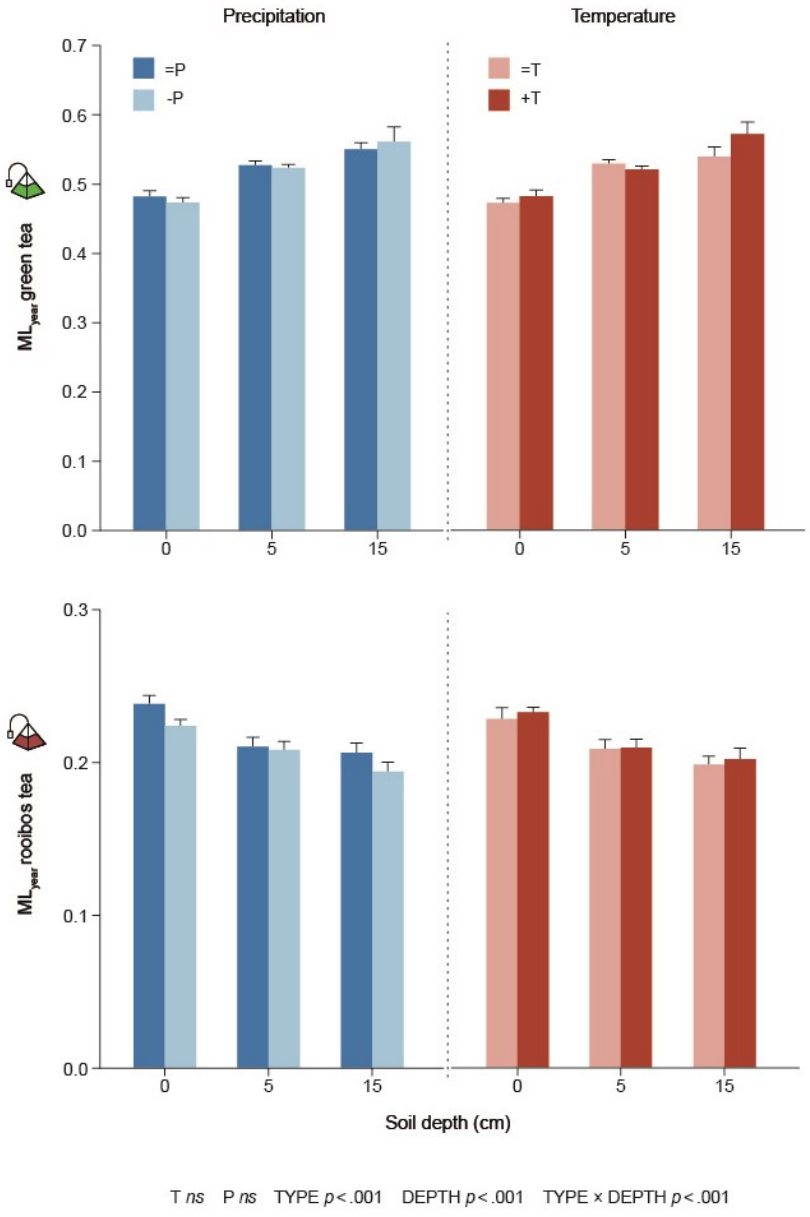


Fig. 5.3: Mean mass loss proportion of standard litter (TYPE: green and rooibos tea) after 1 year of incubation (ML_{year}) at three depths (DEPTH: 0, -5 and -15 cm) under different treatments: precipitation [P: =P, ambient precipitation (C and W plots); -P, precipitation reduction (D and DW plots)] and temperature [T: =T, ambient (C and D plots); +T, warming (W and DW plots)]. Bars indicate standard error (n=10, except for rooibos tea at 0 cm, n=9); the p-value of each fixed effect is reported, with "ns" indicating non-significant terms (p>0.05) excluded during the model selection process.

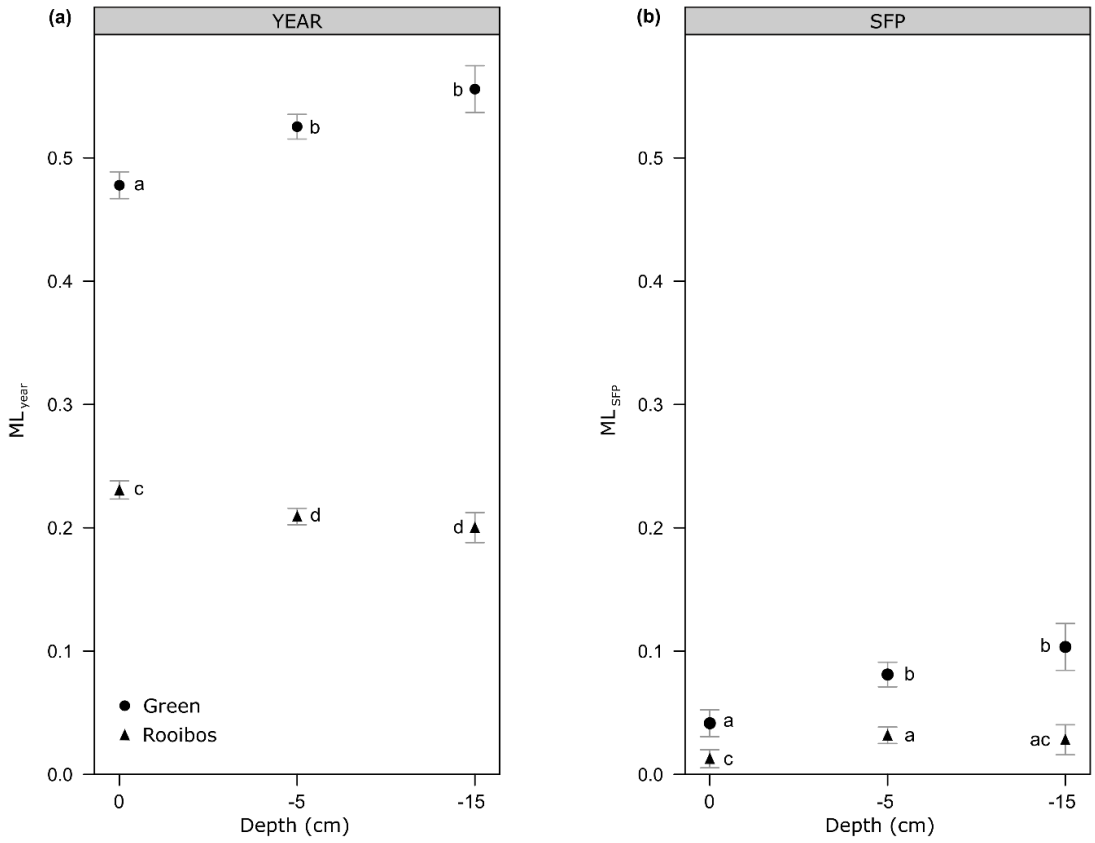


Fig. 5.4: Mean mass loss proportion of standard litter (green and rooibos tea) **(a)** after 1 year of incubation (ML_{year}) and **(b)** during the snow-free period (ML_{SFP}) at three depths (0, -5 cm and -15 cm). Bars indicate the 95% confidence interval. Different letters indicate significant differences (post-hoc Tukey contrast, $p<0.05$).

5.4.3 SCP vs SFP: decomposability of native and standard litter

The incubation period, the type of litter and their interaction significantly influenced both mass loss and the decomposition rate of native and standard litter (**Table 5.5**).

With regard to mass loss (**Table 5.1, Fig. 5.5**), the majority of litter types exhibited higher values during the snow-covered period. Only wood (Wd), the moss *Polytrichum juniperinum* (PJ) and roots (Rts) exhibited the lowest mass loss during the SCP and smaller differences between the two periods (SCP and SFP) compared to other litter types. Regarding native litter incubated on the surface during the SCP, a hierarchical pattern of mass loss could be recognized when grouping litter from different plant species into their respective functional groups: forbs (0.521 ± SE 0.027, n=6) > sedges (0.429 ± SD 0.037, n=1) > grasses (0.410 ± SE 0.076, n=3) > shrubs (0.242 ± SE 0.027, n=3) > mosses (0.052 ± SD 0.008, n=1). Standard litter at all incubation depths exhibited ML_{SCP} values similar to foliage litter of species incubated on the surface rather than to roots incubated belowground; in particular, ML_{SCP} of green tea litter on the soil surface was similar to ML_{SCP} of the dominant sedge *Carex curvula* (CC) (0.436 ± SD 0.006 and 0.429 ± SD 0.037), whereas mass loss of rooibos tea resembled values recorded for shrubs, in particular *Salix herbacea* (SH) (0.217 ± SD

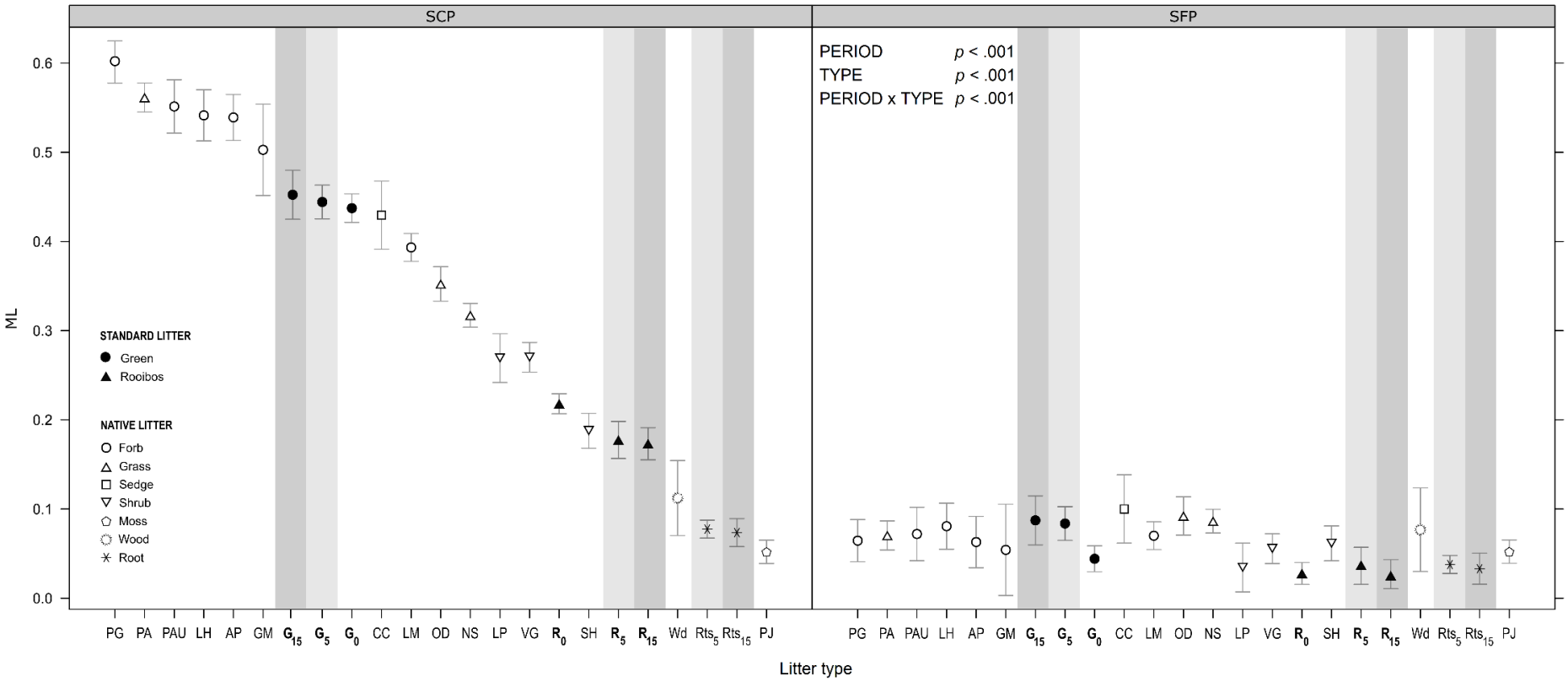
0.005 and 0.188 ± SD 0.006). Overall, similarities in mass loss values between litter types were greater for the SFP (ML range 0.03-0.10) compared to those recorded for the SCP (ML range 0.05-0.60).

Rates of mass loss exhibited patterns similar to those observed for mass loss proportion. Decomposition was generally higher during the SCP for all litter types, except for PJ, wood and roots at both -5 and -15 cm, which exhibited the opposite response, and SH, which showed similar values in both periods (**Fig. 5.6**).

Table 5.5: Results of LMMs testing the effects of the incubation period (snow-covered period and snow-free period) and litter type (23-level factor, native and standard litter at three different depths) on mass loss proportion (ML) and rate of mass loss (RML, % d^{-1}). All variables tested as fixed effects are reported.

	ML			RML		
	df (N,D)	**F-value**	**p-value**	**df (N,D)**	**F-value**	**p-value**
Period	1,172	615.443	< 0.001	1,172	56.3	< 0.001
Type	22,172	425.389	< 0.001	22,172	448.241	< 0.001
Period × Type	22,172	183.329	< 0.001	22,172	29.546	0.001

Fig. 5.5 (p. 115): Mass loss proportion (ML) of different litter types [TYPE: native litter (leaves of 14 different species, wood and roots) and standard litter (green and rooibos tea)] during the two incubation periods [PERIOD: snow-covered period (SCP) and snow-free period (SFP)]. The relative plant functional type of native foliar litter is also indicated. Belowground litter is highlighted with a grey backround (light grey -5 cm; dark grey -15 cm). Bars indicate 95% confidence interval and ID codes are reported in Table 1. Litter types are reported following a descending ML order during the SCP; the p-value of each fixed effect is reported.


SCP
SFP
PERIOD p < .001
TYPE p < .001
PERIOD x TYPE p < .001
ML
0.0
0.1
0.2
0.3
0.4
0.5
0.6
STANDARD LITTER
Green
Rooibos
NATIVE LITTER
Forb
Grass
Sedge
Shrub
Moss
Wood
Root
PG PA PAU LH AP GM G15 G5 G0 CC LM OD NS LP VG R0 SH R5 R15 Wd Rts5 Rts15 PJ
Litter type

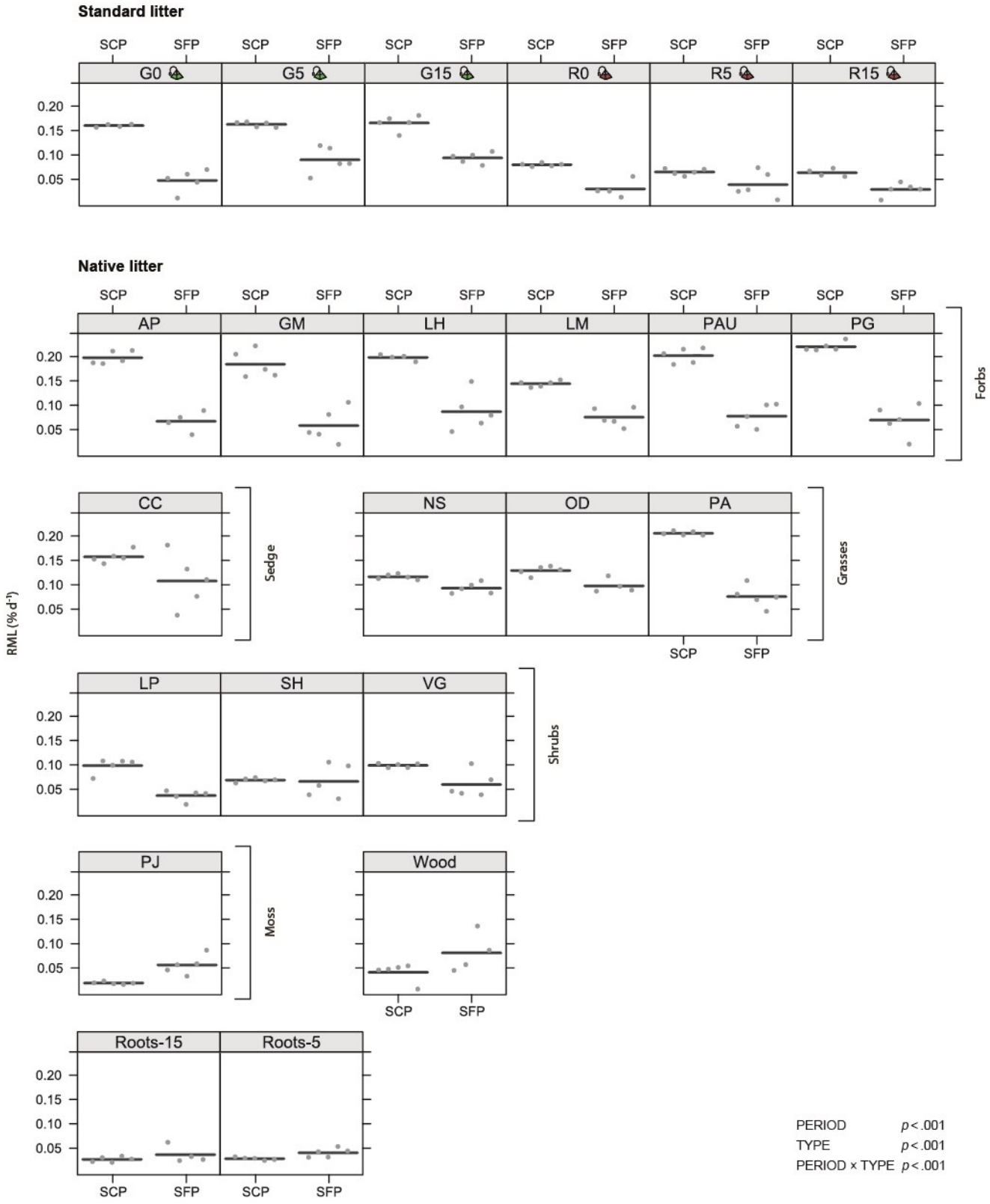


Fig. 5.6: Rate of mass loss (RML, % day^{-1}) for different litter types (native and standard litter) during the snow-covered period (SCP) and snow-free period (SFP). Lines indicate fitted values; the p-value of each fixed effect is reported.

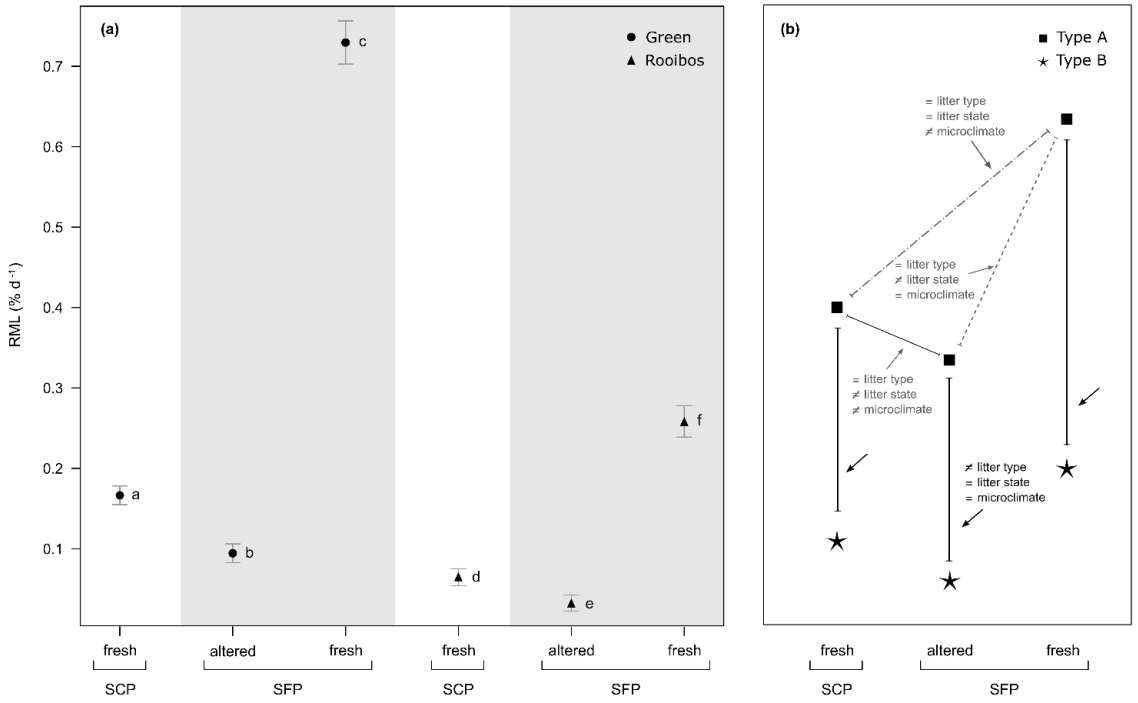
5.4.4 Litter alteration vs microclimate

The degree of litter alteration of standard material – altered and fresh – decomposing during the SFP affected the rate of mass loss ($F_{5,24}$=556.903, p<0.001), while the different depths of incubation were not significant on RML responses (p>0.05).

Within the same litter type, fresh litter decomposed faster than altered litter, not only when the same period was considered (SFP), but also when litter was compared under two different microclimates (SCP vs SFP) (**Fig. 5.7a**). In particular, during the SFP, RML for fresh green tea litter was equal to 0.728 ± SE 0.014 % day^{-1} (n=10) compared to RML of altered litter, which was 0.092 ± SE 0.006 % day^{-1} (n=10). For rooibos tea, RML values of fresh litter during the SFP were equal to 0.257 ± SE 0.008 % day^{-1} (n=10), with 0.034 ± SE 0.007 % day^{-1} (n=10) for altered litter. When the two different microclimates were compared, RML of altered litter for both tea types was lower during the SFP compared to that of fresh litter during the SCP [green tea SFP: 0.164 ± SE 0.003 % day^{-1} (n=10); rooibos tea SCP: 0.064 ± SE 0.002 % day^{-1} (n=9)]. When RML of fresh litter during the SFP is considered, this was higher compared to RML of fresh litter recorded during the winter (**Fig. 5.7a**). Moreover, data revealed that differences in decomposition rates were greater when considering litter with a different state of alteration, incubated during the same period (Δ 0.636 and 0.223 % day^{-1} for green and rooibos tea, respectively) compared to differences between litter sharing the same state of alteration, but decomposing under different microclimates (Δ 0.564 and 0.193 % day^{-1} for green and rooibos tea, respectively).

Finally, when comparing green and rooibos tea during the same incubation period, the difference in RML between the two litters was greater for fresh litter than that for altered litter (Δ Green-Rooibos RML_{SFP} fresh = 0.471 % day^{-1} vs Δ Green-Rooibos RML_{SFP} altered = 0.058 % day^{-1}) (**Fig. 5.7a**).

Fig. 5.7: (a) Rate of mass loss (RML, % d^{-1}) during the snow-covered period (SCP, white background) and snow-free period (SFP, grey background) of standard litter (green and rooibos tea), characterised by different degrees of alteration: "altered" – litter that has already undergone winter decomposition; and "fresh" – intact, unaltered litter that has not undergone any prior decay process. Bars represent 95% confidence interval. Different letters indicate significant differences (post-hoc Tukey contrast, $p<0.05$). **(b)** Schematic diagram in support of the interpretation of data displayed in graph (a), with comparison made on two levels: the first level focuses on RML responses within the same litter type, characterised by different combinations of diverse degrees of alterations (litter state: fresh and altered) and microclimate conditions (SCP and SFP); the second level illustrates RML responses between litter types sharing the same litter state and microclimatic conditions.



5.5 DISCUSSION

High-elevation ecosystems are experiencing drier summers in various regions of the world (Basistha et al., 2009; Yang and Gong, 2010; Brugnara and Maugeri, 2019). With regard to the Alps, such a trend, together with warming, is expected to intensify by the end of the 21st century (Gobiet et al., 2014). The aim of the present study is to improve understanding of how decomposition processes in alpine environments will be affected by these changes and, at the same time, identify the underlying mechanisms which could go towards explaining the observed responses. Results reveal that the long snow-covered season is the period during which the earlier, more climate-dependent phases of decomposition occur. In all likelihood, this period represents one of the key elements enabling short-term decomposition processes in alpine ecosystems to exhibit a certain degree of resistance to changes in temperature and precipitation during the summer.

5.5.1 Warming and precipitation reduction: effects on standard litter decomposition

As was set down in the initial hypothesis, no significant effect of higher temperatures and/or reduced precipitation during the growing season was observed on either the annual or summer mass loss of standard litter in the target alpine grassland (**Fig. 5.3**). The lack of promoting effects of warming on decomposition rates has often been explained as the result of low soil moisture levels (Aerts, 2006; Blok et al., 2016; Christiansen et al., 2017; Brigham et al., 2018; Sarneel et al., 2020; Liu et al., 2021), which are known to reduce microbial activity under severe drought conditions (Bloor and Bardgett, 2012; Sanaullah et al., 2012b; Allison et al., 2013). In high-elevation ecosystems, in particular, soil moisture is widely recognized as an important factor driving litter decomposition rates across communities (Bryant et al., 1998). This has led to the hypothesis that drier summers would directly affect decaying processes in these environments (Gavazov, 2010). In the present study, however, standard litter outcomes were not in line with such predictions, but corroborated results which found no responses, or only weak ones, of litter decomposition over one year to summer climate manipulations in subalpine and alpine ecosystems (Benot et al., 2014; Carbognani et al., 2014a; Bernard et al., 2019). A possible explanation of such an outcome could be related to the important contribution of the long, snow-covered incubation period, as suggested by Carbognani et al. (2014a) in a study on decomposition dynamics within an alpine snowbed. Even though there is evidence that a great amount of litter decay takes place under the snowpack (Hobbie and Chapin, 1996; Baptist et al., 2010), in the study by Carbognani et al. (2014a) the role of winter as opposed to that of growing season manipulations could not be disentangled because mass loss values referred to the whole year.

In detail, results from the present study show that, across all depths, 87% of annual mass loss occurs during the SCP (-86% green tea, -88% rooibos tea) – a period accounting for 75% of the entire incubation period. Contrasting results, such as those obtained by Hagedorn and Joos (2014) who found significant effects of summer drought on litter decomposition in a managed subalpine grassland, may be partially explained by the litter placement on the soil surface immediately prior to the treatment, together with the use of a full-exclusion shelter which might have prevented physical disruption and leaching. In the present study, lower mass loss values were effectively observed for standard litter standing on the soil surface, but differences were not significant. Different types of experimental reduction – i.e. total vs partial rainfall exclusion – could explain these contrasting results.

As far as litter incubated on the soil surface is concerned, the use of partial rain-out shelters might have been able to guarantee a sufficient level of humidity on the surface to maintain litter decomposition levels similar to those recorded in ambient conditions. Moist conditions derive from rain actually falling on the litter surface, together with other "non-rainfall moisture" (Evans et al., 2020), such as water vapour, fog and dew, which could also enable persistent microbial activity (Dirks et al., 2010). Unfortunately, moisture values of litter to verify the degree of difference between samples standing in control and under shelters are not available. Precipitation, however, was frequent during the growing season – more than half the period characterised by rainy days. In addition, when relative humidity (RH) data was checked, classifying "wet hours" as those between the 75% and 90% RH thresholds (Evans et al., 2020), data showed that 51% of the litter incubation period during the SFP was characterised by moist atmospheric conditions. Wet hours were especially concentrated during the night, between 8 pm and 9 am, with over 50% "wet hours". As far as can be ascertained at present, the contribution of non-rainfall moisture on surface litter decomposition has only been investigated in ecosystems naturally characterised by long, warm dry periods without precipitation (Dirks et al., 2010; Evans et al., 2020 and references therein). The extent to which atmospheric humidity, other than rainfall, acts as a buffer against the action of artificially reduced summer precipitation on soil-surface litter decay in cold, high-elevation ecosystems should be a topic of further investigation.

When belowground litter is considered, it cannot be excluded that one of the reasons for the lack of significant effects of reduced precipitation could be the limited treatment effect on soil moisture conditions. The VWC under the shelters was reduced by only -3.2% and -4.1% in D and DW plots, respectively, compared to C (**Table 5.2, Fig. 5.2**); thus, the reduction might not have been severe enough to reach the moisture thresholds which trigger a sharp decrease in microbial activity (Prescott, 2010; Schimel, 2018). Such an explanation is supported by outcomes presented in Chapter 6 (Section 6.3.6) on soil moisture thresholds for ecosystem respiration, a component of CO_2 exchange which depends on microbial metabolic activity; depending on the timing of drought

occurrence, greater declining rates in CO_2 emissions were found to be triggered below VWC values equal to 12% and 25% – corresponding to -0.06 MPa and -0.02 MPa of water potential, respectively. These values were similar to water potential thresholds reported by Orchard and Cook (1983), who found that bacterial activity estimated through respiration declined sharply with WP between -0.05 MPa and -0.30 MPa. In the current study, the range of mean daily VWC at -5 cm recorded under shelters during the summer ranged from 24% (min.) to 36% (max.) in the D treatment and from 25–32% in DW, backing the hypothesis that soil conditions under sheltered plots were not limiting belowground microbial decomposition. Furthermore, the VWC range recorded under shelters appeared to be close to that recorded in ambient conditions (min. 25% – max. 37%), suggesting that the microbial community may have hardly been affected by the shelter-induced reduction in soil moisture, since values were within the range of conditions naturally experienced by microorganisms.

5.5.2 The role of the litter type and incubation depth

When analysing standard litter, the type of litter and incubation depth significantly explained recorded differences in annual and seasonal mass loss values (**Fig. 5.4a, b**). After one year the 51% mass loss of green tea was over twice that recorded for rooibos tea (21%), in agreement with other studies conducted using standard litter on both a regional (Didion et al., 2016; Althuizen et al., 2018; Petraglia et al., 2019; Fanin et al., 2020) and global scale (Djukic et al., 2018). This outcome can be explained by the highly diverse chemical composition of each tea: green tea (high quality litter) exhibits a lower C:N ratio and a water soluble fraction twice that of rooibos tea (low quality litter) (Keuskamp et al., 2013). The water soluble fraction, together with contents of N, lignin and the C:N and lignin:N ratios are known to be directly related to mass loss and nutrient release during decomposition (Aerts et al., 2012; Berg and McClaugherty, 2013). Therefore, abiotic and biotic processes – i.e. leaching and microbial decomposition, respectively – took place at a faster rate when acting on high-quality substrates, as already observed not only for standard litter but also on local substrates (Hobbie, 1996; Cornelissen et al., 2007; Saccone et al., 2013; Carbognani et al., 2014a;). The results obtained in the investigated alpine grassland, therefore, are not surprising and support the paradigm that recognizes climate as the main predictor of decomposition on a large scale, whereas on a local and regional scale, the main actors are litter quality, together with the biotic and abiotic properties of soil (Coûteaux at al., 1995; Aerts, 1997).

Litter decomposition, however, did not appear to be equally constrained along the vertical profile – i.e. soil surface and belowground – with decomposition dynamics differing for the two types of tea. Annual mass loss values showed that while high quality litter decomposed more belowground compared to the soil surface, low quality litter exhibited the opposite pattern (**Fig. 5.4a**). When subtracting values for the snow-covered season from annual mass loss values,

decomposition appeared to be enhanced belowground especially during the SFP, particularly for green tea (**Table 5.4, Fig. 5.4b**). Although buried rooibos tea decayed significantly less belowground during the SCP, such a difference was not significant when analysing annual mass loss (**Table 5.4**) – further evidence suggesting belowground decomposition was enhanced during the summer.

Differences in litter decay found across the vertical profile are in line with results reported for temperate and dry ecosystems (Liu et al., 2015; Margesin et al., 2016) and also with findings using local litter from a tussock tundra, where higher litter decay was observed in the soil compared to the surface (Hobbie and Chapin, 1996). More stable and favourable thermal and moisture conditions in the soil have been recognized as the main factors explaining recorded results (Hobbie and Chapin, 1996; Liu et al., 2015; Margesin et al., 2016).

Values obtained in the present study further illustrate how the dynamics of decomposition depending on the depth of incubation differs across litter types. A regional-scale study on French forests indicated that belowground decomposition was higher for green tea compared to rooibos (Fanin et al., 2020), which is partly borne out by the present study. Fanin et al. (2020) suggested that this result was due to the fact that decomposition of rooibos tea is generally more nutrient-limited compared to high quality litter, where decay is, instead, largely driven by temperature and humidity. Furthermore, when C:N ratios are high, microbial communities have been reported to immobilise N from external sources, such as soil organic matter, in order to degrade low quality material (Prieto et al., 2019 and references therein). Within the target grassland, organic matter is concentrated in the upper soil layers (approx. first 10 cm), with deeper layers presenting higher mineral content. Results from the present study suggest that, during the first incubation period, in this belowground micro-environment the low temperatures during the winter, under which metabolic activity of winter-adapted microorganisms continues (Schinner, 1983; Zinger et al., 2009), combined with nutrient limitation, could have determined a decrease in the efficiency of microbial decomposers on rooibos tea. Mass loss on the surface, instead, may have been driven, for the most part, by leaching during the period preceding snowfall and during snowmelt (Bokhorst et al., 2010). In addition, the similarity observed in decomposition rates during the SCP between green tea and the sedge *Carex curvula* (**Fig. 5.5**) may further suggest that microorganisms could already be adapted to breaking down this particular type of standard litter, as opposed to rooibos tea, given the resemblance of green tea to the major litter input naturally received by the target grassland.

5.5.3 Native and standard litter: decomposability and importance of the snow-covered season

Analysis on standard litter, together with the major litter types characterising the target grassland, gave an overall picture of the actual variability of decomposability present within the

studied ecosystem, again highlighting the relevant contribution of the SCP on the initial phases of litter decomposition.

For the majority of litter types, both ML (**Fig. 5.5**) and RML (**Fig. 5.6**) were greater during the incubation period preceding the growing season, thus confirming the second hypothesis (HP2) put forward in this study. In detail, native litter lost between 50-90% of total annual mass loss during the 273 days preceding the growing season, with values at the lower end of the scale recorded for the moss *Polytrichum juniperinum* (50%), wood (60%) and roots (approx. 70% when averaging values of root mass loss at -5 cm and -15 cm depth). Accounting for the effects that different litterbag mesh-size can have on decomposition rates (Xie, 2020), values from the present study are in line with those reported for the Arctic (72-87% for shrub litter in Blok et al., 2016) and alpine tundra (46-81% for shrub and graminoid litter in Baptist et al., 2010). Unfortunately, by comparing decomposition rates after the snowmelt period – i.e. the end of the SCP – with the initial pre-incubation mass, it was not possible to identify whether faster SCP rates, compared to those in the SFP, were the result of leaching during the 31 days before the onset of snow or during snowmelt (**Fig. 5.2**) or, rather, of actual decomposition under the snowpack; the literature has reported different results on the subject (Moore, 1983; O'Lear and Seastedt, 1994; Bokhorst et al., 2010; Saccone et al., 2013).

The different patterns of decomposition rates – RML during the SCP ≤ RML during the SFP (**Fig. 5.6**) – observed for *Salix herbacea*, *P. juniperinum*, wood and roots could be explained by the high content of recalcitrant compounds that has been reported in the literature for these litter types. For instance, it was interesting to note that similar ML and RML values exhibited by the shrub *Salix herbacea* between the SCP and SFP were in line with previous findings by Baptist et al. (2010), where such an outcome was attributed to high polyphenol concentration in the leaves of *Salix sp.* species. Concerning mosses, findings from the present study, which are in accordance with previous outcomes in tundra ecosystems recorded for this functional group (Hobbie, 1996; Bokhorst et al., 2007; Carbognani et al., 2014a), could be a result of high concentrations of structural, lignin-like, soluble phenolic compounds (Lang et al., 2009 and references therein). The genus *Polytrichum*, in particular, was reported by Banerjee and Sen (1979) as having a pronounced antibiotic activity for this moss group, highlighting how *P. juniperinum* was commonly used for medical purposes in a description from the 1950s by Wren (1956). It could, therefore, be possible that such properties underlie the particularly low mass loss values observed for PJ litter in the present study. In general, the lower RML observed during the SCP compared to the SFP for this particularly recalcitrant litter could be due to the concurrent limitations of low-temperatures and litter quality, with higher temperatures partially releasing climate constraints on microbial activity during the growing season.

By incubating litter from several species, including some of the major *taxa* found in high-elevation grasslands in the Alps, this study provides a comprehensive view of the decomposability spectrum found in typical alpine ecosystems standing on siliceous bedrock. After one year, the native litter of 7 out of 14 species lost more than 50% of its inital mass; with the exception of *Poa alpina* (grass) and *Carex cuvula* (sedge), all species belong to the functional group of forbs. Foliar litter of grasses and shrubs, on the other hand, decomposed less, with ML ranging from 40-44% (excluding PA) to 25-33% for the two functional groups, respectively. Overall, the pattern of decomposition observed across plant functional types within the study site – i.e. forbs > graminoids > shrubs > mosses – is in line with findings from other studies in Arctic and alpine ecosystems (Hobbie, 1996; Cornelissen et al., 2007; Baptist et al., 2010; Carbognani et al., 2014a). Within such a decomposability spectrum, when ML during the SCP is considered, standard litter values resembled those of the dominant sedge *C. curvula* (green tea) and the common dwarf shrub *Salix herbacea* (rooibos tea) (**Fig. 5.5**). As far as can be ascertained at present, this is one of only two studies – the other by Didion et al. (2016) – in which teabags are incubated together with local litter. The preliminary comparison between native and standard litter highlights how the two extremes in terms of litter quality represented by standard litter are unable to capture the entire variability of material decomposing within the target site. This evidences the necessity of using local litter, together with teabags, in order to better understand decomposition processes in diverse ecosystems.

Other results emerging from the study reveal that the decay of fresh, standard litter incubated belowground showed values that were far greater than those exhibited by roots (**Fig. 5.5**). It was only during the SFP, when material had been already partially decomposed, that rates between rooibos tea and roots exhibited similar values, leading to the conclusion that this standard material does not reflect the decomposition dynamic of belowground plant organs during the early stage of decomposition. Root litter, in fact, generally decomposed far less than foliage. When considering annual root ML, averaging values obtained in the field at both incubation depths, this was approx. 4 times lower than that of native leaf litter on the soil surface, and 3.5 times lower than standard leaf litter incubated at the same depth. Highly diverse mass loss between roots and foliage litter have already been highlighted in the literature (Silver and Miya, 2001 and references therein; Freschet et al., 2013) and also observed in other tundra ecosystems (Hobbie and Chapin, 1996; Bryant et al., 1998). Comparison between roots and aboveground litter decomposition rates, however, is not straightforward due to the diversity in environmental conditions where decomposition processes naturally take place – i.e. soil surface for leaves and stems vs belowground for roots, which have more extensive contact with the microbial community (Freschet et al., 2013). Results from this study, where incubation of both above- and belowground litter took place in similar environmental conditions (at -5 cm and -15 cm depth),

thereby overcoming such a difference, lead to the conclusion that differences in litter quality are paramount, driving litter decay dynamics on a local scale.

These results also confirm the important role that belowground organs play as carbon sinks (Bryant et al., 1998) since belowground biomass is estimated to cover a large fraction of total biomass, especially in alpine grasslands, where belowground organs represent a large C pool within soils (Körner and Renhardt, 1987; Grabherr, 1989; Fisk et al., 1998). The generally low decomposition rates found for root litter could have important implications in future climate change scenarios, through the indirect effects that abiotic factors might have on biomass allocation patterns, intended as a shift towards an increased root:shoot ratio or a change in community composition towards deep-rooted species (Zhang et al., 2017; Schmid, 2017; Liu et al., 2018; Liu et al., 2020). On the basis of results from the present research, on the other hand, there is reason to believe that direct effects of altered summer temperatures and moisture conditions on root decomposition will be negligible in the short term in alpine grasslands characterised by generally high mean annual precipitation, given the low decomposition rates observed in ambient conditions and the established secondary role that climate and environmental drivers have on root litter decay (Silver and Miya, 2001).

5.5.4 Litter alteration: its role in the stability of decomposition processes

By cross-comparing the RML of buried standard litter sharing the same state of alteration and/or microclimate – i.e. micro-environment – it was possible to determine to what extent the degree of litter alteration and the environment act on litter decay (**Fig. 5.7a**). When focusing on the same litter type, fresh litter exhibited higher decomposition rates than partially decomposed litter. Not only was this evident for litter decomposing under the same environmental conditions (SFP), but also when compared with litter incubated under different micro-environments (SCP vs SFP). The difference between fresh and altered litter, however, was greater under the same microclimate conditions. This leads to the conclusion that, although environmental conditions during the growing season are more favourable, the action of microorganisms during this period is limited by the state of partial decomposition which characterises litter at the end of the snow-covered season. Defining the early stage of decomposition as the period where the chemical composition of litter and microclimate determine the rates by which the substrate is decomposed, lasting until approx. 40% of mass is lost (Berg and McClaugherty, 2013), it appears that the majority of litter types – including both standard and indigenous litter (**Fig. 5.5**) – undergo this early stage during the fall-winter season; this is especially true for litter types belonging to the most representative species of the target community. Litter approaching the growing season could already be in the late, lignin-dominated phase of degradation, where climate is less influential (Berg and McClaugherty, 2013). Although no chemical analysis was

carried out to verify this hypothesis, it could explain why there seems to be more similarity between different litter types when considering RML during the SFP compared to those recorded during the SCP.

Furthermore, in the case of the present study, when a comparison was made between the decomposition of green tea with that of rooibos, differences between the two litter types were greater when comparing fresh material, rather than partially decomposed litter. The differences in degradation rates are known to be driven by the species-specific amount of organic matter that is not lignified – i.e. leaching of soluble compounds and decomposition of solubles and non-lignified cellulose and hemicellulose – and are mainly visible during the early stage of decomposition (Coûteaux et al., 1995; Berg and McClaugherty, 2013). For instance, in a common garden experiment investigating effects of different precipitation regimes on litter decay and nutrient release, Du et al. (2020) found that mass loss and C release were minimally affected in the second period of incubation (6-12 months), whereas significant responses to treatments were recorded in the earlier phase (0-6 months). The different concentrations of non-lignified organic matter in fresh and altered litter could, therefore, be one of the reasons explaining why manipulation of summer climate variables did not produce any effect in this highly seasonal environment.

5.6 CONCLUSION

Short-term decomposition processes in an alpine ecosystem characterised by strong seasonality were not influenced by summer and reduced precipitation. The important contribution which the long snow-covered period made on the early stage of decomposition confirms, once more, that alpine systems are favourable habitats for early-stage decomposition, even if plant growth in such environments is limited by the harsh conditions (Gavazov, 2010; Carbognani et al., 2014a); during the summer, in fact, the state of litter alteration characterising litter approaching the snow-free season is the main factor constraining decomposition rates under potentially more favourable climate conditions.

In the short term, nutrient and carbon turnover in these environments could, thus, be affected by both changes occurring during the early stages of decomposition in fall and winter, and/or by short-term alterations of litter quality. A further consideration is that litter decay may not be affected by climate change unless climate thresholds for microbial activity are reached (Prescott, 2010).

Since other mechanisms will be influenced by climate change – e.g. alteration of litter input related to shifts in biomass allocation patterns (Freschet et al., 2013; Eziz et al., 2017; Liu et al., 2018) or soil biota community composition and function (Allison et al., 2013; Melillo et al., 2017;

Christiansen et al., 2017) – long-term predictions cannot be made and further studies are needed to account for the effects of such changes on litter decomposition processes. However, it is acknowledged that variations in plant community composition will determine significant alterations in ecosystem functioning, which will involve nutrient cycling together with shifts in the C pool – i.e. quantitative changes, expressed through alterations in plant productivity, and qualitative changes, which are dependent on the diverse set of species present. It seems likely, therefore, that long-term shifts in species identity characterising plant communities will account for major changes in decomposition processes in tundra ecosystems (Cornelissen et al., 2007; Aerts et al., 2012). Finally, when investigating decomposition dynamics, the micro-environment in which decomposition processes take place needs to be evaluated – i.e. surface vs belowground. The simultaneous use of local leaf litter with teabags could also help to clarify results deriving from standard litter, bearing in mind that the use of standard foliage litter may lead to an overestimation of carbon turnover rates with regard to belowground litter decay.

5.7 CONTRIBUTIONS

Alessandro Petraglia and Michele Carbognani initiated the project, designed and supervised the experiment. Roberto Vacchelli, Andrea Comelli, Luca Barchi and Cristian Cavozzi also worked on the design of the shelters and the set-up of the experiment. T'ai G. W. Forte, AP and MC planned the research and led the field sampling. Stefano Meroni, Thai Linh Bacchilega, Paola Chiudinelli, Matteo Maria Maniscalco, Mattia Malerba and Maria Timco also participated in the field sampling and lab work. TGWF and MC performed the statistical analyses. TGWF wrote the manuscript, which was subsequently critically revised by AP and MC.

CHAPTER 6. STUDY III – DROUGHT TIMING: EFFECTS ON PLANT GROWTH AND CO_2 FLUXES IN AN ALPINE GRASSLAND

6.1 ABSTRACT

More extreme precipitation regimes are predicted as a result of global warming. In this scenario, timing of drought represents a crucial aspect in determining the degree to which limited water supply impacts ecosystem functions. Drought-timing effects on alpine mesic grasslands, however, still remain poorly investigated.

A mesocosm experiment using monoliths from an alpine *Carex curvula* grassland was carried out to assess the effects of different drought timings during the growing season on aboveground net primary production (ANPP) and CO_2 fluxes – i.e. ecosystem respiration (ER), gross ecosystem production (GEP) and net ecosystem exchange (NEE). Grassland monoliths were subjected to 3 drought treatments: early-season drought (ED), mid-season drought (MD) – both of which lasted 1 month – and full-season drought (FD) lasting 2 months and implemented to help identifying soil moisture and temporal thresholds triggering different ER and GEP rates. Drought conditions were achieved through full-rainfall exclusion, with no watering taking place. The effects were evaluated by comparing responses from non-watered monoliths with those from controls (C), where watering took place during the entire experimental period.

Data was examined in order to assess: (i) responses to treatments over the whole study period and during each of the two drought periods; (ii) the resistance of ER and GEP within the two drought periods; and (iii) soil moisture and temporal thresholds triggering different rates of ER and GEP.

Over the entire study period, ANPP and CO_2 fluxes were negatively affected by drought, with the greatest impact found in FD. Between the two drought timings, ED exhibited the largest effect on ANPP, ER and NEE, while GEP was equally reduced in both ED and MD. When CO_2 fluxes were analysed considering each drought period separately, response dynamics differed between the two timings: during the early drought period fluxes in non-watered pots significantly differed from those in watered pots after 20 days; during the mid-season drought period it took 13 days for ER to be significantly reduced and 23 days for gross assimilation to exhibit an abrupt decrease; in ED pots, ER and GEP never reached C values after re-watering, whereas NEE equalised C on the last measurement date. GEP was generally less resistant to drought compared to ER, with both processes exhibiting lower resistance to MD than to ED. Threshold analysis revealed that soil moisture and temporal thresholds for ER and GEP were different in each drought timing, with

those in MD being reached at higher moisture level, and after a shorter dry period, compared to thresholds in ED.

Overall, key ecosystem processes in the investigated grassland were found to be significantly impacted by drought, the extent of which varied according to the timing of dry spells during the growing season. The results highlight the existence of different mechanisms underlying diverse ecosystem responses to drought, illustrating the importance of the temporal framework when analysing drought-timing effects. In future, it would seem that mid-season dry spells are more likely to cause abrupt changes in ER and GEP, with thresholds being reached sooner, underlining the fact that timing should be factored in when ecological thresholds are investigated.

6.2 INTRODUCTION

Increased temperatures and altered precipitation patterns can profoundly alter the carbon (C) balance by affecting composition, structure and functions of terrestrial ecosystems (Reichstein et al., 2013; Frank et al., 2015). Drought, in particular, acts on ecosystem functions by decreasing the system's productivity (Zhao and Running, 2010), reducing plant growth and increasing plant mortality (Wu et al., 2011; Frank et al., 2015). Field precipitation experiments have mostly investigated the impact of changed precipitation amounts and frequency (e.g. Harper et al., 2005; Beier et al., 2012; Zeppel et al., 2014; Wilcox et al., 2015; Estiarte et al., 2016), focusing much less on the impact of drought seasonality. The lack of research on this particular aspect is probably the result of the generally held knowledge that the total amount of precipitation is a good predictor of aboveground net primary production (ANPP) changes along both a spatial scale, including different sites with widely differing rainfall amounts, and also a temporal scale, encompassing inter-annual precipitation changes within a single site (Knapp and Smith, 2001; Huxman et al., 2004; Estiarte et al., 2016; Knapp et al., 2017b). However, the process by which plant growth and performance, and overall ecosystem functions, are governed by meteoric water availability is far more complex and goes beyond the exclusive quantitative feature of precipitation regimes (rainfall amount), with the effects of altered temporal distribution of precipitation also needing to be considered (Jentsch et al., 2007; Knapp et al., 2008; Wu et al., 2011). Both inter- and intra-annual variability of precipitation are, in fact, predicted to increase with global warming and, in the face of recent findings, this could lead to important changes in ecosystem functions (Knapp et al., 2008).

As far as drought is concerned, the timing of a dry spell could be a crucial aspect determining the degree to which ecosystem functions are impacted by limited water supply (Sippel et al., 2016; 2018). For instance, productivity was found to be more sensitive to seasonal precipitation, rather than to mean annual precipitation, in 36 plant communities ranging from deserts to forests

(Robinson et al., 2013). Productivity was also observed to be diversely affected in herbaceous ecosystems according to the timing of natural dry spells, with declining effects of drought later in the season (Craine et al., 2012). On a finer spatial scale, spring drought was found to suppress canopy development and peak leaf area in forests (Noormets et al., 2008) and reduce biomass and cover of herbaceous species in a sagebrush steppe (Bates et al., 2006). With regard to temperate and semi-arid grasslands, the few studies available so far have found that drought timing influenced the magnitude of both ANPP response – mid-summer > early summer in Denton et al. (2017); spring > summer in Meng et al. (2019) – and the reproductive output – late summer drought reduced the density of reproductive shoots, whereas spring drought negatively affected seed rain density and richness (Zeiter et al., 2016). In a study on alpine grasslands in Australia, Griffin and Hoffmann (2012) speculated that the occurrence of a long dry period in mid-summer could cause drought-related mortality of tussock grasses observed in areas which usually experience rain-free periods during the autumn.

These findings underline the importance of timing in determining the impact of drought on ecosystem functions, since temperature, soil water availability and stages of plant development are intrinsic, inter-related features which vary not only across different seasons, but also within the same season (De Boeck et al., 2010). These factors, together with the pivotal role of phenology, can determine whether an ecosystem is affected by drought or not (De Boeck et al., 2011). Within the same season, for instance, it is generally assumed that drought in the early part of the growing season will have a greater impact compared to dry spells occurring during the period when plants are approaching senescence (Zeppel et al., 2014; Dietrich and Smith, 2016; Hao et al., 2018; Zhang et al., 2020a). Furthermore, the impact of a dry spell may be more intense when occurring during a critical period for growth and development of the dominant species. For instance, it was observed that ANPP was especially affected by drought during the middle of the summer season, the period coinciding with the flowering stalk initiation of the dominant species, *Andropogon gerardii* (Denton et al., 2017), whose reproductive shoot density and biomass decreased by 94% compared to controls (Dietrich and Smith, 2016). In contrast, Meng et al. (2019) found that productivity of the investigated meadow steppe was more sensitive to spring drought, rather than summer drought, a response driven by the phenological and morphological traits of the dominant species.

Drought timing and ecosystem features – species composition, phenology and microclimate – are therefore crucial in determining the degree of sensitivity to drought. Despite evidence deriving from experimental studies in other grasslands suggesting the importance of timing in modulating the impacts of drought on ecosystem responses (Zeiter et al., 2016; Denton et al., 2017), together with research showing negative effects of reduced amounts of precipitation over the growing season in montane and alpine grasslands (Gilgen and Buchmann, 2009; Stampfli et

al., 2018; Liu et al., 2018), as far as can be ascertained, no study is yet available which analyses the effects of different drought timing in high-elevation, alpine mesic grasslands. There is reason to believe that drought timing could affect ecosystem functions in grasslands located at higher elevations in a different way to that observed when only rainfall amount is taken into consideration.

Snow-dominated ecosystems, such as those present in the alpine life zone, are characterised by a very short growing season and high levels of soil moisture in the first part of the season derived from snowmelt input (Taylor and Seastedt, 1994; Williams et al., 2009; Harpold and Molotch, 2015). Furthermore, alpine communities mainly consist of low statured, perennial herbaceous species with fast development rates, enabling them to cope with the short growing season (Körner, 2003). These characteristics mean that results on drought timing effects derived from lower elevation grasslands, or other ecosystems, cannot simply be extended to those in high elevations. On the one hand, an early-season drought could have a greater impact on alpine ecosystems as it coincides with the most active period of plant growth (Bates et al., 2006), since species in alpine grasslands have almost completed their growth by mid-summer (Bowman and Fisk, 2001; Körner, 2003; Jonas et al., 2008). On the other hand, high levels of soil moisture following snowmelt input at the beginning of the season could lessen the effects of an early-season drought. Consequently, drought occurring during the middle of summer could have a greater impact on the grassland as a result of concurrent high temperatures, lower initial soil water content and higher aboveground biomass (Wang et al., 2007).

Productivity, however, represents only one of the key features of an ecosystem. Experimental studies on the effects of different drought timing on CO_2 fluxes in semi-arid ecosystems found that, in addition to gross ecosystem production (GEP), ecosystem respiration (ER) and, hence, net ecosystem exchange (NEE) were also diversely affected by early-, mid- and late-summer drought; early summer drought was found to produce greater negative effects compared to later dry spells (Liu et al., 2019; Zhang et al., 2019b). Thus, to gain deeper insights into the effects of drought timing on ecosystem functions, the heterotrophic component of the ecosystem also needs to be taken into account, since soil flux represent the second largest CO_2 flux in terrestrial ecosystems (Raich and Schlesinger, 1952). In addition, NEE represents one of the most common metrics employed to assess climate effects on ecosystem carbon gain (e.g. Gerdol et al., 2008; Jongen et al., 2011; Zona et al., 2014; Zhang et al., 2019a), since it describes an ecosystem's source/sink dynamics which is driven by the balance of its two major components – i.e. GEP and ER – both of which are highly dependent on thermal and moisture conditions (Reichstein et al., 2013).

GEP represents the main pool of C intake, driven by both autotrophic assimilation – i.e. photosynthesis – and photorespiration (von Buttlar et al., 2018). Lower water availability can directly affect GEP by reducing the photosynthetic activity of plants, their stomatal conductance

or by altering their structural asset – i.e. leaf area and leaf orientation (Noormets et al., 2008; van der Molen, 2011; Liu et al., 2019). On the other hand, ER expresses the CO_2 output from an ecosystem, which results from both heterotrophic respiration, through soil fauna and microorganisms, and autotrophic respiration deriving from above- and belowground plant organs (Chapin et al., 2006; von Buttlar et al., 2018). Both the autotrophic and heterotrophic components are tightly linked by their dependency on substrate availability, including photosynthate, litter and soil organic matter (Kuzyakov and Cheng, 2001; Wan and Luo, 2003), which can all be affected by drought. In studies evaluating the effects of lower water input, ER decreased as a result of decreased plant respiration (Darenova et al., 2017; Zhang et al., 2019a) and reduced soil microbial activity via microbial dormancy or death, alteration of nutrient availability, constraint in nutrient diffusion or changes in the structure and composition of microbial communities (Orchard and Cook, 1983; Bloor and Bardgett, 2012; Frank et al., 2015; Schimel, 2018). The balance between ER and GEP determines the sink/source role of an ecosystem through its dependence on the degree of sensitivity of these two processes in response to drought – i.e. the ability to "persist and maintain their functioning during a disturbance", otherwise known as "resistance" (Gessler et al., 2020).

Various studies have reported different sensitivity to low soil moisture on the part of GEP and ER, with GEP being generally more sensitive to drought than ER, therefore reducing the strength of the ecosystem as a carbon sink (Hoover and Rogers, 2016; Liu et al., 2019; Zhang et al., 2019a). Ecosystem CO_2 assimilation and emission, however, might be diversely constrained by water deficit occurring at different times during the growing season (Liu et al., 2019). Such differences are probably related to diverse soil moisture thresholds at which the activity of autotrophs and heterotrophs ceases, with the latter exhibiting much lower water potential values (e.g. up to -36.5 MPa; Manzoni et al., 2012) than the former (-1.5 MPa for herbaceous plant species, corresponding to the permanent wilting point; Lambers et al., 2008). However, as Lichtenthaler (1996) illustrated when examining the stress concept in plants, thresholds depend on both the nature of the stress and the identity of the organism. Extending this concept to drought and CO_2 fluxes, soil moisture thresholds for GEP and ER might differ not only between each other, due to the identity of the organisms involved in each component, but could also differ in response to the timing of a dry spell, as a consequence of the particular phenological state of the ecosystem at the beginning of such an event. If soil moisture thresholds differ between different drought timings, presumably the length of the dry period necessary to reach them will also differ. Diverse sensitivity of photosynthetic and respiration processes has been observed under natural conditions (Lund et al., 2012; Liu et al., 2019) and in manipulation experiments (Hao et al., 2018; Zhang et al., 2019b), but differences in the length and/or severity of dry spells produce a certain level of uncertainty. Furthermore, as far as can be ascertained, no study has yet investigated

potential differences in soil moisture and temporal thresholds under two different drought timings. To this aim, experiments in highly controlled environment are therefore needed to clearly identify mechanisms involved in responses to different drought timing, coupled with field experiments enabling to upscale responses under natural, more complex conditions (Kreyling et al., 2014).

The main goal of the present research is to understand how extreme drought affects plant growth and CO_2 exchange – GEP, ER and NEE – of an alpine primary grassland and whether such responses are sensitive to an approx. 1-month drought occurring in two different periods within a single growing season – early-season drought vs mid-season drought. In order to gain a more detailed understanding of responses to different drought timing and assess thresholds triggering different ER and GEP response rates, a further full drought treatment of approx. 2 months was also implemented within the study. Manipulation experiments were conducted to evaluate plant growth and CO_2 responses to the same experimental drought timing both in the lab, within a mesocosm ("bounded and partially enclosed outdoor experimental setups"; Odum, 1984), and in the field.

In the present chapter, methods and results from the mesocosm experiment conducted in winter-spring 2020 on grassland monoliths at the garden site of the University Campus of Parma are presented and discussed; methods and outcomes from the field experiment which took place in spring-summer 2020 are described in Chapter 7. The data was examined in order to: (a) assess ANPP and CO_2 exchange over the whole study period (February-April) to evaluate overall effects of early- and mid-season drought; (b) investigate CO_2 flux responses during each of the two drought periods to obtain a deeper understanding of the mechanisms driving ecosystem dynamics with respect to water shortage; (c) determine whether resistance of GEP differs from that of ER within the two drought periods, and whether GEP and ER exhibit different degrees of sensitivity – i.e. resistance – to early and mid-season drought; and, finally, (d) identify soil moisture and temporal thresholds triggering different rates of CO_2 emissions and assimilation.

6.3 MATERIALS AND METHODS

6.3.1 Study site and experiment design

The mesocosm experiment was set-up in the garden site of the University Campus of Parma (Department of Chemistry, Life Sciences and Environmental Sustainability, 72 m a.s.l., 44°46'6.07" N, 10°18'52.69" E).

At the beginning of September 2019, 42 small monoliths of the target *Carex curvula* grassland were collected from the field site (**Fig. 6.1a**, see Chapter 2 for a detailed description of the site) and transplanted into pots (diameter 12 cm, depth 15 cm) which were then left to overwinter in

an incubator for 5 months at 0 °C within large containers filled with expanded clay and covered with a layer of non-woven fabric to insulate the plants and prevent soil freezing. At the beginning of February (07 February 2020), when daily air temperatures of the location site – i.e. Parma – resemble those recorded in June at the reference site – i.e. Gavia Valley – pots were removed from the incubator, left to settle, weighed and subsequently saturated with distilled water to simulate soil saturation following snowmelt water input at the beginning of the growing season (**Fig. 6.1b**). The attainment of water potential (WP, kPa) at field capacity was assessed through direct measurements on 3 replicates during the saturation procedure (-10 kPa). Subsequently, each pot was weighed to obtain an indirect estimate of soil water status at the beginning of the experiment, after which it was randomly assigned to one of the four treatments – control and drought treatments – constituting the experiment. The drought treatments consisted of three different manipulations (**Fig. 6.2**), as described below:

- Early-season drought (ED): no watering imposed during the first part of the growing season, immediately after simulated snowmelt (8 February, day 0 of experiment); re-watering after 32 days (11 March).

- Mid-season drought (MD): watering during the first part of the growing season, followed by no watering during the second part (11 March, day 1 of drought for this period) until the end of the experiment (10 April).

- Full-season drought (FD): no watering for 62 days, a period including ED and MD; treatment started immediately after simulated snowmelt (8 February, day 0 of experiment) and ended on the same date as MD (10 April).

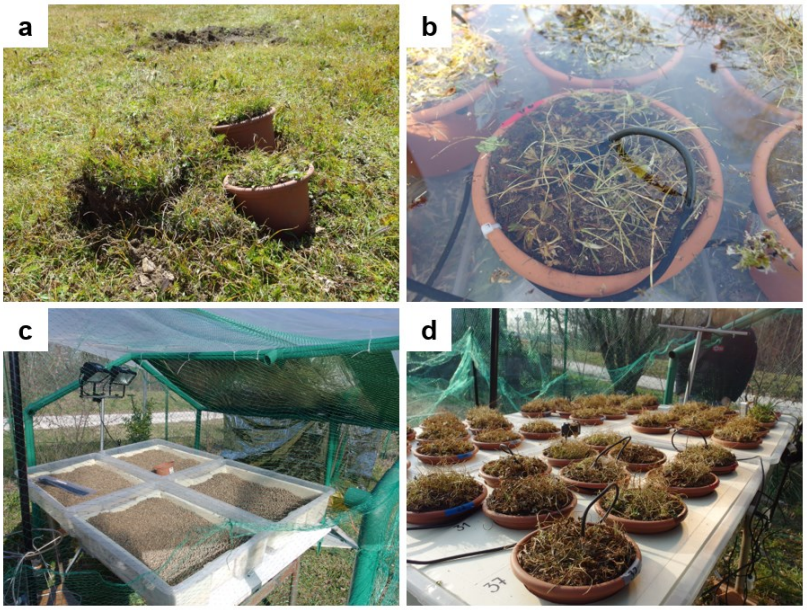


Fig. 6.1: (a) Monoliths of the target *Carex curvula* grassland collected from the field site on 4 September 2019 and transplanted into pots; **(b)** pot saturation with distilled water (and sensor of water potential) prior to the beginning of experimental treatments; **(c)** basins used for pot placement under the shelter, filled with expanded clay, together with halogen lamps at the side; **(d)** pot disposition within the experimental area under the shelter, with those employed for soil moisture measurements in the foreground.

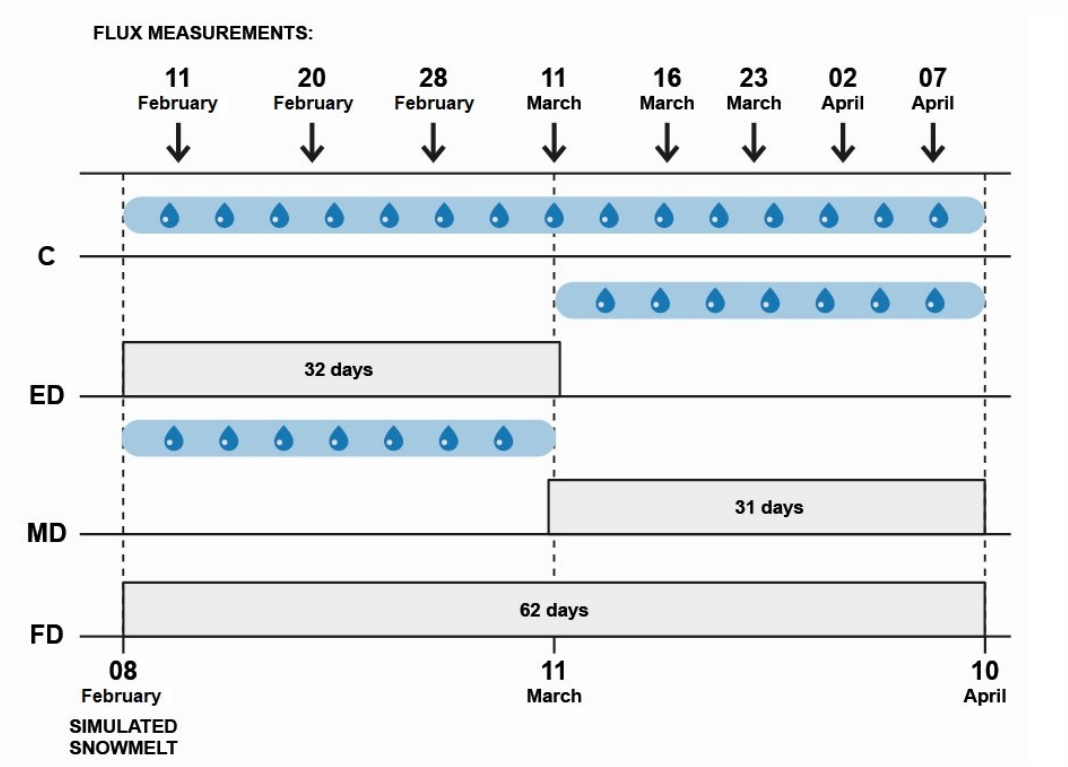


Fig. 6.2: Scheme illustrating the experimental design to test the effects of drought timing and duration carried out on *Carex curvula* grassland pots at the garden site of the University Campus of Parma. The four treatments are reported (C – control, no manipulations; ED – early-season drought; MD – mid-season drought; FD – full-season drought) together with drought-period length; the CO_2 flux measurement dates are specified at the top, while the simulated snowmelt date together with the set-up and end-of-treatment dates are indicated at the bottom.

While the ED and MD treatments were performed to gain insights into ecosystem responses to different drought timings, the FD treatment was carried out to identify thresholds triggering different rates of CO_2 flux.

Drought conditions were achieved through passive full-rainfall exclusion, using a 4 m × 6 m transparent, removable polythene foil covering the entire experimental area under which pots were placed. Control pots, together with ED and MD depending on the period, were watered twice a week with distilled water, in a quantity that mimicked average daily rainfall amounts during the summer – i.e. 3 mm per day considering the average over June-September obtained from the historical records of a weather station near the alpine field site (Careser Diga, see Chapter 3, Section 3.1).

Each treatment consisted of 7 replicates, except for controls, which had 8 replicates; 6 pots were assigned to microclimate measurements (see below) while 7 pots underwent further treatment, which is not part of the present study. Once assigned to treatments, all pots, including controls, were placed under the shelter and settled in basins completely insulated with polystyrene boards, including the top, and filled with expanded clay to prevent the pots from overheating (**Fig. 6.1c**). The bottoms of the containers were pierced to allow ventilation and avoid water stagnation and basins, each of which contained 11 pots (**Fig. 6.1c, d**), were placed at approx. 1.20 m from the ground. Two pairs of halogen lamps were placed at both sides of the table and lit the basins two hours before sunrise and after sunset to improve solar radiation and photosynthetic active photon flux density (PPFD) and also to simulate a photoperiod similar to the one experienced by the target vegetation in June (**Fig. 6.1c**). All pots were randomly re-located twice a week, after each watering date, to avoid bias due to pot location within the mesocosm.

6.3.2 Microclimate

Climate data was recorded every hour. To assess the effects of the transparent, polythene shelter on microclimate, measurements of solar radiation (W m^{-2}) and photosynthetic active photon flux density (PPFD, µmol m^{-2} s^{-1}) were taken both below and outside the shelter (HOBO Micro Station Data Logger with S-LIB-M003 Solar Radiation (Silicon Pyranometer) Smart Sensor and S-LIA-M003 Photosynthetic Light (PAR) Smart Sensor), together with records of air temperature gathered at 1.20 m aboveground, the same height as the pots (Spectrum Technologies Inc., Chicago, IL, USA). On the other hand, to assess the effects of treatments on microclimate, records of soil moisture, together with temperature measures, were taken in C and FD pots in the 0-5 cm soil layer. Volumetric water content (VWC, %; WaterScout SM100, Spectrum Technologies, Inc.) was measured in one replicate per treatment (C and FD); soil water potential (WP, kPa; Teros21, Decagon Devices, Inc.) was recorded in two replicates in C and in one replicate in FD, after the second replicate for this treatment had been excluded due to instrumental failure.

To monitor the soil water status within each pot, before re-watering was carried out, all pots were weighed every 2-3 days during the whole experimental period. At the end of the experiment, after phytomass clipping, all pots were oven-dried at 100 °C until constant mass was reached; the final mass was then recorded to quantify the amount of soil originally present within each pot. Subsequently, from each of the 24 dates when pot mass was recorded, soil mass values obtained for each pot were subtracted to estimate the pot-specific water mass during the entire experimental period. A linear regression model was then fitted to water mass values (explanatory variable)) and VWC (response variable) measured for those pots for which instrumental measures were available. Data from both treatments, C and FD, was used after checking that there was no significant difference between the two treatments – i.e. non-significant interaction between water mass and treatment ($F_{1,46}$=0.001, p=0.969). The model thus obtained was then applied to estimate for each pot the VWC in each weighing session during the experiment.

6.3.3 Aboveground net primary production (ANPP)

ANPP was estimated by harvesting aboveground phytomass (hereafter biomass) for each pot, including those where instruments were installed (10 April, corresponding to 62 days from simulated snowmelt). Clipping of vascular plants was carried out above the soil surface. The detached, dead plant material from the previous year (litter), identified by its grey colour and major fragmentation, was separated from the current year's production, which was then sorted into species – in the present study only total ANPP value is reported. Plant material was subsequently oven-dried at 65 °C for 48 hours and dry mass was recorded and reported in g m^{-2}.

6.3.4 CO_2 fluxes

CO_2 fluxes were recorded weekly using a PP System EGM-4 infrared gas analyzer (IRGA) connected to a transparent cylindrical chamber sealed over each pot (volume 2 l, area 102 cm^2) (**Fig. 6.3**). To prevent an excessive temperature increase inside the chamber, ER measurements were performed first. ER was recorded in dark conditions by covering the chamber with an opaque sheet. Subsequently, under natural light conditions, measurements of NEE were carried out. Records of CO_2 concentrations were set every 5 s for 60 s; measurements were performed during the central hours of the day (10:30 – 16:00), with CO_2 fluxes in control pots, together with ED and MD treatments depending on the period, always recorded prior to the re-watering procedure. Recordings started on 11 February, 3 days after the beginning of the experiment, and ended on 7 April, 3 days before the end of the experiment. Measurements were taken for a total of 8 dates but, of these, only 7 dates were kept for analysis after the first date was excluded in order to achieve complete stabilization of the system after soil saturation. Three measurement

sessions, therefore, fell into the first, early-growing season, while the remaining 4 dates were included in the second, mid-season period.



Fig. 6.3: Transparent cylindrical chamber connected to the EGM-4 infrared gas analyser used for CO_2 flux measurements (photo by Michele Carbognani).

Using the ideal gas law, the rate of CO_2 change inside the chamber was employed to estimate the flux of CO_2 (g m^{-2} h^{-1}). Estimates of ER and NEE were used to calculate GEP, computed by subtracting for each pot and date the mean ER rate from the corresponding light measurements – i.e. GEP= NEE - ER. A linear regression model was fitted to each flux data to obtain ER, NEE and GEP fluxes for each pot. Since NEE represents CO_2 fluxes going from the ecosystem into the atmosphere (Chapin et al., 2006), in the present study negative NEE (and GEP) values indicate a net ecosystem uptake of CO_2, whereas positive values indicate a net CO_2 release.

6.3.5 Resistance

The sensitivity of GEP and ER to diverse types of drought imposed in the study was used as an estimate of resistance, following Hao et al. (2018):

$$Sensitivity\ (\%) = \frac{(Flux_{dr} - \overline{Flux}_{ctr})}{\overline{Flux}_{ctr}} * 100$$

where $Flux_{dr}$ corresponds to the CO_2 flux rate measured in each pot and $Flux_{ctr}$ indicates the average CO_2 flux rate in controls. For GEP, values >0 due to error associated with rate estimates [0.04 ± SD 0.04 g (CO_2) m^{-2} h^{-1}] were set equal to 0. For the three drought treatments, resistance was obtained based on CO_2 fluxes recorded on the last measurement date for each drought

period. The lower the sensitivity values, which represent changes in CO_2 fluxes of drought plots compared to controls, the lower was the resistance.

6.3.6 Data analysis

Statistical analyses were carried out in R (R Core Team, 2020). Linear mixed effects models (LMMs) [R package "nlme" (Pinheiro et al., 2020)] were employed in all the analyses, following approaches described by Zuur et al. (2009) and Crawley (2012). Model selection was carried out using the maximum likelihood (ML), step by step excluding non-significant terms until a final, parsimonious model was obtained which retained only significant terms ($p<0.05$). At each step, linear model assumptions were verified by visually checking the residual patterns, with the inclusions of a variance structure to deal with heterogeneity of residuals whenever it was necessary. Model goodness of fit and selection of the best random effect and variance structure was done by means of the Akaike Information Criteria (AIC). The final model was then fitted using the restricted maximum likelihood (REML) to obtain estimates of the effects of factors tested and their significance (Zuur et al., 2009). When necessary, a multiple comparison of significant terms was performed by means of post-hoc Tukey contrasts using the R package "lsmeans" (Lenth, 2016) and the "multcomp" package was used for letter display (Hothorn et al., 2008).

Analysis of CO_2 fluxes was carried out separately for each flux component (GEP, ER and NEE). Plant cover percentage, visually estimated for each pot at the end of the experiment, was included as a covariate in the models together with the dry mass of soil to account for variability among pots. Previously, the non-parametric Kruskal-Wallis test had been performed to examine differences in plant cover between treatments (4-level categorical), while ANOVA had been carried out to examine soil mass values. With regard to plant cover, the test highlighted a significant difference across treatments (Kruskal-Wallis Chi-squared=10.484, p=0.015, df=3), due to significantly lower cover % in ED pots (raw data, 83 ± standard deviation (SD) 16%) compared to C (96 ± SD 4%). However, it seemed reasonable to assume that this difference was not due to the treatment applied, but rather derives from the plant abundance originally present within the transplanted grassland patches, since mean value of FD pots (93 ± SD 2%), which received a more extreme drought treatment than ED, did not significantly differ from that recorded in C. On the other hand, analysis revealed that soil mass was not significantly different between treatments ($F_{3,25}$=1.501, p=0.238).

To test the response of CO_2 fluxes over the entire study period, all flux measurements were considered and tested by setting treatments as a 4-level categorical variable; pot and day were set as random crossed effects while variance of treatments was homogenised for all models, including cover for LMM regarding NEE.

As for drought timing, a separate analysis was performed for each experimental period – early and mid-season – to account for the fact that treatments were not constant over time. In particular, to assess the effects of drought during the early period, the four treatments characterising the experimental design were reduced to a 2-level categorical variable, grouping together pots sharing the same treatment – "ambient", including watered treatments (C and MD); "drought", including non-watered pots (ED and FD). Analysis of fluxes during the mid-season, on the other hand, were performed by considering treatments separately (4-level categorical variable). The day of measurements was further inserted as an independent variable (3- and 4-level categorical variable for early and mid-period, respectively) and its interaction with treatment was tested. The pot was set as a random effect in all LMMs testing drought during early- and mid-season; cover variance was homogenised in LMMs for ER, while variance of treatments and days was corrected in LMMs for GEP and NEE.

Analysis of resistance was performed on GEP and ER to evaluate differences between processes within the same drought treatment (2-level categorical: ER and GEP) and differences between treatments within the same flux component (2-level categorical: ED and MD; ED also includes CO_2 fluxes in FD during the first period). LMMs were employed to test the difference in sensitivity between flux components during early-season drought, with pot as a random effect, and to analyse ER resistance between drought timings, homogenising variance of treatments. On the other hand, since linear model assumptions were not fulfilled, the Wilcoxon test was carried out to analyse the difference in sensitivity between GEP and ER during mid-season drought and to assess GEP resistance between treatments.

A further analysis of thresholds that triggered different GEP and ER response rates was carried out; thresholds were assessed both for soil moisture – using estimated VWC values – and for time – i.e. number of days without precipitation. Although soil WP is a direct measure of water availability for plants, this parameter was not used to directly assess soil moisture thresholds, since the WP gradient was not adequately covered by data – i.e. most values lay towards one extreme, making it difficult to identify and estimate numerical thresholds.

For each flux component – GEP and ER – and each threshold type – VWC and time – separate analyses were carried out. In addition, to account for differences in ecosystem phenology – i.e. drought starting immediately after snowmelt vs drought starting approx. 1 month later – two separate analyses were performed for each dry spell onset: first period, considering all measured values in ED (until 11 March) and FD (until end of experiment); and second period, considering values recorded in MD during drought (from March to April). To avoid bias, due to natural undeveloped vegetation coupled with high soil moisture values, the first two measurement dates were excluded from the GEP threshold analysis. In addition, GEP values >0 were set equal to 0. Two-phase generalized linear models (GLMs) were employed using the R package "chngpt" (Fong

et al., 2017). Selection of the most parsimonious model was carried out by means of the likelihood ratio test [package "lmtest" (Zeileis and Hothorn, 2002)] by comparing different models, including hinge, upper hinge, segmented and stegmented models (**Fig. 6.4**) and simple linear models; random structure was excluded from all models after being tested. Confidence intervals were estimated by means of permutations (n=1000) and a visual check of residual patterns was performed to assess linear model assumptions. Discontinuous ("stegmented") GLMs were, finally, selected as the best-fit moisture and temporal threshold models for both ER and GEP, with the exception of GEP and its VWC-threshold during the early growing season period, for which a continuous ("hinge") model was selected. Finally, the WP corresponding to each VWC threshold obtained was estimated by fitting a smooth spline to the WP-VWC relationship, built by using direct WP records and VWC estimates in the pots where WP had been recorded during the experiment.

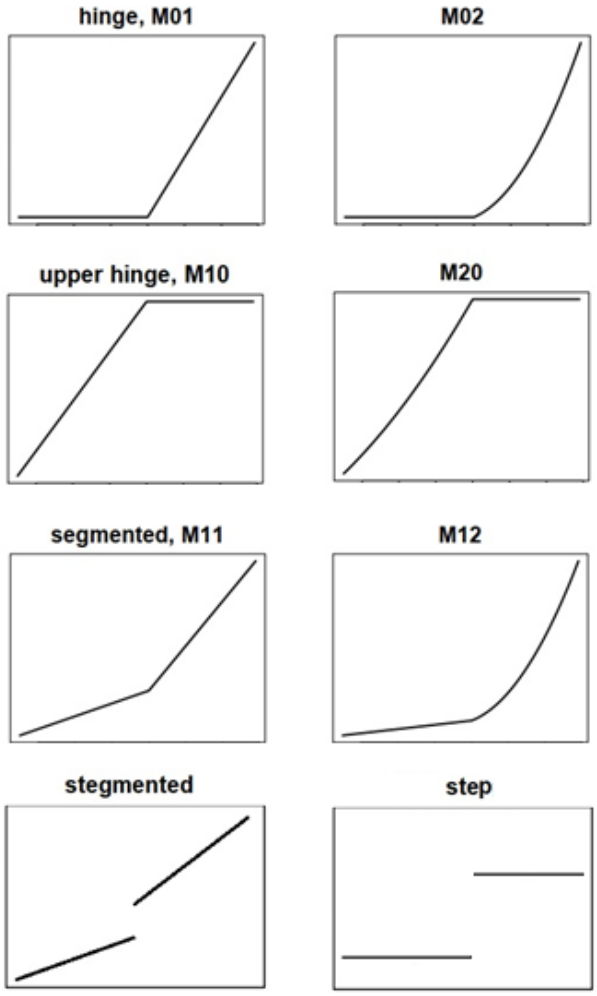


Fig. 6.4: Types of threshold models supported in the R package *chngpt* and employed in the threshold analysis [figure modified from Fong (2019)].

6.4 RESULTS

6.4.1 Microclimate

When evaluating the effects of the shelter on PPFD and SR, data revealed that mean daily PPFD measured under the shelter where all pots had been placed was 16% lower compared to values recorded outside (242.15 ± standard error (SE) 14.19 µmol m^{-2} s^{-1} vs 288.73 ± SE 17.83 µmol m^{-2} s^{-1}; n=56), while SR was reduced by 10% (129.64 ± SE 6.71 W m^{-2} vs 144.17 ± SE 8.92 W m^{-2}; n=56). With regard to air temperature, over the entire study period mean daily values recorded under the shelter were similar to those measured outside, whereas comparison between drought periods showed that temperatures during the second period were 2 K higher compared to the first one (9.95 ± SE 0.39 °C for the mid-season vs 7.87 ± SE 0.32 °C for the early season; n=2) (**Fig. 6.5**).

Concerning treatment effects on microclimate, mean daily soil temperature in the 0-5 cm profile over the entire study period was similar between control and full-drought pots, while there was a difference of 3 K between early- and mid-season values, with means equal to 10.95 ± 0.09 °C during the early period and 14.27 ± SE 0.24 °C during the mid period (n=2) (**Fig. 6.5**).

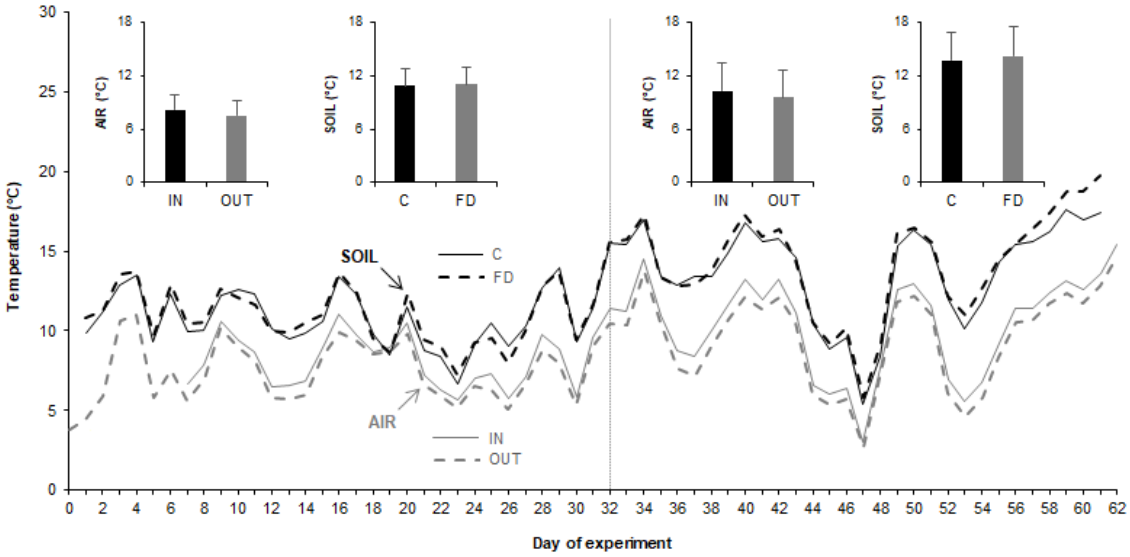


Fig. 6.5: Daily air temperature (°C) measured under (IN) and outside (OUT) the shelter at +120 cm (grey lines) and daily soil temperature (°C) recorded within control pots (C) and full-drought pots (FD), all placed under the shelter, at 0-5 cm depth (black lines). Time is reported as the number of days from the beginning of manipulations (8 February, day 0). The vertical grey line indicates the end of ED and beginning of MD (11 March, day 32). Internal panels illustrate the daily mean air measured in and ouside the shelter and and daily mean soil temperature recorded within each treatment during the first (early) and second (mid) drought period; bars represent standard deviation.

Concerning soil moisture, the different watering regimes led to different VWC dynamics in the respective treatments (**Fig. 6.6a).** During the early period, ED and FD pots started to exhibit reduced VWC as soon as the experiment began, with mean values measured at the end of the

early period being equal to 17.00 ± SE 1.13% for ED and 16.70 ± SE 0.93% for FD (-32% compared to watered pots); analysis on effects of drought on VWC over the entire first period revealed significant differences between watered and dry pots ($F_{3,32}$=19.191, p<0.001) (**Fig. 6.6b**). During the second period (**Fig. 6.6a**), VWC in MD rapidly decreased, reaching values which were only 3% higher than those recorded in FD by the end of the experiment, where water content still kept decreasing after the first period (6.24 ± SE 0.77% vs 3.49 ± SE 0.60%). On the other hand, VWC in ED gradually increased after re-watering, reaching by the end of the study period soil moisture values similar to C (26.46 ± SE 2.16% in ED vs 28.35 ± SE 1.00% in C). When analysing soil moisture across treatments over the second period ($F_{3,32}$=79.033, p<0.001), ED still exhibited significantly lower values compared to controls (p<0.05), not statistically different from those recorded in MD which, in turn, was significantly moister than FD (**Fig. 6.6.5c**).

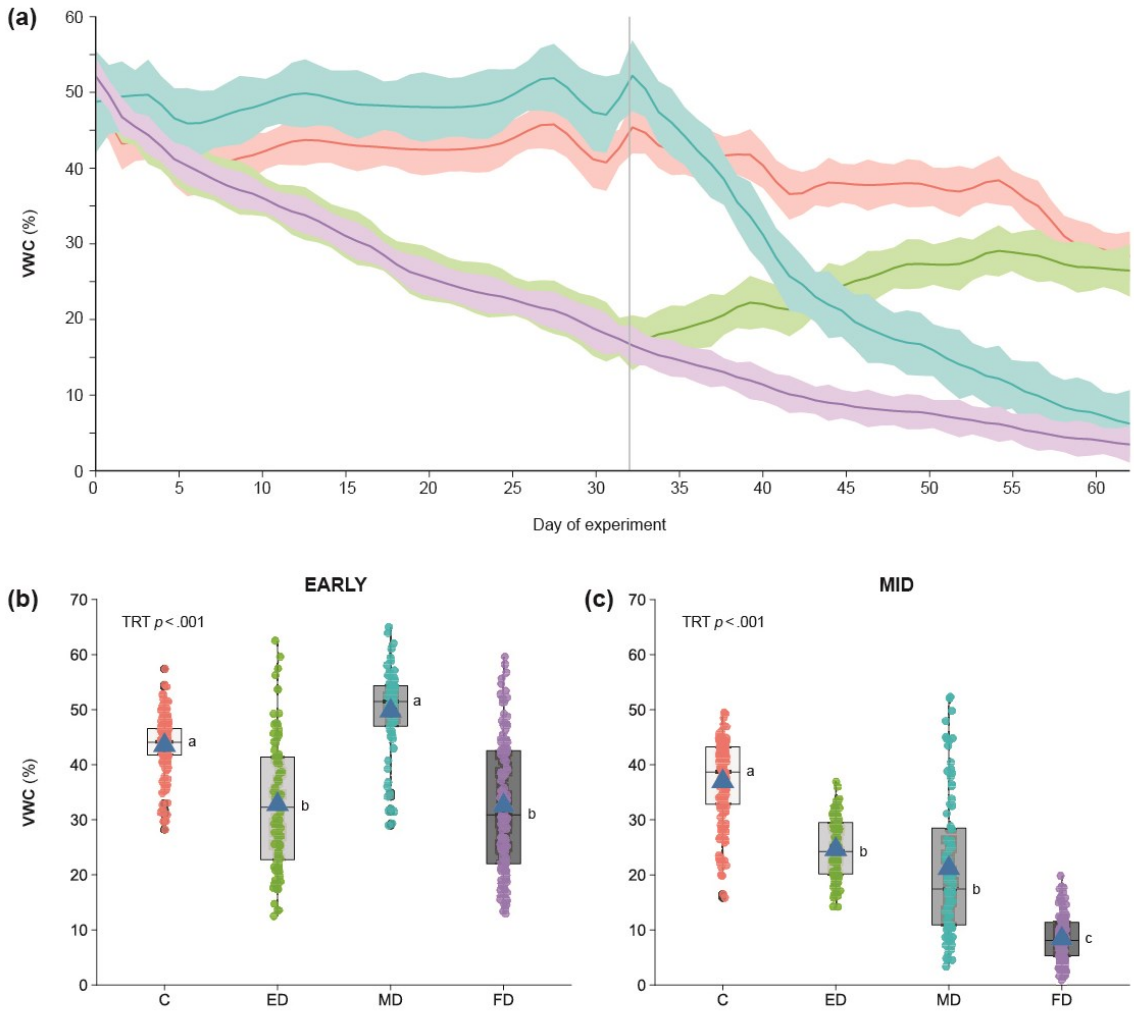


Fig. 6.6: Volumetric water content (VWC, %) for the 0-5 cm soil layer: **(a)** Dynamics of VWC in the 4 treatments TRT [C – control, ED – early-season drought; MD – mid-season drought; FD – full-season drought] estimated on 24 dates during the study period. Time is reported as the number of days from the beginning of manipulations (8 February, day 0). The vertical grey line indicates the end of ED and beginning of MD (11 March, day 32). Shaded areas represent the standard error (n=10 for C; n=7 for ED and MD; n=10 for FD). **(b)** VWC recorded in the 4 treatments during the first period (early) and **(c)** during the second period (mid), with coloured dots indicating single VWC values measured in each pot for each treatment and blue triangles referring to mean values.

6.4.2 ANPP

ANPP estimated on the last day of the experiment was significantly affected by treatments ($F_{3,31}$=16.134, p<0.001) (**Fig. 6.7**). Pots experiencing the full-drought treatment (FD) exhibited the lowest values, which were 48% lower than controls, followed by ED (-28%) which, in turn, did not exhibit statistically different ANPP values from those recorded in both FD and MD. Pots experiencing drought during the second part of the growing season (MD) had significantly higher ANPP compared to FD, with values not statistically different to those recorded in C.

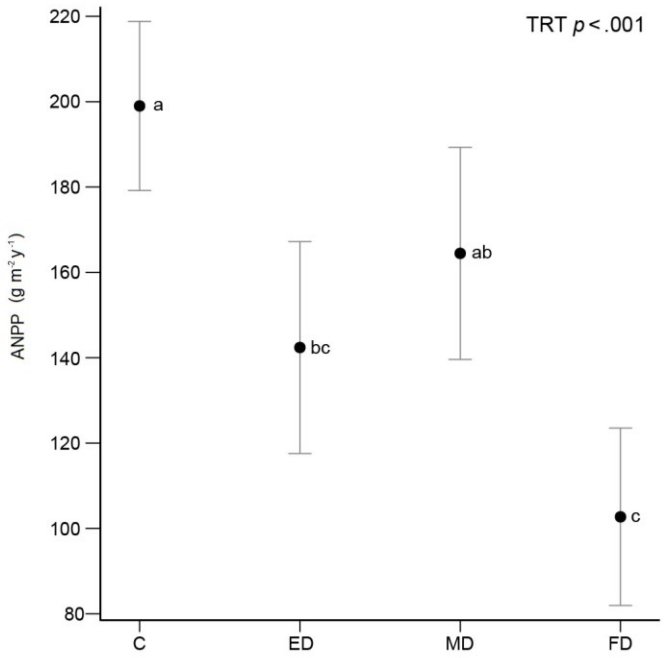


Fig. 6.7: Mean ANPP (g m^{-2} y^{-1}) estimated for each treatment at the end of the experimental period [C – control, ED – early-season drought; MD – mid-season drought; FD – full-season drought]. Bars represent the 95% confidence interval (C, n=11; ED and MD, n=7; FD, n=10).

6.4.3 CO_2 fluxes: seasonality

Analysis of CO_2 exchange during the experimental period revealed that drought treatments significantly influenced ER, GEP and NEE, with the percentage of plant cover also resulting as a significant factor in explaining differences in GEP and NEE values (**Table 6.1**). On the other hand, soil mass did not influence any of the CO_2 exchange processes considered, after being excluded during the model selection process (**Table 6.1**).

Table 6.1: Results of LMMs testing the effects of drought on ecosystem respiration (ER), gross ecosystem production (GEP) and net ecosystem exchange (NEE) during the entire treatment period (February-April). Fixed effects: treatments (TRT), % of plant cover (COVER) and soil mass ($MASS_{soil}$); "ns" refers to non-significant terms excluded during model selection (p>0.05). Pot was set as a random effect for all LMMs, while the variance structure included in the final model is specified.

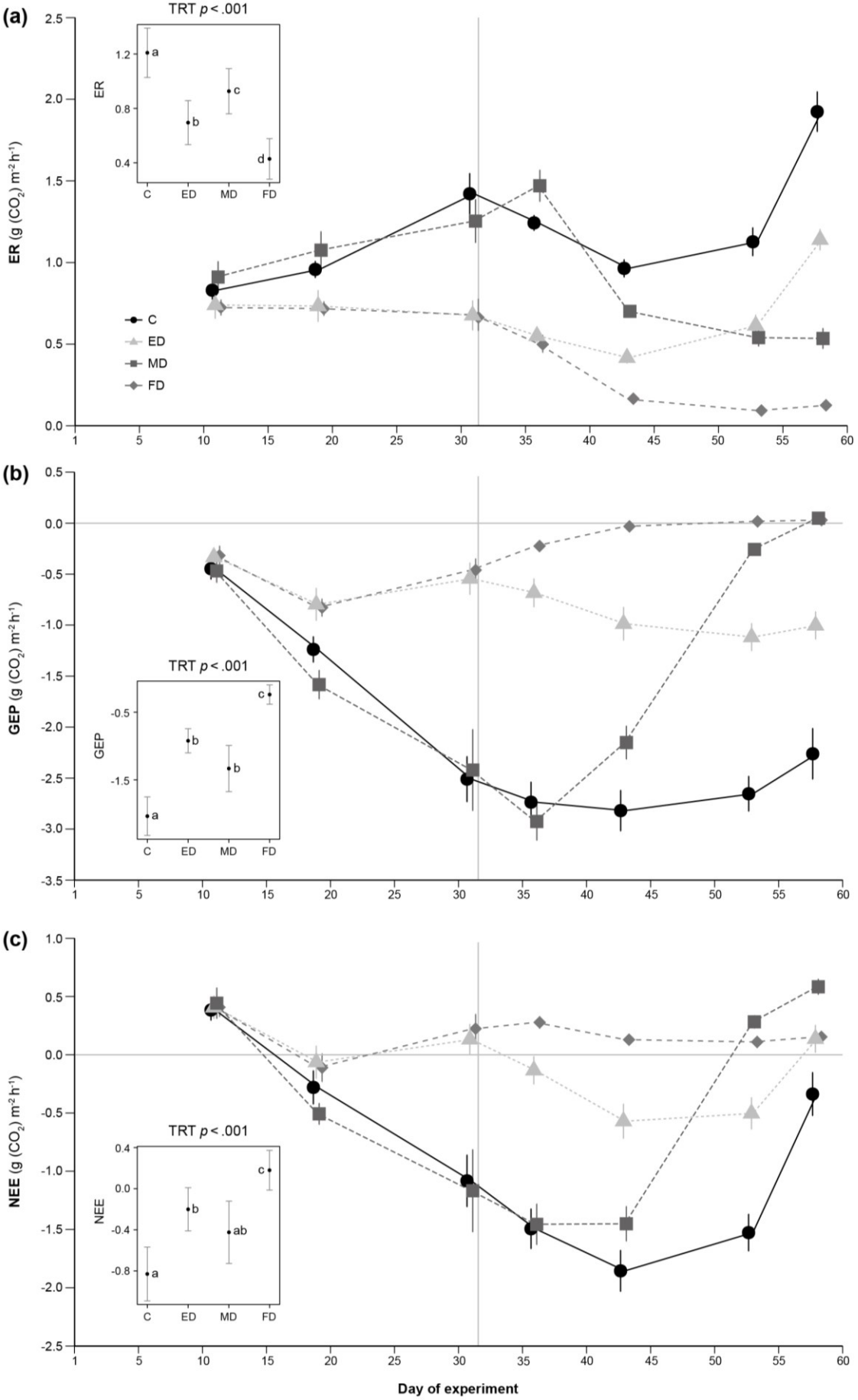
CO_2 fluxes	Fixed effects	Variance	df (N,D)	F-value	p-value
ER	TRT		3,198	61.967	<0.001
	COVER	trt	ns	ns	ns
	$MASS_{soil}$		ns	ns	ns
GEP	TRT		3,197	83.938	<0.001
	COVER	trt	1,197	16.738	<0.001
	$MASS_{soil}$		ns	ns	ns
NEE	TRT		3,198	31.540	<0.001
	COVER	trt, cover	1,198	16.840	<0.001
	$MASS_{soil}$		ns	ns	ns

With regard to ER (**Fig. 6.8a**), the highest, significant reduction (64%) compared to controls was found for FD plots. For pots exposed to different drought timings, ER was significantly reduced by 42% in ED and by 23% in MD compared to controls.

Concerning GEP (**Fig. 6.8b**), analysis revealed a seasonal pattern among treatments that was similar to that found for ER, with the lowest gross assimilation in FD, 88% lower than C, followed by ED (-55%) and MD pots (-35%); the two timing treatments resulted as being statistically equivalent to each other. The increase of ER per unit of plant cover percentage was equal to -15.47 mg (CO_2) m^{-2} h^{-1}.

Finally, NEE exhibited the highest (positive) values in FD, while ED and MD pots represented a CO_2 sink during the entire experimental period, although NEE values in ED pots were significantly higher than those recorded in controls (**Fig. 6.8c**). Overall, values were -12.02 mg (CO_2) m^{-2} h^{-1} per unit of plant cover percentage.

Fig. 6.8 (p. 146): (a) Ecosystem respiration (ER), **(b)** gross ecosystem production (GEP) and **(c)** net ecosystem exchange (NEE) measured in treatments TRT [C – control, ED – early-season drought; MD – mid-season drought; FD – full-season drought] during the experimental period (February-April). Bars indicate the standard error (n=7; n=8 for C). Time is reported as the number of days from the beginning of manipulations (9 February, day 1). The vertical line indicates the end of ED and beginning of MD (11 March, day 32). For each process, internal panels illustrate the LMM output (estimated means and 95% confidence interval), with different letters indicating significant differences (post-hoc Tukey contrast, p<0.05).


(a)
TRT p < .001
ER
a
b
c
d
C
ED
MD
FD
ER (g (CO2) m-2 h-1)
C
ED
MD
FD
(b)
TRT p < .001
GEP
a
b
b
c
GEP (g (CO2) m-2 h-1)
(c)
TRT p < .001
NEE
a
b
ab
c
NEE (g (CO2) m-2 h-1)
Day of experiment

6.4.4 CO_2 fluxes: drought timing

Drought during the early part of the growing season did not significantly affect ER, GEP and NEE, although interaction between treatments and days was found to be significant (**Table 6.2; Fig. 6.9**); under watered conditions CO_2 emission (**Fig. 6.9a**), gross assimilation (**Fig. 6.9b**) and net assimilation (**Fig. 6.9c**) increased across measurement dates, whereas under drought CO_2 flux dynamics varied, resulting in an increasing difference between treatments over time. Moreover, with regard to ER, the percentage of plant cover was found to significantly influence values observed [+5.59 mg (CO_2) m^{-2} h^{-1} per unit of plant cover percentage] (**Table 6.2**). In contrast, soil mass did not influence any of the CO_2 exchange processes examined.

In detail, on the first measurement date – i.e. 12 days after the beginning of drought treatments – overall CO_2 fluxes were not significantly different between dry and watered pots, with low ER (**Fig. 6.9a**) and GEP (**Fig. 6.9b**) values, and NEE exhibiting positive values (**Fig. 6.9c**). On the following date – i.e. 20 days after the beginning of treatments – ER and GEP were significantly higher than the first date and CO_2 fluxes in dry pots started to significantly differ from values recorded in watered pots, with 24% lower CO_2 emissions (**Fig. 6.9a**) and -28% gross assimilation (**Fig. 6.9b**) compared to pots in watered conditions. In contrast, the resulting NEE did not statistically differ across treatments, with mean negative values for both dry and watered pots (**Fig 6.9c**). After 32 days from the start of the experiment – i.e. the last measurement date for the early drought period – there was still a significant increase in ER (**Fig 6.9a**) and GEP (**Fig 6.9b**) in watered pots, whereas non-watered pots exhibited the opposite pattern. Carbon dioxide emissions were 56% lower compared to watered conditions and significantly lower (-22%) than the previous date (**Fig 6.9a**). Gross assimilation was reduced by 80% in dry pots and values were not significantly different from fluxes measured 12 days before (**Fig 6.9b**). As a result, by the end of the early drought period, non-watered pots exhibited positive NEE values [estimated value 0.18 ± 95% confidence interval (CI) 0.24 g (CO_2) m^{-2} h^{-1}], in contrast with those recorded for watered pots [-1.12 ± CI 0.33 g (CO_2) m^{-2} h^{-1}], which exhibited the lowest (most negative) values during the period under analysis (**Fig. 6.8c**).

Table 6.2: Results of LMMs testing the effects of drought on ecosystem respiration (ER), gross ecosystem production (GEP) and net ecosystem exchange (NEE) during two different periods (early and mid- season). Fixed effects: treatment (TRT), date of measurement (DAY), % of plant cover (COVER) and soil mass ($MASS_{soil}$); "ns" refers to non-significant terms excluded during model selection (p>0.05). Pot was set as a random effect for all LMMs, while the variance structure included in the final model is specified in the table.

CO_2 fluxes	Fixed effects	Variance	df (N,D)	F-value	p-value
Early season					
ER	TRT		1,26	0.935	0.343
	DAY		2,53	25.604	<0.001
	COVER	cover	1,26	5.260	0.030
	$MASS_{soil}$		ns	ns	ns
	TRT × DAY		2,53	52.811	<0.001
GEP	TRT		1,27	1.809	0.190
	DAY		2,53	73.390	<0.001
	COVER	trt, day	ns	ns	ns
	$MASS_{soil}$		ns	ns	ns
	TRT × DAY		2,53	33.635	<0.001
NEE	TRT		1,27	0.002	0.967
	DAY		2,54	47.937	<0.001
	COVER	trt, day	ns	ns	ns
	$MASS_{soil}$		ns	ns	ns
	TRT × DAY		2,54	18.175	<0.001
Mid season					
ER	TRT		3,24	52.998	<0.001
	DAY		3,75	31.948	<0.001
	COVER	cover	1,24	5.149	0.032
	$MASS_{soil}$		ns	ns	ns
	TRT × DAY		9,75	33.170	<0.001
GEP	TRT		3,24	99.503	<0.001
	DAY		3,75	6.951	<0.001
	COVER	trt, day	1,24	14.853	0.001
	$MASS_{soil}$		ns	ns	ns
	TRT × DAY		9,75	70.780	<0.001
NEE	TRT		3,24	57.99	<0.001
	DAY		3,75	25.427	<0.001
	COVER	trt, day	1,24	18.193	<0.001
	$MASS_{soil}$		ns	ns	ns
	TRT × DAY		9,75	58.755	<0.001

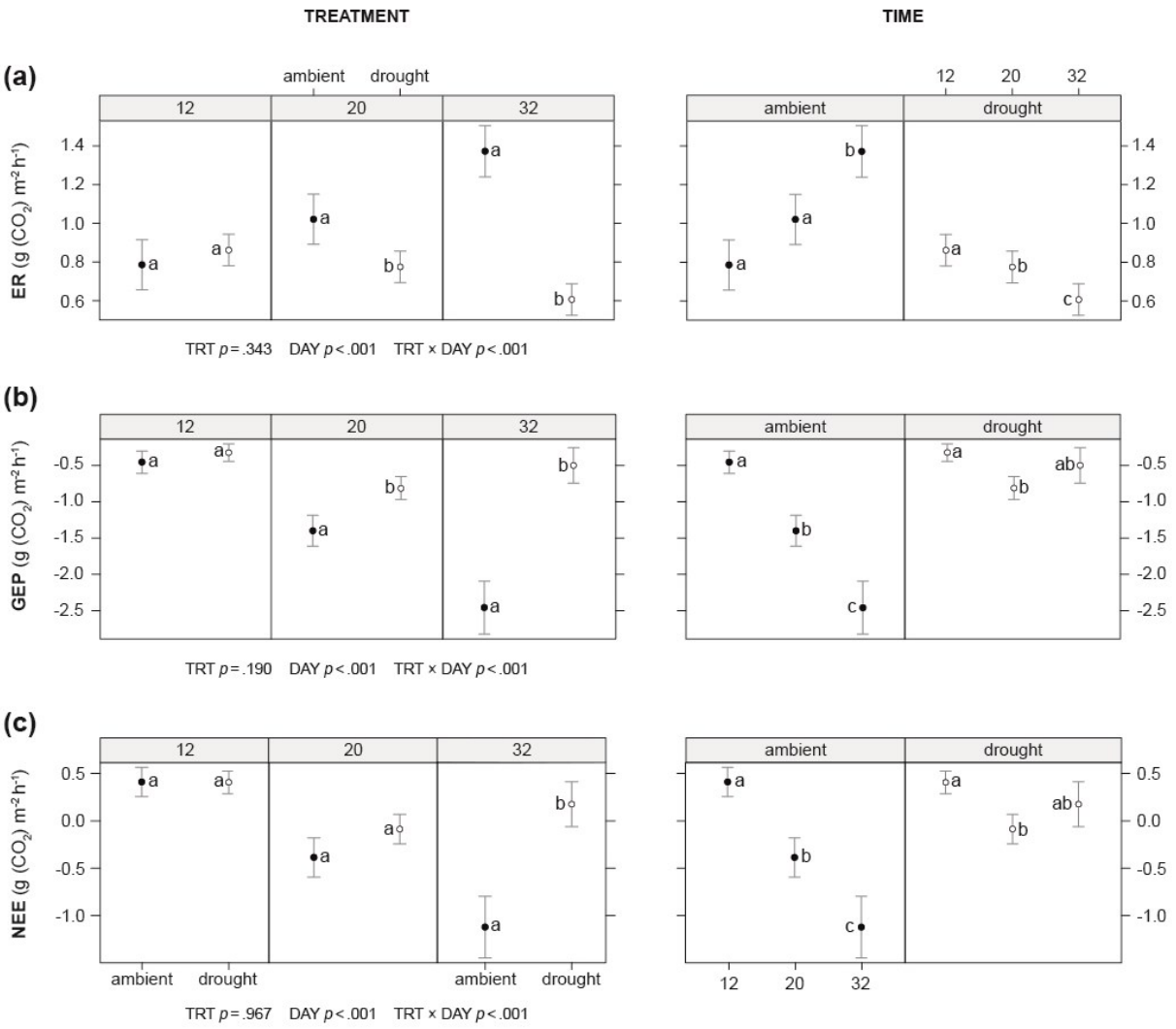


Fig. 6.9: LMMs output (estimated mean and 95% confidence interval) for **(a)** ecosystem respiration (ER), **(b)** gross ecosystem production (GEP) and **(c)** net ecosystem exchange (NEE) measured during the early growing season in 2-level treatment TRT ["ambient", control (C) and mid-season drought (MD); and "drought", early-season drought (ED) and full-season drought (FD)]. For each process, left-hand graphs illustrate differences between treatments on the same measurement date (DAY), whereas right-hand graphs indicate differences between measurement dates within the same treatment. The p-value of fixed effects is specified, with different letters indicating significant differences (post-hoc Tukey contrast, p<0.05). Measurement dates are reported as the number of days from the beginning of manipulations (9 February, day 1).

During the second experimental period (mid-season drought period), CO_2 fluxes were significantly different across treatments, which exhibited inconsistent effects over time – i.e. significant interaction between treatment and day (**Table 6.2; Fig. 6.10**). In particular, ER and GEP were quite constant in FD, whereas the dynamics of both CO_2 fluxes differed between watered and non-watered pots: ER showed increasing values over time under wet conditions (C and ED) and a decreasing trend under dry conditions (MD) (**Fig. 6.10a**); on the other hand, gross assimilation, which was almost constant in C, showed a marked reduction over time in MD and a slight increase in ED (**Fig. 6.10b**). In addition, plant cover significantly influenced ER [+3.73 mg (CO_2) m^{-2} h^{-1} per unit of plant cover percentage], GEP [-23.55 mg (CO_2) m^{-2} h^{-1} per unit of plant

cover percentage] and NEE [-19.86 mg (CO_2) m^{-2} h^{-1} per unit of plant cover percentage]. None of the CO_2 exchange processes considered were affected by soil mass.

In detail, with regard to ER (**Fig. 6.10a**) the highest value was recorded in C on the last measurement date. CO_2 emissions in ED pots were significantly lower compared to controls during the entire second period during which ED pots were re-watered. Values in ED measured on the first two dates – i.e. DAY 37 and DAY 44, respectively 6 and 13 days after the beginning of re-watering – were statistically equivalent, being 52% and 53% lower, respectively, compared to C pots. Furthermore, values recorded 6 days after re-watering (DAY 37) were still statistically equivalent to those recorded in FD, whereas they were significantly higher after 13 days (DAY 44). On the third date of measurement – i.e. DAY 54, 23 days after the beginning of re-watering – CO_2 emissions in ED pots were still 42% lower compared to C and, although significantly higher on the last measurement date – i.e. DAY 59, 28 days of re-watering – values still remained 39% lower compared to C. Concerning the MD treatment, 6 days after the beginning of the treatment (DAY 37) CO_2 emissions were still statistically equivalent to controls, whereas they were 28% lower after 13 days. On the two following dates (DAY 54 and 59), ER fluxes measured in MD were not statistically different from each other, and were 53% and 73% significantly lower, respectively, compared to C, statistically equivalent to ED on the third date (DAY 54) and significantly lower on the last date (DAY 59), but always significantly higher compared to FD. In contrast, CO_2 emissions in FD pots, which were the lowest during the entire second period, significantly decreased from the first date to the second and were, respectively, 60% and 83% lower compared to C. On the two last measurement dates – i.e. 54 and 59 days after the beginning of drought – ER values in FD were not statistically different from those measured on the previous date, and were statistically equivalent when compared with each other, showing a reduction of 92% on DAY 54 and 94% on DAY 59 compared to CO_2 emissions in controls.

As far as GEP is concerned (**Fig. 6.10b**), patterns across treatments were similar to those observed for ER. Gross assimilation recorded in ED was always significantly lower compared to C, despite pots being re-watered, and gradually increased with time. By the end of the experiment, however, GEP was similar to that recorded at the beginning of re-watering. Six days after the end of the dry period (DAY 37) gross assimilation in ED was still 66% lower compared to C, but significantly higher compared to FD. After 13 days (DAY 44) from the beginning of re-watering, GEP was reduced by 55% compared to controls and was statistically equivalent to values recorded on the two following dates, where gross assimilation was, nevertheless, still 50% and 54% lower compared to C pots. With regard to MD, GEP was not statistically different from that recorded in C after 6 days (DAY 37) and 13 days (DAY 44) from the beginning of drought, although GEP was significantly reduced by 27% from the first measurement date to the second. After 23 days from the beginning of drought (DAY 54), gross assimilation was 93% lower compared to C and almost

null after 28 days (DAY 59); values on these last two dates were not statistically different from those recorded in FD. Pots subjected to drought during the entire experimental period (FD) still showed little assimilation 37 days after the beginning of drought (-93% compared to C). On the following date – i.e. after 44 days of drought – gross assimilation was almost null, statistically equivalent to values recorded on the following two measurement dates, after 54 and 59 days.

Net ecosystem exchange (**Fig. 6.10c**) in ED pots maintained negative values all through the second period, being statistically equivalent to C only on the last measurement date of the experiment. On the other hand, NEE in MD pots was not statistically different to controls on the first two measurement dates of the second period, exhibiting significantly higher, positive values compared to those in C on the last two measurement dates of the experiment – i.e. 23 days (DAY 54) and 28 days (DAY 59) of drought. On these two last dates, NEE in MD was not statistically different from that recorded in FD after 54 and 59 days of drought. In this treatment (FD), NEE values were positive and approaching zero, remaining stable over the entire second period of drought.

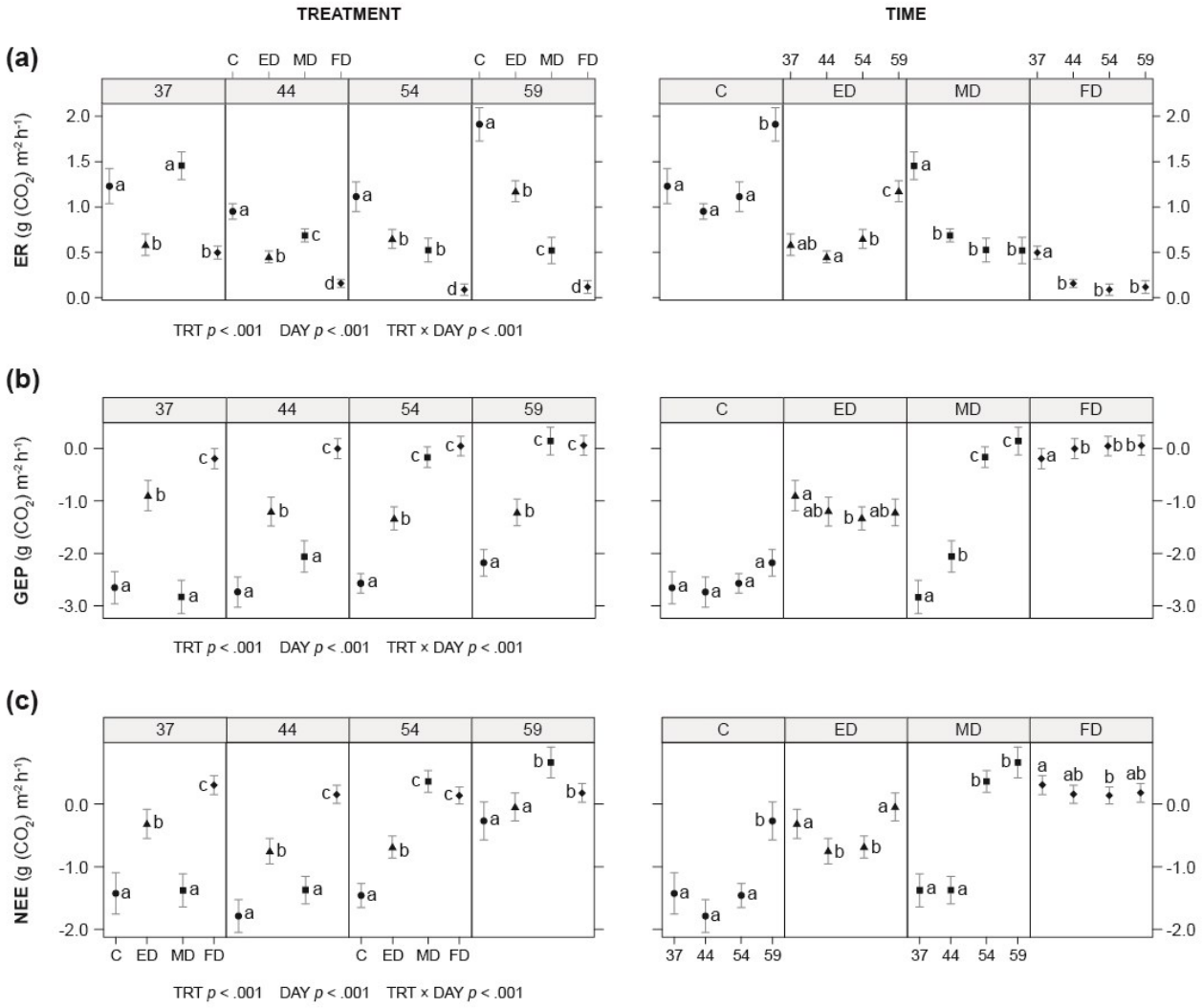


Fig. 6.10: LMMs output (estimated mean and 95% confidence interval) for **(a)** ecosystem respiration (ER), **(b)** gross ecosystem production (GEP) and **(c)** net ecosystem exchange (NEE) measured during the mid-growing season in 4-level treatment TRT [C – control, ED – early-season drought; MD – mid-season drought; FD – full-season drought]. For each process, left-hand graphs illustrate differences between treatments on the same measurement date (DAY), whereas right-hand graphs indicate differences between measurement dates within the same treatment. The p-value of fixed effects is specified, with different letters indicating significant differences (post-hoc Tukey contrast, p<0.05). Measurement dates are reported as the number of days from the beginning of manipulations (9 February, day 1).

6.4.5 Resistance

Sensitivity during drought – i.e. drought resistance – calculated for CO_2 fluxes at the end of each treatment period, was significantly different between ER and GEP when comparison was carried out between the two processes within the same drought period (early-season drought: $F_{1,13}$=37.771, p<0.001; mid-season drought: Z=3.202, p=0.001), with GEP being more sensitive to drought both in the early- and mid-growing season (**Fig. 6.11a**). Resistance also significantly differed across the two drought periods (ED and MD) for both ER ($F_{1,19}$=12.930, p=0.002) and GEP (Z=3.529, p<0.001), with the most negative values in MD for both ecosystem processes (**Fig. 6.11b**).

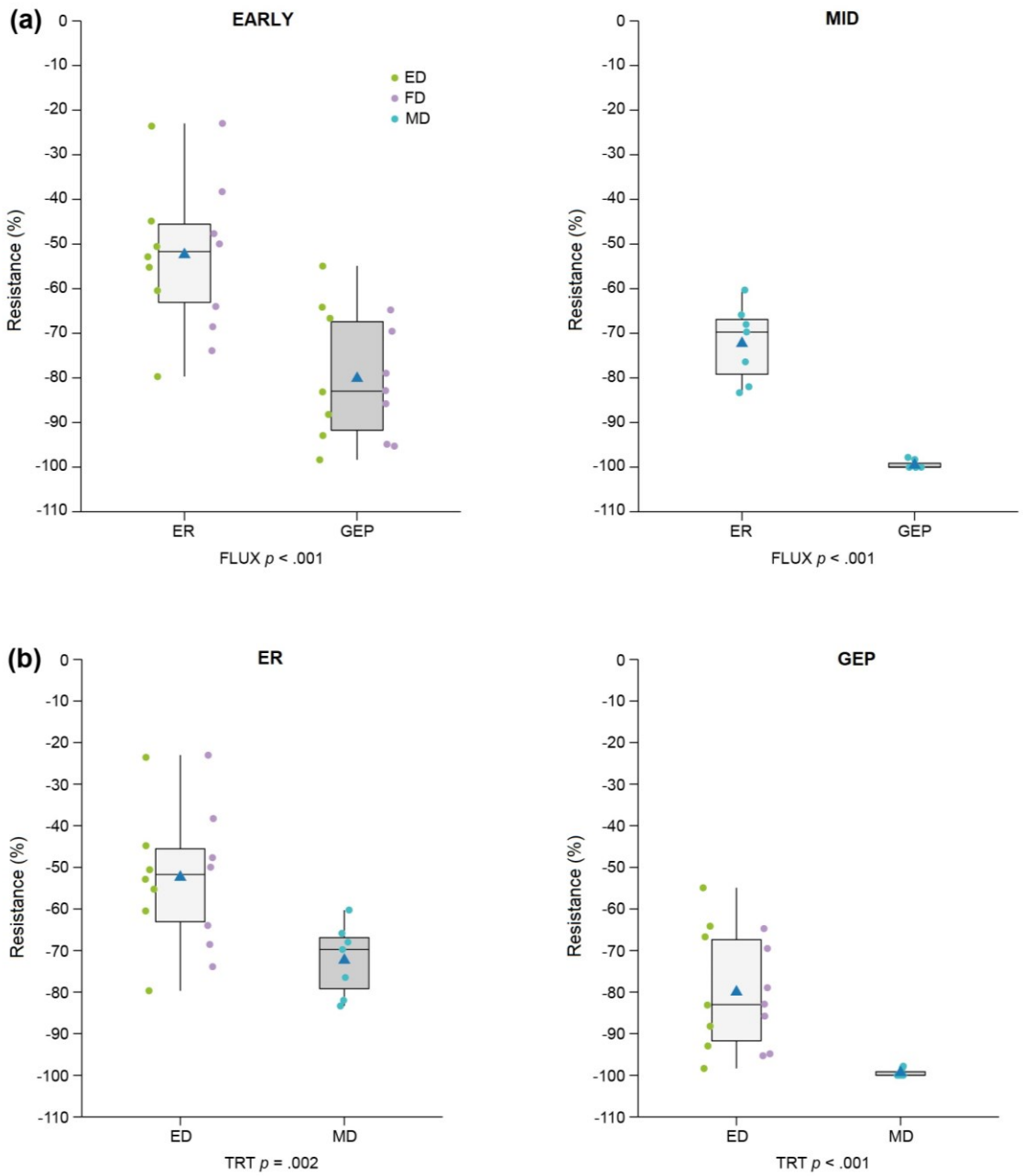


Fig. 6.11: Resistance to drought (% of change in drought pots cf. controls) of ecosystem respiration [ER, g (CO_2) m^{-2} h^{-1}] and gross ecosystem production [GEP, g (CO_2) m^{-2} h^{-1}] **(a)** within the same drought period (EARLY – early growing season; MID – mid-growing season) and **(b)** across the two different drought periods [ED – early-season drought; MD – mid-season drought]. Together with ED, values for full-season drought (FD) were also included in the early period. Coloured dots refer to single values for each treatment (TRT).

6.4.6 ER and GEP thresholds

Results from threshold analyses are reported in **Table 6.3**. As for soil moisture thresholds, values for monoliths experiencing drought from the beginning of the growing season were lower for both ER (12.42 ± 3.77%, estimated WP -57.72 kPa) and GEP (5.89 ± 1.06%, WP -1115.00 kPa) compared to values for monoliths subjected to a dry period from the middle of the growing season (ER threshold: 25.25 ± 8.87%, -19.87 kPa; **Fig. 6.12a**; GEP threshold: 15.26 ± 3.19%, WP -39.39 kPa; **Fig. 6.12b**). On the other hand, temporal thresholds – i.e. the number of days without precipitation triggering a different CO_2 response rate – were observed after 37 ± 6 days for ER (**Fig. 6.13a**) and 32 ± 3 days for GEP (**Fig. 6.13b**) in monoliths which underwent a dry event immediately after snowmelt. These thresholds were 24 and 21 days earlier than that estimated for monoliths where the dry period began one month after snowmelt (13 ± 0 days).

Table 6.3: Two-phase linear model parameter estimates (Est) of soil moisture thresholds (volumetric water content, VWC, %) and temporal thresholds (days without precipitation, DWP) for ecosystem respiration (ER) and gross ecosystem production (GEP). Calculation of thresholds was based on drought starting in two different periods: at the beginning of the growing season (EARLY) and on the middle of the growing season (MID). Estimates before the threshold point (Pre_{thr}), and after ($Post_{thr}$) are reported. For Pre_{thr} "Intercept" refers to estimated value of CO_2 flux [g (CO_2) m^{-2} h^{-1}] at VWC, or DWP, equal to zero, whereas for $Post_{thr}$ "$Intercept_{thr}$" indicates CO_2 flux at the VWC, or DWP, threshold value; "Slope" refers to changes in CO_2 flux [g (CO_2) m^{-2} h^{-1}] per unit change of VWC ($Slope_{VWC}$) or DWP ($Slope_{DWP}$).

		EARLY			MID		
		Est	SE	p-value	Est	SE	p-value
ER							
Soil moisture							
Pre_{thr}	Intercept	-0.035	0.080	0.662	0.417	0.214	0.052
	$Slope_{VWC}$	0.034	0.015	0.026	0.012	0.023	0.587
$Post_{thr}$	$Intercept_{thr}$	0.624	0.191	0.220	1.258	1.917	0.784
	$Slope_{VWC}$	0.008	0.015	0.087	0.014	0.607	0.998
Time							
Pre_{thr}	Intercept	0.896	0.054	<0.001	2.129	0.175	<0.001
	$Slope_{DWP}$	-0.009	0.006	0.170	-0.110	0.014	<0.001
$Post_{thr}$	$Intercept_{thr}$	0.175	0.208	0.055	0.550	0.203	0.458
	$Slope_{DWP}$	-0.003	0.006	0.386	-0.001	0.021	<0.001
GEP							
Soil moisture							
Pre_{thr}	Intercept	-0.005	0.012	0.690	0.227	0.315	0.472
	$Slope_{VWC}$	0.000	-	-	-0.036	0.036	0.320
$Post_{thr}$	$Intercept_{thr}$	-0.005	0.012	0.690	-1.786	0.671	0.029
	$Slope_{VWC}$	-0.041	0.005	<0.001	-0.047	0.034	0.735
Time							
Pre_{thr}	Intercept	-1.330	0.271	<0.001	-3.589	0.390	<0.001
	$Slope_{DWP}$	0.026	0.010	0.009	0.111	0.037	0.003
$Post_{thr}$	$Intercept_{thr}$	-0.223	0.110	0.012	-0.747	0.225	<0.001
	$Slope_{DWP}$	0.009	0.012	0.156	0.049	0.038	0.105

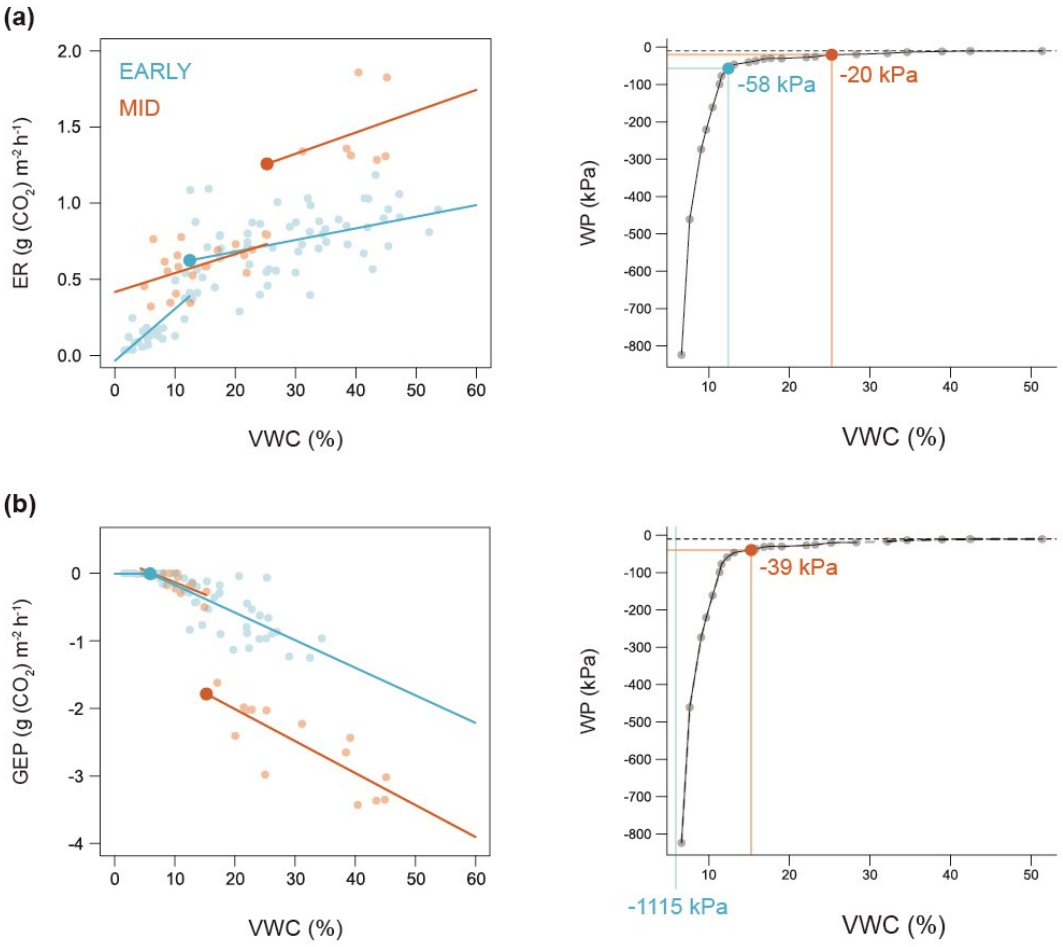


Fig. 6.12: Two-phase linear models of thresholds of soil moisture (volumetric water content, VWC %) for **(a)** ecosystem respiration (ER) and **(b)** gross ecosystem production (GEP). Calculation of thresholds was based on drought starting in two different periods: at the beginning of the growing season (EARLY) and in the middle of the growing season (MID). For each CO_2 flux, right-hand graphs indicate the estimated water potential (WP, kPa) for each drought period; horizontal, black dashed line indicates WP at field capacity (-10 kPa).

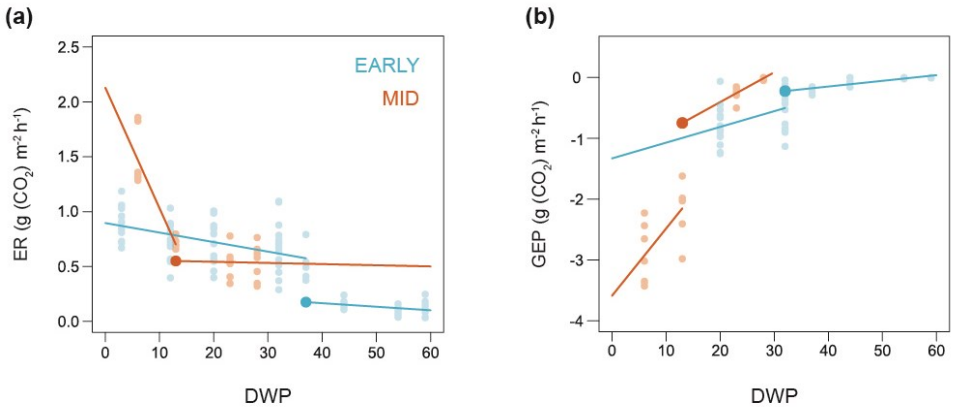


Fig. 6.13: Two-phase linear models of temporal thresholds (days without precipitation, DWP) for **(a)** ecosystem respiration (ER) and **(b)** gross ecosystem production (GEP). Calculation of thresholds was based on drought starting in two different periods: at the beginning of the growing season (EARLY) and in the middle of the growing season (MID).

6.5 DISCUSSION

Regions all over the world are facing shifts in drought timing and duration (Breinl et al., 2020). This, together with future increases in inter- and intra-annual variability of precipitation, could result in significant structural and functional changes in ecosystems, especially if dry spells occur at a critical moment of the growing season (Knapp et al., 2008; Reichstein et al., 2013; Sippel et al., 2016). Results from the present study reveal that timing is, in fact, crucial in determining the degree to which alpine mesic ecosystems respond to a limited water supply. Additionally, soil moisture and temporal thresholds for ER and GEP were found to differ between early and mid-season drought, highlighting the diverse mechanisms driving ecosystem responses to drought occurring at different moments during the growing season.

6.5.1 Drought effects during the study period

One of the aims of the present study was to assess CO_2 exchange responses to early- and mid-season drought over the entire study period. Overall, results showed that drought negatively affected ANPP and CO_2 fluxes. This outcome is in line with findings in grasslands which have reported reduced ANPP in response to water shortage (Gilgen and Buchmann, 2009; Bloor and Bardgett, 2012; Darenova et al., 2017; Zhang et al., 2020b), together with lower net CO_2 assimilation resulting from reductions in ER and GEP (Darenova et al., 2017; Zhang et al., 2019a; von Buttlar et al., 2018). Not surprisingly, the 2-month long drought period impacted the grassland to a greater extent than a 1-month dry event, thus confirming the importance of drought duration in determining the magnitude of ecological responses (von Buttlar et al., 2018; Sippel et al., 2018).

With regard to drought timing, over the entire study period early-season drought had a greater effect on ANPP production and CO_2 exchange compared to mid-season drought: monoliths undergoing ED exhibited 28% lower biomass compared to C (**Fig. 6.7**), a significant greater reduction in ER (-42%, **Fig. 6.8a**) and GEP (-55%, **Fig. 6.8b**), producing a significant reduction in NEE (**Fig. 6.8c**). However, it was interesting to note that, considering the average NEE for the entire study period, grassland monoliths, although shifting towards positive NEE values by the end of the period, maintained their C-sink level under both drought-timing treatments, while exhibiting overall negative NEE values – it was only in the most extreme drought, with no watering during the entire experiment, that grassland patches were a C-source status.

A greater impact of early-season drought compared to that occurring later in the season has been observed in other field studies (Hao et al., 2018; Liu et al., 2019; Meng et al., 2019; Zhang et al., 2019b), which support the hypothesis that drought effects on plants are most severe during the most active growing period (Bates et al., 2006; Craine et al., 2012; Darenova et al., 2017). In

the present study, ANPP data revealed that this period was concentrated in the early-season period, approximately one month after snowmelt: although ANPP in MD monoliths was reduced (-17%), this did not significantly differ from C (**Fig. 6.7**), indicating that the peak of biomass had already been reached after 32-40 days. This figure could also explain the lower reduction in ER and GEP in MD compared to ED. During the early period, high levels of CO_2 exchange were found in MD monoliths (**Fig. 6.8a, b**), which might have been sufficient to compensate for ER and GEP reduction during subsequent drought. In fact, conditions prior to a dry event have been recognized as a key factor in seasonal and inter-annual carbon exchange responses (Kwon et al., 2008; Sippel et al., 2018). For instance, a regional-scale study on drought effects in the USA found that a warm spring preceding summer drought was able to make up for 78% of drought-induced reduction in net carbon uptake (Wolf et al., 2016). In the current study, high levels of ER and GEP during the first part of the growing season might, therefore, have been sufficient to determine a lower CO_2 flux reduction in MD compared to ED.

With regard to ANPP recorded in ED, this was significantly reduced compared to controls, with values not statistically different from those recorded in the full drought treatment (**Fig. 6.7**). This outcome demonstrates that, although starting from a condition of soil saturation which simulated natural snowmelt conditions, the grassland monoliths underwent significant drought stress in the first part of the season. Several studies have reported reduced leaf area as a response to water shortage (Jung et al., 2014; Rosbakh et al., 2017; Wellstein et al., 2017; Vitra et al., 2019) and changes in physiological and morphological plant traits as a result of altered precipitation patterns during the early season (Zeppel et al., 2014 and references therein). In the present study, reduced plant growth observed in the ED treatment could be related to the direct impact of water shortage on cell formation – i.e. cell expansion and differentiation – which is reported as being highly sensitive to losses in turgor – i.e. high leaf water potential (-0.5 MPa to -0.8 MPa) needed to inhibit meristems (Körner, 2015 and references therein). Reduced ANPP could explain the GEP reduction in ED (Rogiers et al., 2005; Suttle et al., 2007; Noormets et al., 2008; van der Molen, 2011) which may also have influenced ER both directly (Darenova et al., 2017; Zhang et al., 2019a) and indirectly through the reduction of heterotrophic respiration dependent on newly assimilated carbon (Wan and Luo, 2003; Burri et al., 2018). Lower plant growth during the early season could in fact provide an explanation for CO_2 fluxes recorded during the days following drought, in which ED monoliths never returned to C values, despite re-watering taking place for one month after the treatment: by the end of the study period ER and GEP were still 39% and 54% lower in ED, respectively, compared to values recorded in C. Detrimental effects on CO_2 fluxes as a result of reduced leaf area following a dry period during the early part of the season have been reported for forest ecosystems (Noormets et al., 2008). There could be other causes, however, for GEP and ER reductions in ED later in the season. It cannot be excluded that flux reduction, especially of

GEP, following re-watering could also have resulted from “stress memory”, an ecophysiological mechanism by which plant-performance modifications persist after stress ceases, potentially improving plant performance and resistance under recurrent stressful events (Walter et al., 2013). “Drought-memory” has been effectively reported for grasses (Walter et al., 2011), however such a hypothesis could not be tested within the present study since no comparison between single-drought monoliths and recurrent-drought monoliths were carried out; further studies specifically aimed at addressing this topic need, therefore, to be developed. As far as ER reduction during the second period is concerned, this might also be explained by lower soil moisture values still present after re-watering (**Fig. 6.6a**). In fact, inhibition of the microbial physiological activity is known to take place with soil dehydration as a result of diverse mechanisms – loss of cell turgor, lack of a solvent for water soluble substrates and of a medium for nutrient transport (Schimel, 2018). Progressive drying starting from the topsoil layer (Borken and Matzner, 2009 and references therein) probably reduced microorganism activity, which also determined the ER reduction during both drought timings. In should be noted that VWC recorded in monoliths only refer to the 0-5 cm soil profile (**Fig. 6.6a-c**); the soil right at the surface may have been even drier (see Chapter 3, Section 3.3.2), closer to the values reported by Cook and Orchard (1983), who found reduced microbial respiration for water potential <-0.05 MPa.

To summarise, outcomes from the entire study period can be identified as the result of two mechanisms: (a) the compensating effect of CO_2 exchange in the first part of the season, which reduced seasonal impacts of drought on CO_2 emission and assimilation during the mid-season drought period; and (b) reduced plant growth in the early part of the season and related carry-over effects during the month following the early-season dry event. It is important to note, however, that the temporal frame in this study does not allow a complete assessment of effects of different drought timings on ecosystem functions, with two reasons being identified for this: (a) lack of data on grassland recovery after a drought event in the second part of the growing season; and (b) no data regarding plant mortality under different drought timings. Furthermore, the mesocosm approach and the consequent use of pots makes a comparison of results with outcomes of field experiments difficult to implement (Brilli et al., 2011).

With regard to the first point concerning the temporal frame of the study, the investigated period did not cover the entire growing season, meaning that potential recovery of MD following an equally-long period of re-watering, could not be assessed. It could be the case that, during the remaining part of the growing season, plants are already undergoing senescence as *Carex curvula* leaves start to turn brown early in the season (Erschbamer and Winkler, 1995). This could result in a lower potential recovery than that exhibited by plant communities experiencing water stress during the early period (De Boeck et al., 2011) as stomatal conductance and assimilation capacity is lower in senesced leaves (Zhang et al., 2020a).

As far as detecting plant mortality is concerned, this cannot be captured by ANPP and CO_2 fluxes over a single season, but it can be partially identified when exploring CO_2 responses during drought in greater detail. Furthermore, as pointed out by Frank et al. (2015), assessment of drought needs to distinguish "concurrent" vs "lagged" impacts according to their differences in response time – i.e. during and after the dry event. Data from the current study only allows the detection of immediate, short-term responses during the season following an early, spring drought. A comparison between short-term responses to mid-season drought and long-term effects of both drought timings is needed, since lagged effects might occur when changes in plant and soil characteristics take place. These include reduction in carbohydrate reserves in plants, changes in soil microbial communities, higher soil nitrogen availability following drought and mortality (van der Molen et al., 2011; Sippel et al., 2018). The extension of drought effects beyond the dry period – i.e. legacy effects – have been reported in several studies (e.g. Sala et al., 2012; Zona et al., 2014; Hoover and Rogers, 2016; De Boeck et al., 2018b) and assessing how different drought timings may affect alpine grassland ecosystem functions in subsequent years will, therefore, be a topic for further investigation.

Finally, with regard to the use of pots and the difficulty in comparing results from the present study with those obtained in field experiments, mesocosm experiments, while guaranteeing a high level of control, are recognised as being unable to capture the complexity of the ecosystem under natural conditions (Kreyling et al., 2014). Pot experiments not only represent a confined environment where biotic interactions are altered, but the small pot size itself can determine a set of biological constraints which are not so evident – or even absent – in the field. These restrictions include: limited soil quantity, reduced water and nutrient availability, reduced photosynthesis and reduced root growth (Poorter et al., 2012 and references therein). It is, therefore, likely that both the magnitude of responses and the rate at which stressful conditions occur will be greater in a pot experiment such as the present one compared to responses observed in field studies.

6.5.2 Drought effects within each drought period

As a response to the second aim of the study, which was to achieve a more detailed understanding of the mechanisms driving ecosystem dynamics under drought, CO_2 fluxes were investigated within each drought period. The dynamics of CO_2 fluxes, and the strength of the response, appeared to be diversely affected by drought occurring immediately after snowmelt compared to those observed in monoliths experiencing a dry period one month later.

In detail, ED effects on CO_2 fluxes (**Fig. 6.9**) were only evident 20 days after the beginning of the treatment as a result of both reduced ER and GEP in dry monoliths compared to those that had been watered. By the end of the ED period – i.e. 32 days after snowmelt – ER and GEP in dry

pots were reduced by -56% and -80%, respectively, compared to those in watered conditions; grassland patches functioned as a carbon source (positive NEE values) in contrast with watered monoliths, which acted as a carbon sink (negative NEE). On the other hand, MD monoliths (**Fig. 6.10**) exhibited significantly reduced ER after 13 days and GEP was found to be significantly reduced compared to controls (-93%) 23 days after the beginning of drought. By the end of the MD treatment, there was a 73% reduction in ER compared to controls, while GEP was almost null and NEE statistically equivalent to values recorded in FD pots experiencing a 62-day long drought.

Differences in the strength of response to diverse drought timing are usually reported as being dependent on the initial soil water status, the temperature and the phenological phase characterising the investigated ecosystem at the time of drought occurrence (De Boeck et al., 2011; Zeppel et al., 2014; Vitra et al., 2019; Yang et al., 2020b). In the current study, a difference in soil moisture at the beginning of the dry period can be excluded as a factor influencing the magnitude of the response, since MD pots were watered and maintained moist all through the first period until the start of the drought treatment (**Fig. 6.6a-c**). With regard to mean temperature, on the other hand, this was effectively higher in the MD period compared to the earlier part of the season – i.e. by 2 K and 3 K in the air and soil, respectively (**Fig. 6.5**). In comparison with natural temperatures experienced by the grassland community, average air temperatures at the University Campus garden were similar to those occurring under field conditions (see Chapter 3, Section 3.3.2). On the other hand, soil temperature during the second period was approx. 2 K higher compared to mean values recorded between July-August in the field. Such a difference was probably the result of both faster soil drying and the confined environment represented by the pot itself – two factors which contribute to making mesocosm conditions difficult to compare with those in the field (Brilli et al., 2011). The co-occurrence of drought with higher temperatures recorded under natural dry events (De Boeck et al., 2010) and the higher temperatures which usually characterise the middle part of the growing season represent an intrinsic environmental feature which cannot, therefore, be disentangled from the impact of drought timing.

On the basis of the discussion so far, the most probable explanation for the different dynamics in CO_2 fluxes observed within the two drought periods can be identified in the phenological stages of the biocoenosis. When considering GEP, results could depend on the amount of ANPP characterising grassland patches at the beginning of each drought period: plants in ED were characterised by lower initial biomass compared to those in MD, which had already achieved almost the peak of ANPP when the mid-season drought treatment was applied. As already explained in the previous section, ANPP measured in MD pots at the end of the experimental period was, in fact, statistically equivalent to that measured in C (**Fig. 6.7**). Previous studies have demonstrated that the extent to which climate extremes impact a community is positively related

to the amount of plant biomass – a result of a greater depletion of water resources through plant absorption and evapotranspiration (Wang et al., 2007; van Ruijven and Berendse, 2010). This also holds true at the species level, with species characterised by a larger leaf area more affected by drought (De Boeck et al., 2016). The larger amount of biomass present at the beginning of the mid-season drought could, therefore, have led to a greater demand for water in order to maintain plant turgor and sustain water loss due to greater evapotranspiration. This explanation is supported by the more rapid (**Fig. 6.6a**) and higher (**Fig. 6.6b**) soil moisture reduction in MD compared to ED monoliths. The inability to satisfy water demand probably triggered a variety of physiological responses in MD monoliths resulting in the wilting of aboveground organs (Reddy et al., 2004; Farooq et al., 2012); wilted plants were observed in MD pots by the end of the experiment, expressed as null GEP values in the data – i.e. GEP approaching 0. In contrast, grassland patches experiencing an early-season drought had a lower initial biomass, due to the intrinsic phenological stage of the plant community which, together with lower temperatures experienced during this period (**Fig. 5**), might have led to more limited water depletion (**Fig. 6.6a, b**) as a result of both lower water demand and evapotranspiration rates.

Ecosystem respiration appeared to follow the same trend as GEP, supporting the explanation that the reduction of ER in the MD treatment was mainly related to the greater reduction in gross assimilation. This connection was probably the result of wilted aboveground plant organs, potentially lower root respiration and/or the consequent reduction of labile organic compounds produced by photosynthesis (Kuzyakov and Cheng, 2001; Liu et al., 2019; Zhang et al., 2019a). Additionally, the faster depletion of soil moisture resources during the second drought period (**Fig. 6.6a**) might have led to the faster reduction observed for ER compared to GEP during the MD treatment, through decreased water-filled pore spaces able to constrain nutrient diffusion and determine a decreased microbial activity (Schimel, 2018).

6.5.3 ER and GEP: differences in drought resistance

Results highlighted that, after 1-month, under both early- and mid-summer drought, GEP was significantly less resistant – i.e. more sensitive – to soil drying than ER (**Fig. 6.11a**). This outcome is in line with previous findings under both natural climate extremes (Noormets et al., 2008; Schwalm et al., 2012; Liu et al., 2019) and experimental conditions (Hoover and Rogers, 2016). With regard to differences across the two drought timings, results demonstrated that ER and GEP exhibited a different resistance in ED and MD, with a greater decline for both processes at the end of the mid-season dry event (**Fig. 6.11b**). By comparing two drought periods of equal length and magnitude, outcomes from the present study support the hypothesis put forward by studies analysing naturally occurring drought events, which states that CO_2 flux sensitivity may differ between dry events occurring in different periods (Lund et al., 2012; Liu et al., 2019).

Manipulation experiments carried out on semi-arid ecosystems have also found that CO_2 fluxes exhibit diverse resistance to drought timing (Hao et al., 2018; Zhang et al., 2019b), with drought having a greater effect on ecosystem processes in the early part of the growing season. In these studies, however, responses were mediated within the drought period and did not permit an assessment of the mechanism underlying the differences observed. Furthermore, precipitation experiments in the field are usually subjected to a covariation between treatments and controls which can change the magnitude of the treatment effect, making it difficult to assess ecological responses in the short-term (Hoover et al., 2018). By conducting an experiment in a controlled environment, where the water amount received by treatments and controls was regulated, it was possible to overcome this issue and assess soil moisture and temporal thresholds for ER and GEP.

6.5.4 ER and GEP thresholds: differences between early and mid-season drought

Soil moisture thresholds

The outcome of analysis on soil moisture thresholds revealed that the same process exhibited different threshold levels in response to water stress depending on the timing of the dry spell onset. During a mid-season drought, higher VWC values were required to trigger different rates of both ER and GEP compared to values observed when drought coincided with the beginning of the growing season. In detail, for mid-season drought, ER rates exhibited an abrupt change at VWC of c. 25% (estimated WP c. -20 kPa, **Fig. 6.12a**) whereas for early-season drought sharply declining rates were found at VWC of c. 12% (estimated WP c. -58 kPa, **Fig. 6.12a**). Similarly, rates of GEP were abruptly reduced at VWC of c. 15% (estimated WP c. -39 kPa, **Fig. 6.12b**) under mid-season drought, whereas under early-season drought there was a steep, continuous reduction until c. 6% of VWC (estimated WP -1115 kPa, **Fig. 6.12b**) was reached, under which GEP became null.

A closer examination of VWC-threshold models for GEP revealed different patterns, evidencing a sharp discontinuity under mid-season drought as opposed to the continuous reduction in the early-season model. The pattern observed in MD could reflect the strategy for water-use adopted by plants within the community – i.e. acquisitive vs conservative, a concept which has been widely investigated in studies on plant-water relations (e.g. Tardieu and Simonneau, 1998; Martínez-Vilalta et al., 2017). As clearly explained by Belluau and Shipley (2017), plant species with an acquisitive strategy tend to exhibit a high stomatal conductance (g_s) and photosynthetic rate (A), but low water use-efficiency (WUE, given by the ratio between A and g_s) under field-capacity moisture conditions, showing a sharp decline in g_s and A when soils become drier, with plants finally wilting at relatively high values of soil moisture availability, while still exhibiting high g_s. Species adopting a conservative strategy, on the other hand, are characterised by low g_s and A, and high WUE, even under moist conditions. These plants are less sensitive to variations in soil moisture, exhibiting lower rates of decline of A and g_s as soils become drier, thus wilting at

relatively low soil moisture values while exhibiting low g_s. Acquisitive strategies have been described as being typical of plant species inhabiting wet soils, while in drier environments a conservative strategy is more commonly adopted (Belluau and Shipley, 2017).

In the present study, the discontinuous decline detected in the threshold model for GEP during the second period (**Fig. 6.12b**), together with the mesic conditions of the investigated ecosystem, suggests that this sedge-dominated community might be generally characterised by plants adopting a water acquisitive strategy, with gas exchange processes taking place until the occurrence of severe water deficit. Under natural conditions, in fact, the investigated plant community develops in an environment in which water does not act as a limiting resource on growth, with water supplies guaranteed by snowmelt input and abundant rainfall during the summer (Körner, 2003). The presence of moist conditions is supported by soil moisture records gathered at -5 cm within the field site, showing that under non-manipulated conditions VWC was generally ≥30% (see Chapter 3, Section 3.3.2) – i.e. values near the WP field-capacity (-10 kPa) according to the WP-VWC model presented in the current study. Acquisitive strategies under water shortage have also been identified in a study examining g_s and A in four mountain grassland species, where gas exchange processes were found to be limited at VWC <10% (Brilli et al., 2011). In another study, Körner et al. (1980) found that *Carex curvula* and *Festuca halleri* exhibited very little variation in leaf conductance even under high leaf water potential variations reaching values as low as -1.8 MPa. On the whole, these studies support the results from the present study which, however, do not refer to a single species but encompass a complex of organisms and plant functional types, each exhibiting different water-use strategies and traits which enable some species to cope with drought better than others (Oberbauer and Billings, 1981; Belluau and Shipley, 2017). Investigating species-specific responses under different drought timing could, therefore, help in the identification of traits underlying drought resistance and, at the same time, lead to an improved understanding of mechanisms driving responses at a community-level.

As for the pattern exhibited by the VWC threshold model for GEP under drought starting from the beginning of the growing season (**Fig. 6.12b**), the continuous declining trend could represent the reduced growth which was detected in ED monoliths (**Fig. 6.7**). The lower water amount necessary to sustain aboveground organs, as a consequence of reduced biomass, probably explains the lower VWC threshold necessary for assimilation to cease. The hypothesis that GEP decline during the two different drought timings was driven by different mechanisms – i.e. reduced plant growth and green leaf area in the first period vs the wilting of larger biomass with a greater leaf area in the second period (see Section 6.5.2) – is therefore supported by VWC threshold models for GEP.

With regard to ER, soil moisture thresholds detected identified values at which CO_2 emissions started to decline at a faster rate, thus not representing the threshold of ceased processes which,

instead, was found by VWC-threshold models for GEP. Such a difference is probably related to diverse mechanisms underlying threshold values of ER and GEP: the former is regulated by both autotrophs and heterotrophs, whereas the latter is uniquely driven by autotrophs. ER components have been found to exhibit different sensitivities to drought (Sanaullah et al., 2012a). In particular, a recent field-based study investigating drought effects over precipitation gradients has found that autotrophic respiration is generally more affected by drought than heterotrophic respiration, and aboveground respiration is more sensitive than that of roots (Zhang et al., 2019a). Threshold values for ER, estimated in the present study, are in line with outcomes reported by Zhang et al. (2019a): the higher gap observed in the VWC threshold under mid-season drought (**Fig. 6.12a**) supports the hypothesis that reduced ER was strongly driven by reduced plant respiration, both above- and belowground, and consequent reduction in photosynthates (see Section 6.5.2). This is also confirmed by temporal thresholds estimated on the same data, which were equal for both processes under mid-season drought– i.e. 13 days without precipitation needed to abruptly reduce both GEP and ER (**Fig. 6.13a, b**). In contrast, the difference between VWC threshold of ER and GEP in mid-season drought – i.e. ER threshold (at 25% VWC) > GEP threshold (at 15% VWC) – and early-season drought – i.e. ER threshold (at 12% VWC) > GEP threshold (at 6% VWC) – could be the result of reduced metabolic activity of microorganisms located immediately below the soil surface.

In the light of these considerations, therefore, it is probable that CO_2 emissions below VWC thresholds for each drought period (Pre_{thr}, **Table 6.3**) largely reflect the heterotrophic component of ER, which appeared to respond differently to drought timing when the slope and the intercept for each period were considered: (a) the slopes ($Slope_{VWC}$) revealed that the ER decline in pots experiencing early-season drought was faster when compared to mid-season, post-threshold rates, and (b) the intercept displayed near-zero values – i.e. cease of respiration processes – only in monoliths subjected to a two-month long drought period (**Fig. 6.12a, Fig. 6.13a**).

The difference in post-threshold slopes could be due to the higher soil moisture levels characterising the post-threshold period for the middle of the season, compared to early-season moisture levels, which probably favoured higher rates of microbial metabolic activity (Schimel, 2018). As far as the post-threshold intercept is concerned, respiration processes appeared to cease only in monoliths which had experienced a two-month long drought immediately after snowmelt, even though MD reached equivalent VWC to that recorded in FD (**Fig. 6a, c**). This could be explained by the extreme nonlinear relationship between VWC and WP (Papendick and Campbell 1981 and **Fig. 12**, right-hand graphs) evidencing that, when soils are dry, even a slight change in VWC can determine a large change in soil water potential. Although VWC approached near-zero values, the corresponding water potential might still not have been low enough to completely inhibit ER in MD.

In fact, soil microbial communities are known to exhibit very low moisture thresholds (Manzoni et al., 2012; Schimel, 2018 and references therein). The meta-analysis by Manzoni et al. (2012) evidenced water potential thresholds at which heterotrophic respiration ceased as low as -13.8 MPa in mineral soils and -36.5 MPa in surface litter, in some cases reaching a minimum of -1580 MPa. As for plant species, which are known to exhibit different levels of tolerance to drought (e.g. Belluau and Shipley, 2017), microorganisms have been found to respond differently to water shortage according to their functional group (Schimel, 2018), with fungi generally able to withstand drier conditions better than bacteria (Schimel et al., 2007; Manzoni et al., 2012; de Vries et al., 2018). However, biotic processes are known to exhibit distinct phenological cycles (Sippel et a., 2018), with microbial communities in alpine ecosystems undergoing natural, temporal shifts in their composition during the growing season (Lipson et al., 2002; Schmidt and Lipson, 2004). The phenology of the heterotrophic community may, therefore, represent a further factor influencing ER responses to drought timing, but further investigation on microbial dynamics under different seasonal drought timings is needed in order to separate the different processes contributing to ER and identify the major drivers. In addition to this, the contribution of the autotrophic component – including both above- and belowground plant organs − to soil CO_2 efflux also needs to be assessed in order to shed light on the outcomes of the present study.

Temporal thresholds

The number of days without precipitation necessary to trigger changes in ER and GEP rates differed between the two drought timings (**Fig. 6.13a, b**). In detail, data showed that when drought started one month after the beginning of the growing season, it took 13 days for monoliths to exhibit almost null GEP and faster declining rates of ER. This resulted in thresholds being reached on average 3 weeks earlier in MD compared to thresholds in monoliths experiencing drought from the beginning of the growing season, where it took on average 37 ± 6 days for ER to almost cease, and 32 ± 3 days for GEP to effectively reach null values.

To identify whether equivalent dry periods could actually occur in the field, the climate variability of the area where the target grassland is located (Rhaetian Alps) was assessed. This was done by analysing the precipitation data for the last two decades (2000-2019) provided by the nearest weather station to the study site, located at a similar elevation (Careser Diga, 2600 m a.s.l.; see Chapter 3, Section 3.1). For each year, the longest period without precipitation between June and August was recorded, as this interval matched the growing season period in the present study.

Historical data showed that, in the last two decades, the average longest period without rainfall lasted 8 ± 3 days, with a maximum of 15 days in 2006 and a minimum of 4 in 2014. Analogous figures were recorded by another weather station at a lower elevation (Passo Tonale, 1780 m a.s.l.; see Chapter 3, Section 3.1). This data provides evidence that drought events of a long enough duration to significantly impact CO_2 fluxes could, in all probability, occur in the future. The results from the present study underline the additional fact that if these dry spells occur in the middle of the growing season, when alpine grasslands have almost completed their seasonal growth, there is a greater chance of thresholds for ER and GEP being reached. Furthermore, the higher frequency and longer duration of drought events reported in some areas in Europe (Hartfield et al., 2018; Breinl et al., 2020) together with the frequent concurrence of heat waves and drought (De Boeck et al., 2010; De Boeck and Verbeeck, 2011) may further increase the risk of important functional impacts on ecosystems which are similar to that studied in the present research (De Boeck et al., 2016).

6.6 CONCLUSION

Findings from the present study underline the crucial aspect of timing when assessing drought impact on alpine ecosystem functions within the growing season. When looking at the entire study period, early-season drought was found to reduce ANPP and CO_2 fluxes to a greater extent compared to mid-season drought. Further investigations on ecosystem process dynamics during drought revealed that ED never returned to C values after 1 month of re-watering and that a dry

spell occurring in the middle part of the growing season, when the plant community had already reached almost the peak of its biomass, had a more detrimental effect on photosynthetic and respiration processes. Threshold analysis also revealed that under mid-season drought both higher soil moisture values and a lower drought length were necessary to trigger different ER and GEP rates – conditions which the grassland might well experience in the future.

These results highlight how different mechanisms underlie diverse ecosystem responses to drought – i.e. reduced plant growth under early-season drought vs wilting of aboveground plant organs under mid-season drought – stressing the importance of the temporal framework considered when analysing the effects of drought timing: short-term responses may be transient, leading to incomplete conclusions regarding long-term responses (Kreyling et al., 2014). Further investigations are, therefore, needed to accurately assess potential carry-over effects of different drought timing.

Moreover, differences in soil moisture and temporal thresholds in response to drought confirm that timing is an additional aspect which should be factored in when studies aimed at identifying ecological thresholds in response to climate extremes are set up. The phenological state characterising the ecosystem when drought occurs represents the "organism's identity" and, together with the nature of drought stress, can modify the threshold (Lichtenthaler, 1996). Drought timing should, therefore, be included in gradient-based experiments aimed at identifying non-linear relationships between ecological variables and water resources (Beier et al., 2012; Kreyling et al., 2014; Estiarte et al., 2016; Knapp et al., 2017b).

In conclusion, mesocosm experiments are useful in pinpointing drought response mechanisms in controlled conditions, but they are unable to capture the complexity of the ecosystem under natural conditions (Kreyling et al., 2014), producing, for instance, unrealistic biotic interactions and belowground soil moisture dynamics, or a reduced rooting-depth capacity. To achieve a comprehensive understanding of alpine ecosystem responses to water shortage, therefore, a complementary field-based study would be needed to upscale the lab-based findings (Kreyling et al., 2014).

6.7 CONTRIBUTIONS

Alessandro Petraglia, Michele Carbognani and T'ai G. W. Forte initiated the project, designed and supervised the experiment. T'ai G. W. Forte, AP and MC planned the research and led the field sampling. Giorgio Chiari and Francesca Arici also participated in the experimental set-up, data collection and lab work. TGWF and MC performed the statistical analyses. TGWF wrote the manuscript, which was subsequently critically revised by AP and MC.

CHAPTER 7. STUDY IV – PLANT GROWTH AND CO_2 FLUXES UNDER DIFFERENT DROUGHT TIMING AND DURATION: ALPINE GRASSLAND RESPONSES FROM A FIELD MANIPULATION EXPERIMENT

7.1 ABSTRACT

Timing and duration of drought events represent key aspects which can determine the degree to which ecosystem functions are affected by dry spells. Responses of plant growth and CO_2 exchange in a typical alpine mesic grassland to different drought timing and duration were assessed in a controlled, mesocosm study. However, the effect on these processes in the field still had to be assessed.

To fill this knowledge gap, a complementary field-study was carried out on a *Carex curvula* grassland during the 2020 growing season (June-August). This consisted of a manipulation experiment to assess effects of a 1-month long drought during the early- (ED) and mid-growing season (MD), and of a 2-month long dry event (FD) on aboveground net primary production (ANPP) and CO_2 fluxes – i.e. ecosystem respiration (ER), gross ecosystem production (GEP) and net ecosystem exchange (NEE). To detect potential drought effects at the species level, morphometric and chlorophyll fluorescence measurements for the major grassland species were also recorded. Resistance and recovery of GEP and ER within the two drought periods were also evaluated.

Results revealed that drought duration, rather than timing, negatively affected CO_2 fluxes and the photosynthetic efficiency of *Carex curvula*, whereas ANPP and plant growth were relatively stable under all drought treatments. Only ER was significantly affected under both drought timings; a further analysis on drought resistance, however, revealed no difference in GEP and ER sensitivity to drought and an equivalent sensitivity of both processes to different timings and durations. All CO_2 fluxes showed complete and equivalent recovery values across treatments.

Overall, findings revealed that the alpine grassland is particularly resistant to pulse drought events in the short-term in the field, with key ecosystem functions, such as ANPP and net ecosystem exchange, being particularly stable in the face of dry spells. Data further highlighted the high soil moisture levels characterising the alpine ecosystem, which did not reach drought stress thresholds identified for ER and GEP in the mesocosm experiment. Results confirm that field experiments are necessary to assess to what extent mechanisms identified in controlled studies actually occur under more complex, natural conditions.

7.2 INTRODUCTION

The framework of the current research is illustrated in the "Introduction" of Study III (see Chapter 6, Section 6.2). This study shares the same main objectives as the mesocosm experiment presented in the previous chapter – i.e. to understand how extreme drought affects plant growth and CO_2 exchange (ER, GEP and NEE) in a primary alpine grassland and evaluate the degree to which such processes are sensitive to an approx. 1-month dry spell occurring in two different periods of the growing season (early and mid-season drought, ED and MD). A further full drought treatment of approx. 2 months (FD) was also implemented within the study to assess whether the length of drought was a determining factor in affecting ecosystem processes.

The experiment was carried out in the field to evaluate ecosystem responses under natural environmental conditions replicating the same experimental lab set-up (see Chapter 6, Section 6.3.1). In contrast to the mesocosm experiment, in the field certain adaptations were incorporated into the experimental procedure: ANPP was estimated indirectly using the non-destructive point-intercept method at the peak of the growing season; morphometric measurements were also taken on the 6 most abundant species to gain deeper insight into drought effects on plant growth; measurements of chlorophyll fluorescence – an eco-physiological indicator of drought stress in plants (Lichtenthaler, 1996; Maxwell and Johnson, 2000) – were also obtained at the end of each drought period for the two most abundant species (*Carex curvula* and *Alchemilla pentaphyllea*); and, finally, a further set of CO_2 flux measurements were gathered after drought treatments had ended to assess recovery after drought – i.e. the ability to "return to the undisturbed ecosystem state and functioning following a disturbance" (Gessler et al., 2020).

The study took place during the summer of 2020 (June-August). The effects of treatments on microclimate, including precipitation exclusion, air and soil temperature, and soil moisture, were recorded. The data was then analysed in order to: (a) assess ANPP, species performance and CO_2 exchange over the entire study period in response to different drought timing and duration; (b) investigate CO_2 flux responses during each of the two drought periods so as to better understand mechanisms driving seasonal patterns; and (c) determine whether ER resistance differed from that of GEP within the two drought periods – i.e. resistance as the capacity to "persist and maintain [ecosystem] functioning during a disturbance" (Gessler et al., 2020) – and, in this context, to verify whether ER and GEP exhibited different resistance and recovery to early- and mid-season drought.

Based on outcomes from the mesocosm experiment and, therefore, assuming that drought would impact the investigated response variables, the following hypothesis were formulated: (a)

when taking into consideration the whole study period, the longest drought treatment (FD) would produce the greatest effects on plant growth and CO_2 fluxes, and ED would have a more negative impact than MD; (b) when taking into consideration each separate drought period, MD would have a greater impact on CO_2 fluxes than ED; and (c) GEP would be less resistant than ER to drought and both processes would be generally less resistant to FD and MD. No lab-based hypothesis could be formulated regarding recovery since these measures had not been carried out in the mesocosm, but a prediction could be made that ER would exhibit higher recovery values than GEP (Borken and Matzner, 2009) and that FD and MD treatments would exhibit a lower recovery only in the case of soil moisture thresholds being reached.

7.3 MATERIALS AND METHODS

7.3.1 Study site

A detailed description of the study site is provided in Chapter 2.

7.3.2 Experiment design

The drought timing experiment was set-up in June 2020, following a randomized block design which, along with controls, included three drought treatments (**Fig. 7.1**), as described below:

- Early-season drought (ED): total rainfall exclusion imposed during the first part of the growing season. Shelters were placed above the assigned plots 17 days after snowmelt (20 June) and removed after 28 days (18 July).

- Mid-season drought (MD): total rainfall exclusion imposed during the second part of the growing season. Shelters were placed above the assigned plots 45 days after snowmelt (18 July) and removed after 29 days (16 August).

- Full-season drought (FD): total rainfall exclusion imposed for 57 days, a period equal to the sum of ED and MD. Shelters were removed on the same end date of MD (16 August).

Drought periods corresponding to ED and MD were calculated in order to simulate an approx. 40% reduction of summer precipitation over the growing season (see Chapter 3, Section 3.1) which was considered as the period between the snowmelt date and the peak of productivity (mid-August) – i.e. the most biologically meaningful time for plant growth (Winkler et al., 2016 and references therein).

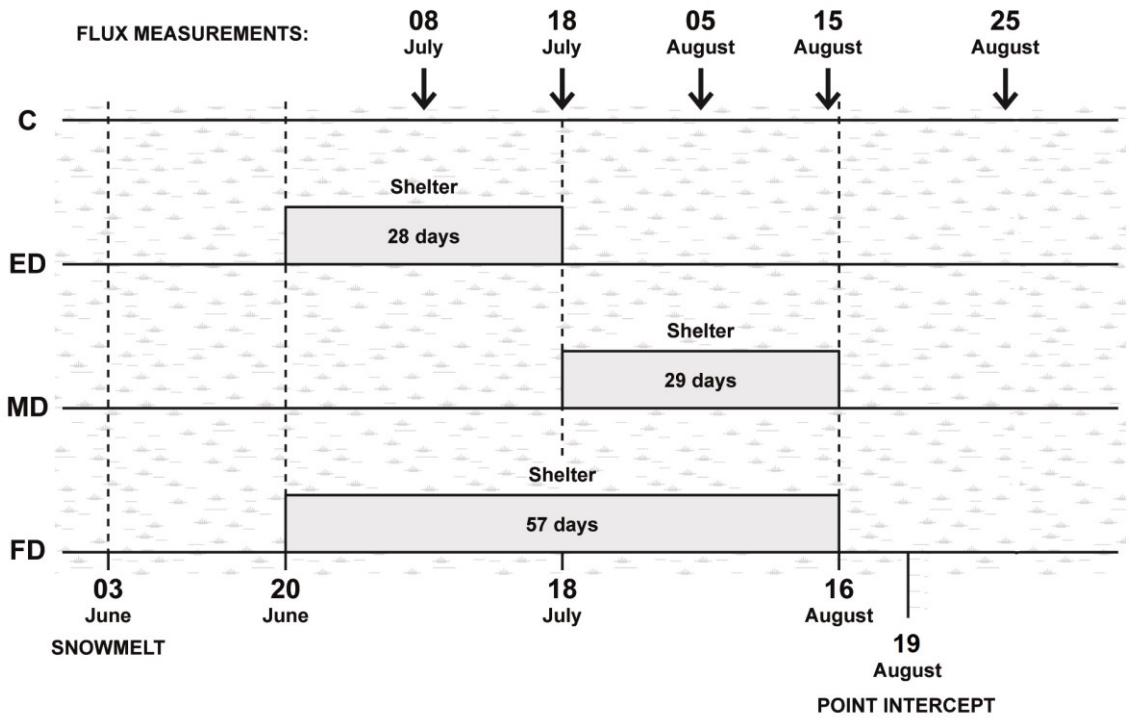


Fig. 7.1: Scheme illustrating the experiment design for drought timing and duration carried out in a *Carex curvula* alpine grassland during summer 2020. The 4 treatments are reported (C – control, no manipulations; ED – early-season drought; MD – mid-season drought; FD – full-season drought) together with the length of each drought period; the CO_2 flux measurement dates are specified at the top, while the simulated snowmelt date together with the set-up and end-of-treatment dates, and the date in which ANPP was estimated through the point intercept method, are indicated at the bottom.

Treatments were imposed on a 1.20 × 1.80 m^2 area, over which fixed, total rain-out shelters were placed (**Fig. 7.2a**). Sample areas, where measurements were taken, were located in the centre of the plots, leaving a buffer zone of approx. 20 cm from the roof edge in order to avoid margin effects. Each shelter was constructed using aluminium frames, sustaining 3 mm thick, transparent sheets of poly-methyl methacrylate (PMMA); roofs were placed at approx. 40 cm above the canopy. In each drought treatment, belowground incoming water flow was minimized by digging a trench, 20 cm deep and 10 cm wide, around the whole plot, with a 20 cm buffer zone between the trench and the area underneath the shelter (**Fig. 7.2b**). Water-flow isolation was further insured by lining each trench with plastic before filling it with the material previously excavated to ensure trench stability. Finally, each trench was covered with a layer of white bubble wrap to prevent over-heating (**Fig. 7.2a**). To avoid bias in results due to root damage, cuts were made in the soil around the control plots.

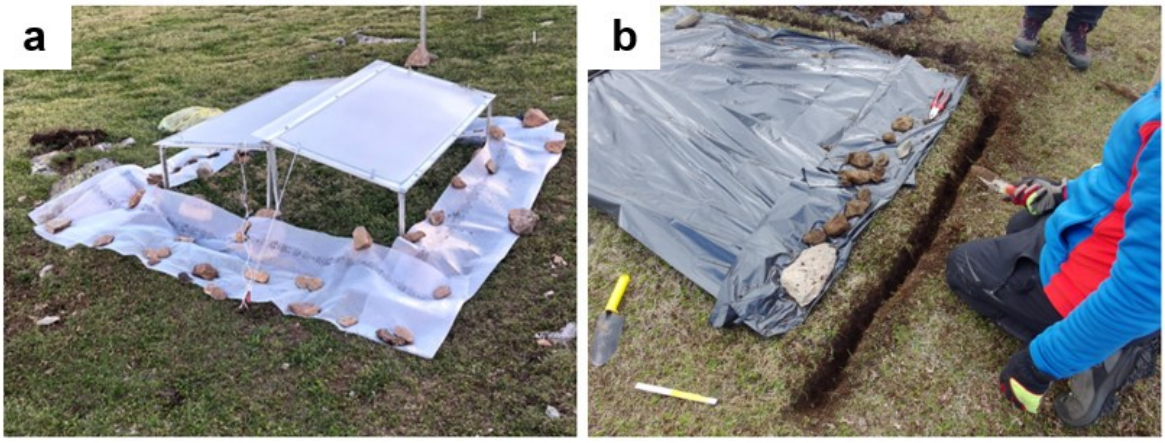


Fig. 7.2: (a) Full-exclusion rainout shelters employed to simulate extreme drought within the study site (photo by Giorgio Chiari); **(b)** trench-digging surrounding the area under the shelter to prevent lateral belowground water flow.

7.3.3 Microclimate

Soil moisture was monitored by measuring both soil water potential (WP, kPa) and volumetric water content (VWC, %). WP was measured hourly at -5 cm (Teros21, Decagon Devices, Inc.) for 2 replicates per treatment. VWC measurements were made using a portable instrument (TDR 100, Spectrum Technologies Inc., Chicago, IL, USA) at the 0-3.8 cm and 0-7.6 cm soil layers located near the subplots where CO_2 fluxes were recorded. Measurements took place on 4 different dates: 18 July, end of ED; 5 and 16 August, middle and end of MD, respectively; and 26 August, 10 days after the end of MD and FD. On each sampling date, four records per soil layer in each plot were gathered, and then averaged to obtain the mean VWC for each plot. In addition, soil temperature was measured at -5 cm in 2 replicates per treatment (Teros21, Decagon Devices, Inc.), while air temperature and relative humidity were measured at approx. 5 cm above the canopy in one block of treatments (ATMOS 14 Decagon Devices, Inc.). Within the site, ambient measurements of photosynthetic active photon flux density (PPFD, µmol m^{-2} s^{-1}) were gathered (HOBO Micro Station Data Logger with S-LIA-M003 Photosynthetic Light (PAR) Smart Sensor), while ambient precipitation records were collected from a snowbed site located approx. 1 km from the target grassland (Rain Collector, Decagon Devices, Inc.). The mean value for each microclimate variable was combined with CO_2 flux measurements based on when records were taken – i.e. the precise hour and minute each incubation started and ended.

7.3.4 Vegetation parameters: ANPP, traits and chlorophyll fluorescence

Aboveground phytomass (hereafter aboveground net primary production – ANPP, g m^{-2}) was indirectly estimated using the point intercept method (Jonasson et al., 1988) once during the peak of the growing season (19 August). The first step consisted in placing a frame with a 5 cm-square grid on top of the 900 cm^2 permanent subplot where flux measurements were taken. The number

of contacts for each vascular species in each of the 36 pins constituting the sampled area was then recorded. Subsequently, plant biomass present in each plot was indirectly estimated using a mean biomass value calculated for each species, which had been previously obtained by calibrating, for each species, the number of contacts with the biomass value measured on N samples of 20 cm × 20 cm harvested in 2019 (see Chapter 4, Section 4.2.3 for details on calibration method).

To gain a major insight into plant performance in response to drought, in C and FD plots both vegetative and reproductive height, together with leaf length, were measured at the end of the experiment for the 6 most abundant species within the grassland: vegetative and reproductive height were measured from the base to the tallest point, without extending the plant, while leaf length was recorded for the largest leaf (including the petiole); the length of reproductive structures (hereafter reproductive length) was also measured for *Alchemilla pentaphyllea* due to its creeping inflorescence. For each trait, individuals were selected randomly from each plot, with a maximum of 10 measured for each species. The list of species and number of individuals measured for each trait and treatment are reported in **Table 7.1**.

Table 7.1: Number of individuals measured for each species and each plant trait in control (C) and full-season drought (FD) treatments. Species names are standardised following Pignatti (1982) followed by the relative ID code used as a reference for each species.

Species	ID	Height				Leaf length		Reproductive length	
		Vegetative		Reproductive					
		C	FD	C	FD	C	FD	C	FD
Alchemilla pentaphyllea L.	AP	50	50	39	31	50	50	39	31
Carex curvula All.	CC	50	50	32	28	50	50	NA	NA
Leontodon helveticus Mérat	LH	32	33	23	14	32	33	NA	NA
Ligusticum mutellina (L.) Crantz	LM	45	50	16	19	45	50	NA	NA
Phyteuma hemisphaericum L.	PH	39	38	25	22	39	38	NA	NA
Poa alpina L.	PA	45	30	20	12	45	30	NA	NA

Furthermore, as an indicator of drought stress, measurements of chlorophyll fluorescence were taken for the two dominant species, *Carex curvula* and *Alchemilla pentaphyllea*, using a Handy PEA chlorophyll fluorimeter (Hansatech Instruments ltd). The F_v/F_m ratio was recorded – i.e. the maximum quantum efficiency of photosystem II – where F_v is variable fluorescence from dark- adapted leaves and F_m is the maximum fluorescence (Maxwell and Johnson, 2000).

Measurements were made on two dates, corresponding to the end of the two drought periods: 18 July, end of ED; and 16 August, end of MD and FD. Three individuals from each species were selected within each plot and measured on both dates. At the end of the first period, data was collected across all treatments (4 replicates) while, during the second period, measurements

were carried out only in C and FD (5 replicates) due to time constraints. Prior to measurements, *A. pentaphyllea* and *C. curvula* leaves were left to dark adapt in the cuvette for 20 and 30 minutes, respectively.

7.3.7 CO_2 fluxes

Ecosystem CO_2 fluxes were measured during the central hours of the day (10:30 – 16:00) on 5 dates under clear-sky conditions (**Fig. 7.1**). A PP System EGM-4 infrared gas analyzer (IRGA) was employed, which was connected to a transparent, cubic 900 cm^3 chamber made of 3 mm PMMA placed over a permanent subplot for each treatment and replicate (**Fig. 7.3a**). At the beginning of the growing season, a 2 cm deep cut was made along the perimeter of the subplot to ensure a flat base between the soil surface and the flux chamber. Records of CO_2 concentrations were set every 5 s for 60 s. To prevent temperature increase inside the chamber during measurements, it was equipped with a cooling system consisting of both an active (Peltier cell, **Fig. 7.3a**) and passive mechanism (ice packs) together with a fan to ensure homogeneous air mixing. Airtight sealing was obtained using memory foam material placed inside and on top of the subplot perimeter cut. Measures of ecosystem respiration (ER) and net ecosystem exchange (NEE) were gathered for each subplot, with ER dark measurements always preceding NEE in light conditions, in order to prevent air heating inside the chamber. Dark measurements were obtained by placing an opaque thermal silver sheet over the chamber (**Fig. 7.3b**).

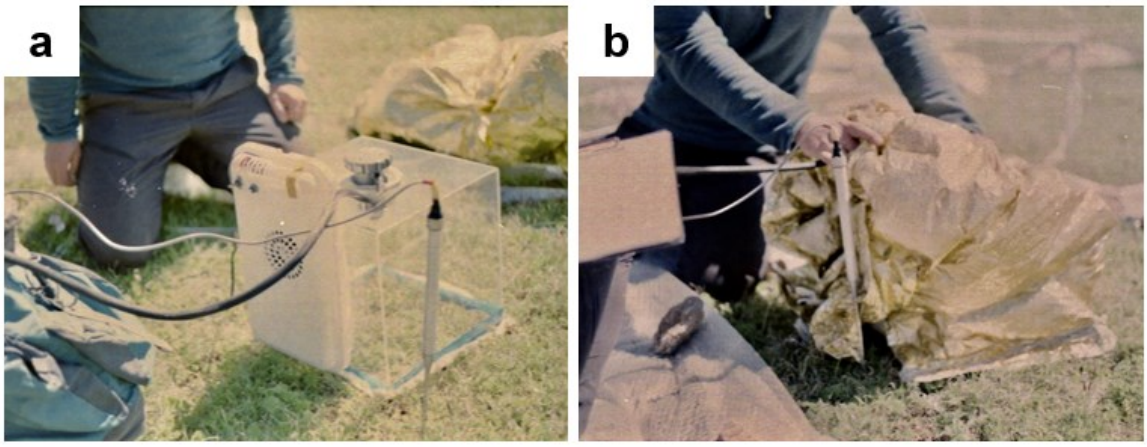


Fig. 7.3: (a) Transparent cubic chamber employed to measure ecosystem CO_2 fluxes, with the active cooling system (Peltier cell) visible in the foreground; **(b)** opaque thermal silver sheet placed on the chamber for measurements of ecosystem respiration (photos by Francesca Arici).

Using the ideal gas law, the rate of CO_2 change inside the chamber was employed to estimate the flux of CO_2 (g m^{-2} h^{-1}). Direct estimates of ER and NEE were used to calculate gross ecosystem production (GEP), computed by subtracting the instantaneous plant, microbial and soil fauna

respiration from the total net ecosystem exchange (GEP= NEE - ER). A linear regression model was fitted to each data to obtain ER, NEE and GEP estimates for each plot, with mean R^2 values equal to 98.5 (ER), 99.5 (GEP) and 97.7 (NEE). Since NEE represents CO_2 fluxes going from the ecosystem to the atmosphere, in the present study negative NEE (and GEP) values indicate a net ecosystem uptake of CO_2, with positive values indicating a net CO_2 release (Chapin et al., 2006).

7.3.8 Resistance and recovery

The sensitivity of ER and GEP to diverse types of drought imposed in the study was used as an estimate of resistance and recovery, following Hao et al. (2018):

$$Sensitivity\ (\%) = \frac{(\overline{Flux}_{dr} - \overline{Flux}_{ctr})}{\overline{Flux}_{ctr}} * 100$$

where $Flux_{dr}$ corresponds to the average CO_2 rate, over time, for each replicate in drought plots and $Flux_{ctr}$ indicates the average rate in controls. Resistance was obtained by considering CO_2 fluxes during drought – 2 dates for ED and MD, 4 dates for FD – while recovery was estimated using measurements from the first date following the end of drought – 18 days after the end of drought for ED (5 August) and 9 days after the end of drought for MD and FD (25 August). The lower the sensitivity values, which represent changes in CO_2 fluxes of drought plots compared to controls, the lower were the resistance and recovery.

7.3.9 Data analysis

Statistical analyses were carried out in R (R Core Team, 2020). Linear mixed effects models (LMMs) followed the procedure described in Chapter 6 (Section 6.3.6) and the same R packages were used for analysis and graphical outputs.

Analysis on microclimate data was performed exclusively on VWC data to test for: treatment (4-level categorical variable: C, ED, MD and FD); time (4-level categorical variable: 4 measurement dates); their two-way interaction; and depth (2-level categorical variable: 0-3.8 cm and 0-7.6 cm). Plot was set as a random effect.

The difference in ANPP between treatments was also tested, with block as a random effect. Regarding vegetative and reproductive traits, the effect of treatment (2-level categorical variable: C and FD) on each trait was assessed separately for each species. Plot was set as a random effect, while variance was homogenised across treatments for the following response variables and species (ID in **Table 7.1**): vegetative height of CC, LH and LM; reproductive height for AP, CC, LH and LM; and leaf length for AP and CC. Where necessary, response variables were square-root

transformed for analysis – i.e. vegetative height of PH, reproductive height of LM and leaf length of LH.

Chlorophyll fluorescence (F_v/F_m) response to drought was also assessed separately for each species (AP and CC) and for each drought period (early- and mid-season). Analysis on the responses during early-season drought were carried out by coding the four treatments into a 2-level categorical variable by grouping together plots sharing the same treatment (ambient: C and MD, no shelters; drought: ED and FD, with shelters). For the early-season period, block and plot were set as a random effect for AP and CC, respectively, while treatment variance was homogenised for both species. For analysis of mid-season responses, plot and block were set as a random effect for AP and CC, respectively, with no variance structure included in LMMs for either species.

With regard to CO_2 fluxes, LMMs were carried out separately for each flux component (ER, GEP and NEE) and the total vascular plants biomass of each plot was included as a covariate in the fixed effects to account for spatial variability between plots. The natural temporal variation in biomass values during the growing season was accounted for by correcting peak biomass values estimated from point intercepts for each CO_2 flux measurement date. In detail, corrections were made based on species-specific growth dynamics using data gathered during the 2018 growing season. To achieve this ≥30 samples were collected for each of the 5 most abundant species and their morphometric characteristics and biomass assessed (**Table 7.2**). Then, for each species, the relationship between biomass and morphometric characteristics was determined by means of linear models (**Table 7.2**). The biomass models obtained were then used to predict biomass dynamics throughout the growing season, based on non-destructive morphometric measurements taken from 25 individuals for each species in control plots in 2018 (data not shown). The temporal species-specific growth dynamics were then used to correct peak biomass data on each CO_2 flux measurement date by assigning the corresponding percentage values to the biomass of each species in each plot (**Table 7.3**); the biomass of those species for which the biomass model was not available was corrected using the average percentage of biomass obtained from the 5 species sampled (**Table 7.3**).

Table 7.2: Linear models for the relationship between aboveground biomass (mg) and morphometric measures (Xs) for the 5 most abundant grassland species. For each species, the type of transformation applied to shoot biomass is indicated (Y) and the description of predictors (Xs) is reported at the bottom of the table. The following parameters are specified: sample size (N), regression coefficients (Coeff.), standard error (SE), p-value and the % of adjusted R^2 (Adj. R^2).

Species	N	Y	Xs	Coeff.	SE	p-value	Adj. R^2
Alchemilla pentaphyllea L.	72	sqrt(biomass)	intercept	0.207	0.399	0.605	82.98
			no. leaves	0.566	0.111	<0.001	
			leaf length	0.131	0.013	<0.001	
Carex curvula All.	59	sqrt(biomass)	intercept	0.207	0.399	0.700	91.68
			no. tillers	0.551	0.030	<0.001	
			leaf length	0.083	0.007	<0.001	
Leontodon helveticus Mérat	54	sqrt(biomass)	intercept	-0.090	0.503	0.859	79.94
			no. leaves	0.485	0.079	<0.001	
			leaf length	0.079	0.008	<0.001	
Phyteuma hemisphaericum L.	72	sqrt(biomass)	intercept	0.528	0.178	0.004	87.36
			no. leaves	0.221	0.020	<0.001	
			leaf length	0.045	0.004	<0.001	
Soldanella pusilla Baumg.	30	log(biomass)	intercept	0.079	0.235	0.741	81.57
			no. leaves	0.178	0.022	<0.001	
			leaf width	0.182	0.025	<0.001	

no. tillers – total number of tillers constituting the sampled tufts of *Carex curvula*

no. leaves – total number of leaves

leaf length – leaf length of the largest leaf, including the petiole (mm)

leaf width – leaf width of the largest leaf (mm)

Table 7.3: Percentage of biomass estimated for each species on each CO_2 flux measurement date (Date), used to correct biomass indirectly estimated at the peak of the growing season. The days of the growing season from snowmelt are reported (DOGS). Codes refer to *Alchemilla pentaphyllea* (AP), *Carex curvula* (CC), *Leontodon helveticus* (LH), *Phyteuma hemisphaericum* (PH), *Soldanella pusilla* (SP) and other vascular species (Vasc), corresponding with the average biomass % of the 5 species used to correct biomass for those species for which the biomass model was not available.

		Biomass (%)					
Date	DOGS	AP	CC	LH	PH	SP	Vasc
8 July	35	79	86	72	77	71	77
18 July	45	91	100	86	85	81	88
05 August	63	100	100	100	95	94	98
15 August	73	100	100	100	100	100	100
25 August	83	100	100	100	100	100	100

All flux measurements were used to analyse responses over the entire growing season, with treatment set as a 4-level categorical variable; plot and day were set as crossed random effects while ANPP variance was homogenised. With regard to timing, a separate analysis was performed for both the early- and mid-season experimental period to account for the fact that treatments were not constant over time. In detail, to assess the effects of drought during the early period, the four treatments characterising the experimental design were coded as a 2-level categorical variable (see above for analysis on chlorophyll fluorescence). Analysis of fluxes during the second period were, instead, performed considering treatment as a 4-level categorical variable (C, ED, MD and FD). The measurement date was further inserted as an independent variable (2-level categorical variable for each period) and its two-way interaction with treatment was analysed. Plot was set as a random effect in all LMMs.

Finally, a one sample t-test was performed on measures of resistance and recovery for ER and GEP, while ANOVA was used to evaluate differences between the three different drought treatments within the same process and also between processes within the same drought treatment.

7.4 RESULTS

7.4.1 Microclimate

During the entire study period, cumulative rainfall amount in ambient conditions equalled 337 mm (**Fig. 7.4**). This was differently distributed between the two experimental periods, with the first period being drier than the second (102 mm and 236 mm, respectively), resulting in a diverse precipitation reduction for the two drought-timing treatments: ED and MD excluded 30% and 70%, respectively, of the total cumulative rainfall during the study period (**Fig. 7.4**). When all shelters were removed, plots received 17 mm of natural rainfall during the 9 days following the end of the experiment and prior to the last CO_2 flux measurements (day 66).

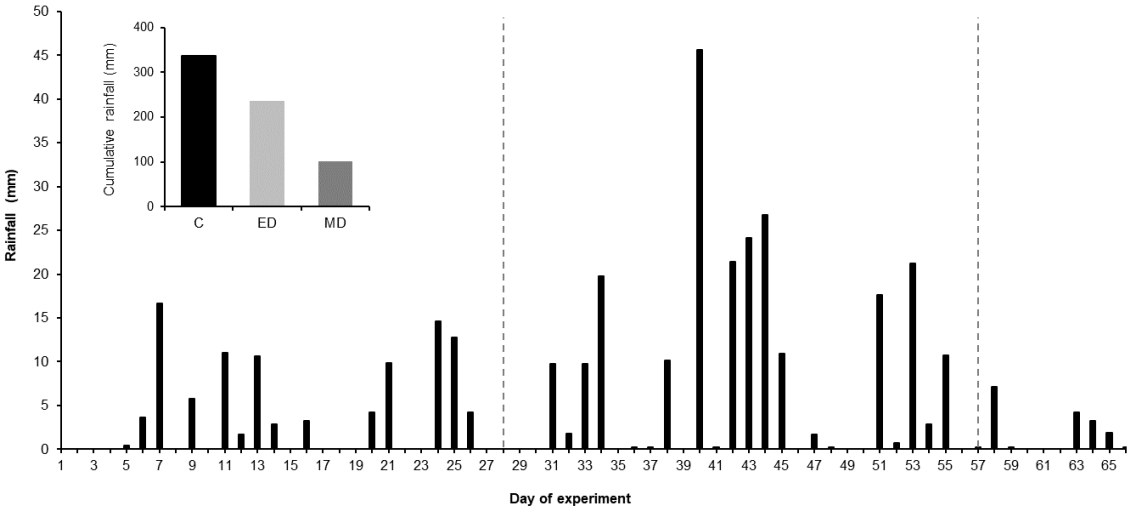


Fig. 7.4: Rainfall distribution (mm) during the study period when CO_2 flux measurements were taken. Time is reported as the number of days from the beginning of the experiment (21 June, day 1). Dashed lines indicate: end of ED (early-season drought) and beginning of MD (mid-season drought) (18 July, day 28); end of MD and FD (full-season drought) (16 August, day 57). The internal panel illustrates the cumulative rainfall (mm) received within each treatment during the experimental period (FD = 0).

With regard to air temperature (**Fig. 7.5a**), values were similar across treatments in both drought periods, with 1.6 K higher temperature during the mid-season period (8.67 ± standard error (SE) 0.09 °C; n=4) compared to the early period (10.29 ± 0.06 °C; n=4). On the other hand, mean daily soil temperatures at -5 cm were approx. 1 K higher under shelters compared to controls in both periods and, in the case of C plots, the difference in soil temperature between the mid- and early-season period was 1.1 K. Two days after the experiment ended, when shelters had been removed, all plots exhibited similar daily average soil temperatures, with 11.95 ± 0.06 °C (n=4) during the following 10 days.

Water potential at -5 cm (**Fig. 7.5b**) exhibited the highest values in control plots, with a daily maximum of -9.5 kPa being recorded. With regard to treatments, WP decreased in ED plots during the early season, reaching a minimum value of -18.1 kPa, but returned to near-control values after the first end-of-treatment rainfall event. Similarly, WP in MD plots declined during the mid-season period as a result of shelter rainfall exclusion, reaching a minimum value of -15.6 kPa and registering lower soil moisture values than other treatments even after shelter removal as a result of the end-of-experiment dry period. Values in FD were never the lowest and, in general, soil moisture fluctuations were evident in all sheltered plots during the entire experimental period.

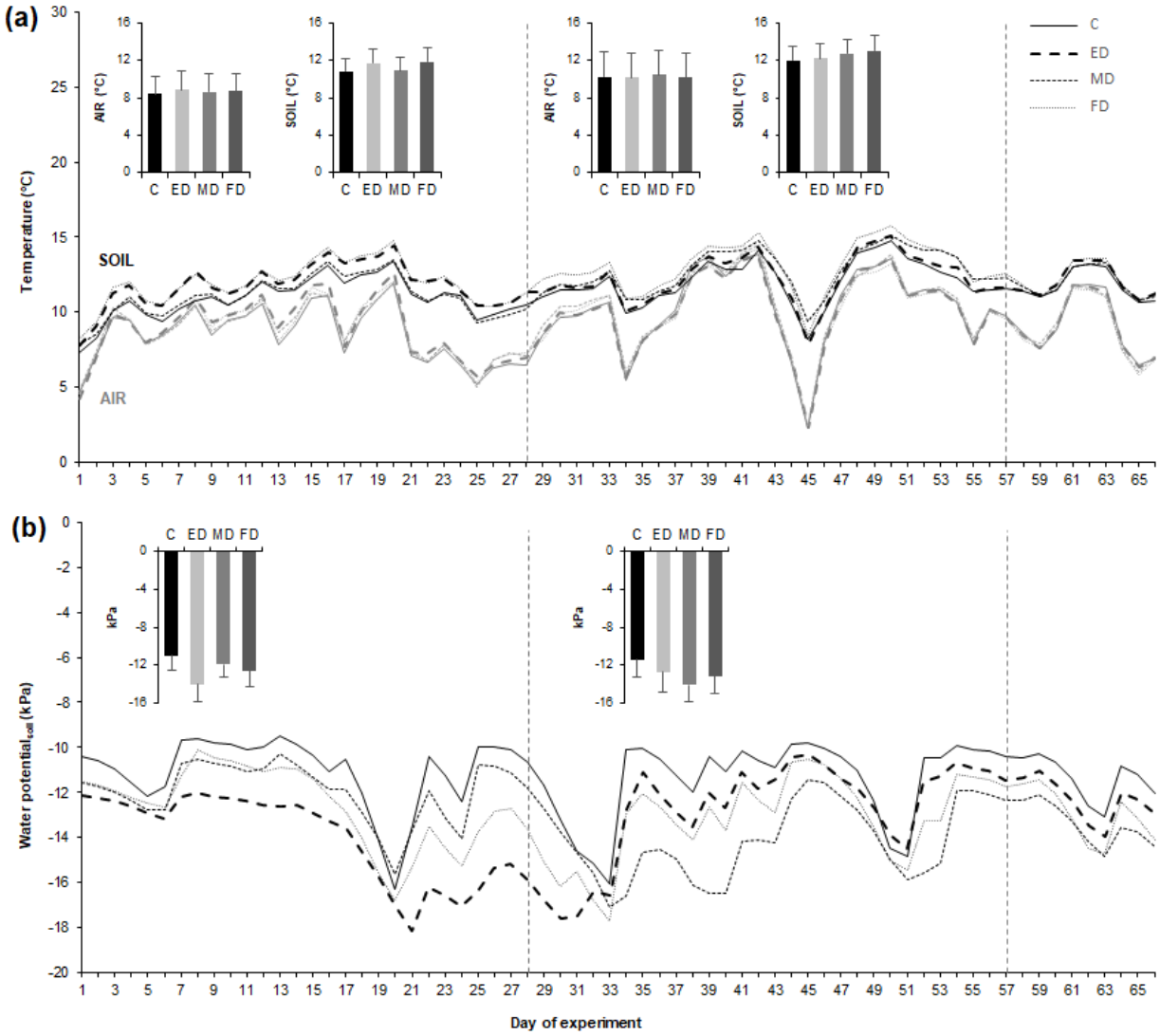


Fig. 7.5: Daily microclimate data measured within treatments [C – control, ED – early-season drought; MD – mid-season drought; FD – full-season drought] during the study period when CO_2 flux measurements were taken: **(a)** air temperature (°C at 5 cm, grey lines) and soil temperatures (°C at -5 cm, black lines); **(b)** soil water potential (kPa at -5 cm, black lines). Time is reported as the number of days from the beginning of the experiment (2 June, day 1). Dashed vertical lines indicate: end of ED and beginning of MD (18 July, day 28); end of MD and FD (16 August, day 57). Internal panels illustrate average daily values measured within each treatment during the first and second experimental period.

When considering VWC measured at two depths (0-3.8 cm and 0-7.6 cm) on 4 dates during the study period, soil moisture values were significantly different between dates ($F_{3,107}$=96.323, p<0.001), soil layers ($F_{1,107}$=46.986, p<0.001) and treatments ($F_{3,16}$=10.065, p=0.001), with VWC in drought plots differing over time depending on the period when rainfall-exclusion was imposed ($F_{9,107}$=12.849, p<0.001) (**Fig. 7.6c**).

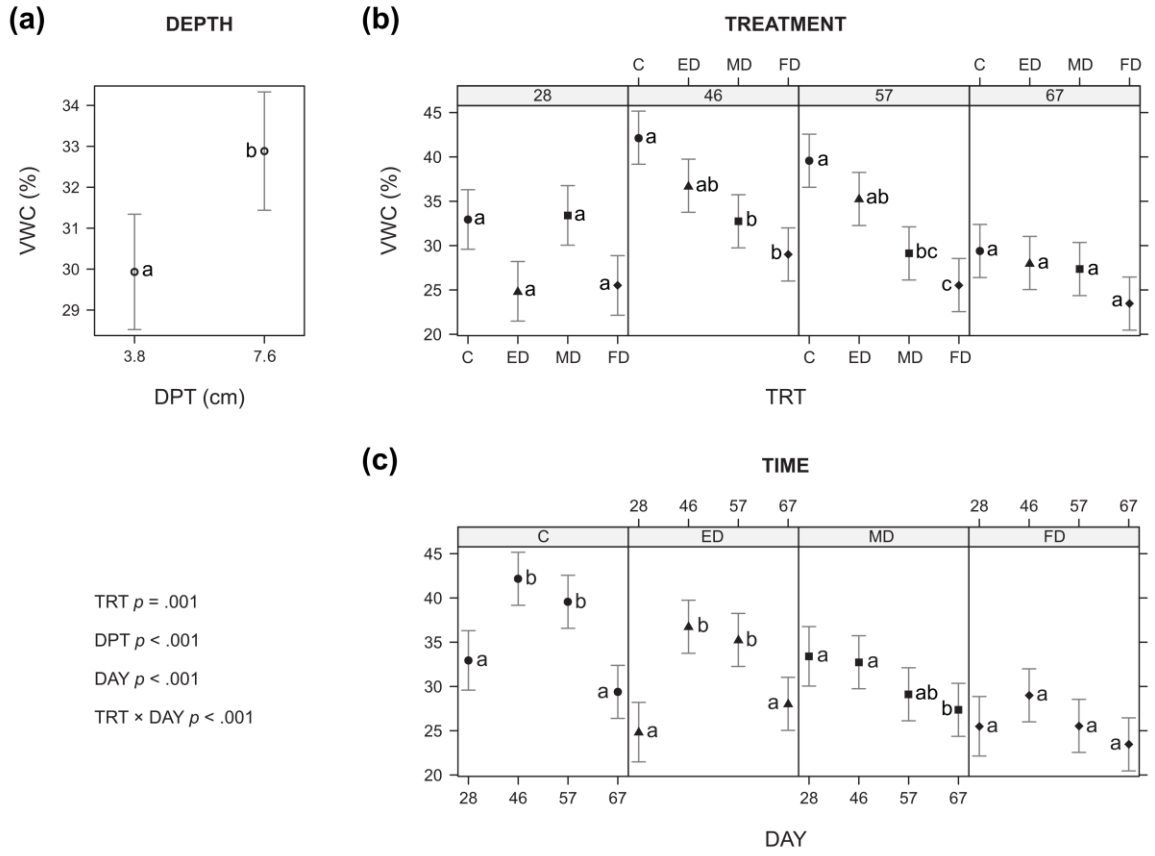


Fig. 7.6: Volumetric water content (VWC, %) measured at 0-3.8 cm and 0-7.6 cm on 4 dates during the study period. LMM output (estimated mean and 95% confidence interval) for **(a)** depth DPT [0-3.8 cm and 0-7.6 cm]; **(b)** treatments TRT [C – control, ED – early-season drought; MD – mid-season drought; FD – full-season drought] on each measurement date; and **(c)** measurement dates (DAY) for each treatment. In particular, time is reported as a categorical variable indicating the number of days from the beginning of manipulations (21 June, day 1): 28 – end of ED (18 July); 46 – middle of MD (5 August); 57 – one day before end of MD and FD (16 August); and 67 – recovery period (26 August). Different letters indicate significant differences (post-hoc Tukey contrast, p<0.05).

Comparison between depths showed that the top layer was drier than the deeper one (**Fig. 7.6a**). With regard to treatments, rainfall exclusion reduced soil moisture depending on the period when manipulations were applied (**Fig. 7.6b**). At the end of the first treatment period (day 28), ED and FD showed a tendency towards lower VWC compared to MD and C, although differences were not significant. Eighteen days after the beginning of the mid-season drought period (day 46), MD and FD exhibited significantly lower VWC compared to C, a difference which was also evident at the end of the experimental period (day 57), when VWC was 10% and 14% lower in MD and FD, respectively, compared to C. Nine days after the end of manipulations (day 67), there was no difference in VWC between treatments, as a result of the significant reduction in C and ED compared to the previous measurement date (**Fig. 7.6c**). In fact, on the last measurement date, VWC was the lowest recorded over the entire period as against the highest values which were recorded on the second measurement date (**Fig. 7.6c**).

7.4.2 Vegetation parameters: ANPP, traits and chlorophyll fluorescence

ANPP estimated at the end of the experimental period was highly variable across plots and did not significantly differ between treatments (p>0.05) (**Fig. 7.7**).

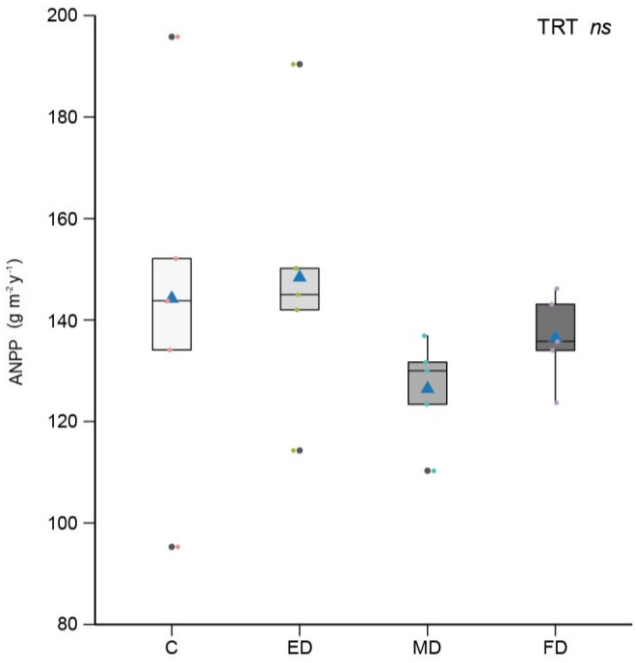


Fig. 7.7: ANPP (g m^{-2} y^{-1}) estimated for each treatment at the end of the experimental period [C – control, ED – early-season drought; MD – mid-season drought; FD – full-season drought]. Mean values are indicated (blue triangles).

Analysis of treatment effects on vegetative traits for each of the most common species revealed that a 2-month drought (FD) did not significantly affect either plant height or leaf length, although the latter exhibited a tendency towards lower values in all species (**Table 7.4**). With regard to reproductive traits, reproductive height was found to be significantly lower in FD compared to control for *Poa alpina*, while reproductive dimensions did not statistically differ between treatments in the rest of the species considered (**Table 7.4**).

Table 7.4: Mean ± standard error [n] of vegetative and reproductive traits measured on the 6 most abundant grassland species in treatments [C – control; FD – full-season drought] at the end of the experimental period (15 August 2020). Results of LMMs (F-value and p-value) for traits and species are reported, with significant values (p<0.05) indicated in bold.

	Treatment				F-value	p-value
	C		FD			
Vegetative height (mm)						
Alchemilla pentaphyllea	32.4 ± 1.5	[5]	29.3 ± 2.6	[5]	1.079	0.329
Carex curvula	85.7 ± 8.2	[5]	74.4 ± 4.6	[5]	1.490	0.257
Leontodon helveticus	31.4 ± 3.0	[5]	25.0 ± 2.4	[5]	2.048	0.190
Ligusticum mutellina	52.3 ± 3.4	[5]	48.3 ± 2.2	[5]	1.761	0.221
Phyteuma hemisphaericum	38.8 ± 2.3	[5]	40.3 ± 6.7	[5]	0.001	0.970
Poa alpina	36.9 ± 3.0	[5]	39.3 ± 5.7	[5]	0.004	0.949
Leaf length (mm)						
Alchemilla pentaphyllea	36.9 ± 1.6	[5]	33.9 ± 2.2	[5]	1.151	0.314
Carex curvula	105.7 ± 10.8	[5]	89.6 ± 4.4	[5]	1.784	0.218
Leontodon helveticus	43.1 ± 2.3	[5]	36.2 ± 2.9	[5]	4.897	0.058
Ligusticum mutellina	65.3 ± 4.0	[5]	60.0 ± 1.5	[5]	2.244	0.172
Phyteuma hemisphaericum	45.8 ± 1.8	[5]	45.2 ± 6.6	[5]	0.369	0.560
Poa alpina	43.4 ± 3.2	[5]	39.9 ± 5.4	[5]	2.715	0.138
Reproductive height (mm)						
Alchemilla pentaphyllea	26.5 ± 0.6	[5]	29.0 ± 2.1	[5]	2.339	0.165
Carex curvula	100.9 ± 12.0	[5]	74.5 ± 5.2	[5]	4.067	0.078
Leontodon helveticus	109.5 ± 8.0	[5]	128.0 ± 10.8	[5]	1.683	0.231
Ligusticum mutellina	153.5 ± 12.1	[4]	132.1 ± 7.8	[5]	2.126	0.188
Phyteuma hemisphaericum	77.6 ± 7.8	[4]	82.6 ± 5.6	[5]	0.251	0.632
Poa alpina	256.7 ± 10.3	[4]	146.3 ± 10.0	[3]	47.306	**0.001**
Reproductive length (mm)						
Alchemilla pentaphyllea	53.1 ± 2.4	[5]	49.1 ± 3.2	[5]	0.985	0.350

When checking for F_v/F_m in the two most abundant community species, values were not significantly different after 28 days of drought between plants under shelters and those in ambient conditions for either *Carex curvula* or *Alchemilla pentaphyllea*, whereas significantly lower F_v/F_m values were found for *C. curvula* in plots subjected to a 2-month drought (**Table 7.5**).

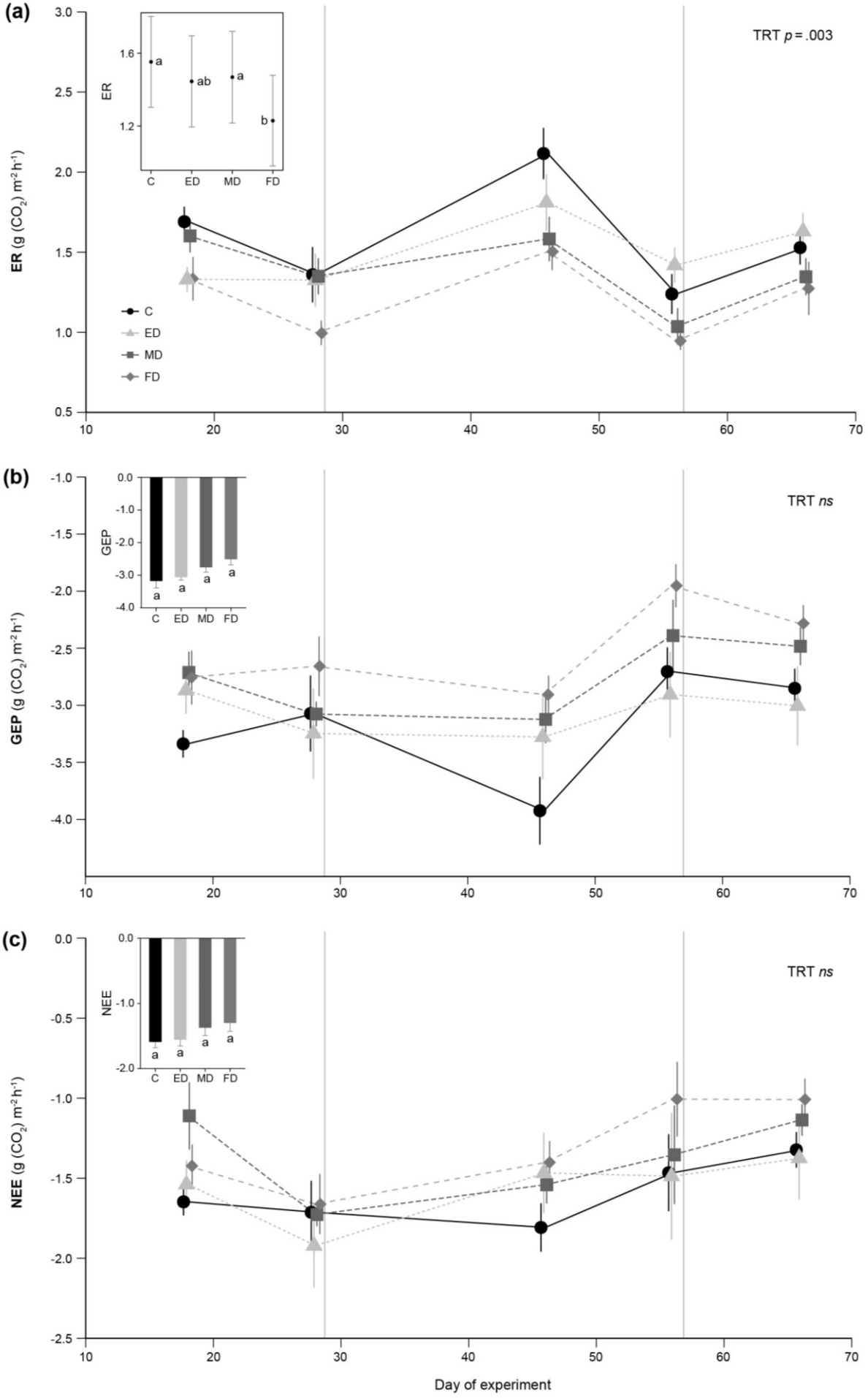
Table 7.5: Mean ± standard error [n] of F_v/F_m measured for the two most common grassland species in treatments at the end of the two drought periods: early season ("ambient", C and MD plots with no shelters; "drought", ED and FD with shelters) and mid season (C – control; FD – full-season drought). Results of LMMs (F-value and p-value) for traits and species are reported, with significant values (p<0.05) indicated in bold.

Early season						
	Ambient		**Drought**		**F-value**	**p-value**
Alchemilla pentaphyllea	0.82 ± 0.01	[8]	0.81 ± 0.01	[8]	0.557	0.460
Carex curvula	0.72 ± 0.01	[8]	0.69 ± 0.02	[8]	1.383	0.247
Mid season						
	C		**FD**		**F-value**	**p-value**
Alchemilla pentaphyllea	0.82 ± 0.01	[5]	0.78 ± 0.03	[5]	2.501	0.152
Carex curvula	0.78 ± 0.01	[5]	0.73 ± 0.01	[5]	15.459	**0.001**

7.4.3 CO_2 fluxes: seasonality

Analysis of CO_2 fluxes across the season revealed a significant effect of treatments on ER ($F_{3,95}$=4.871, p=0.003), while GEP and overall NEE were not significantly affected (p>0.05). In detail, a general reduction in ER was recorded in areas under shelters, with the post-hoc contrast revealing significantly lower values (-21%) in FD compared to C (**Fig. 7.8a**). In contrast, ED and MD, although showing a tendency towards reduced ER during the season compared to ambient conditions, did not significantly differ from C (p>0.05) (**Fig. 7.8a**). With regard to both GEP (**Fig. 7.8b**) and NEE (**Fig. 7.8c**), even in this case fluxes showed a tendency towards a reduction in MD and FD compared to C when the entire growing season was considered, but values were not significantly different from C (p>0.05). Finally, total vascular plant biomass significantly influenced both ER [$F_{1,95}$=20.738, p<0.001, with +7.05 mg (CO_2) m^{-2} h^{-1} per g of biomass] and GEP [$F_{1,98}$=8.716, p=0.004, with -12.38 mg (CO_2) m^{-2} h^{-1} per g of biomass], while resulting as non significant in explaining variance in NEE (p>0.05).

Fig. 7.8 (p. 185): (a) Ecosystem respiration (ER), **(b)** gross ecosystem production (GEP) and **(c)** net ecosystem exchange (NEE) measured in treatments TRT [C – control, ED – early-season drought; MD – mid-season drought; FD – full-season drought] during the 2020 growing season (June-August). Time is reported as the number of days from the beginning of manipulations (30 June, day 10). Vertical grey lines indicate: end of ED and beginning of MD (18 July, day 28); end of MD and FD (16 August, day 57). For ER the internal panel illustrates the LMM output (estimated means and 95% confidence interval), with different letters indicating significant differences (post-hoc Tukey contrast, p<0.05). Internal panels for GEP and NEE illustrate the average values and standard error (n=5) measured for each treatment.


(a)
TRT p = .003
ER (g (CO2) m-2 h-1)
ER
a
ab
a
b
C
ED
MD
FD
C
ED
MD
FD
(b)
TRT ns
GEP (g (CO2) m-2 h-1)
GEP
a
a
a
a
C
ED
MD
FD
(c)
TRT ns
NEE (g (CO2) m-2 h-1)
NEE
a
a
a
a
C
ED
MD
FD
Day of experiment

7.4.4 CO_2 fluxes: drought timing

Drought during the early season significantly reduced ER by 18% compared to fluxes recorded in ambient conditions (**Table 7.6, Fig. 7.9a, b**) whereas neither GEP (**Table 4**, **Fig. 7.9d**) nor NEE (**Table 7.6**, **Fig. 7.9e**) were affected. Ecosystem respiration significantly differed between the two measurement dates, with lower values (-19%) at the end of the early season (18 July) compared to those recorded in the middle (8 July) (**Table 7.6, Fig. 7.9c**); no significant interaction emerged between treatment and day (**Table 7.6**). On the other hand, total vascular plant biomass did not influence ER during the early season ($p>0.05$), but was correlated with GEP and NEE, with -13.52 and -13.40 mg (CO_2) m^{-2} h^{-1} per g of biomass, respectively (Table 7.6).

Table 7.6: Results of LMMs testing the effects of drought on ecosystem respiration (ER), gross ecosystem production (GEP) and net ecosystem exchange (NEE) during two different periods (early- and mid-season). Fixed effects: treatment (TRT), date of measurements (DAY) and total biomass of vascular plants (BM VASC). Only significant fixed effects and interactions retained in the final model are reported, with "ns" indicating non-significant terms ($p>0.05$) excluded during the model selection process. The two-way interaction TRT × DAY, tested in all LMMs, is not reported (non-significant). Plot was set as a random effect for all LMMs, while the variance structure included in the final model is specified.

CO_2 fluxes	Fixed effects	Variance	df (N,D)	F-value	p-value
Early season					
ER	TRT		1,18	10.401	0.005
	DAY	bm vasc	1,19	17.346	<0.001
	BM VASC		ns	ns	ns
GEP	TRT		ns	ns	ns
	DAY	-	ns	ns	ns
	BM VASC		1,19	12.967	0.002
NEE	TRT		ns	ns	ns
	DAY	-	ns	ns	ns
	BM VASC		1,19	30.323	<0.001
Mid season					
ER	TRT		1,16	5.122	0.011
	DAY	day	1,18	57.877	<0.001
	BM VASC		1,18	15.617	0.001
GEP	TRT		1,16	3.622	0.036
	DAY	bm vasc, day	1,18	36.607	<0.001
	BM VASC		1,18	10.684	0.004
NEE	TRT		ns	ns	ns
	DAY	day	ns	ns	ns
	BM VASC		1,19	5.916	0.025

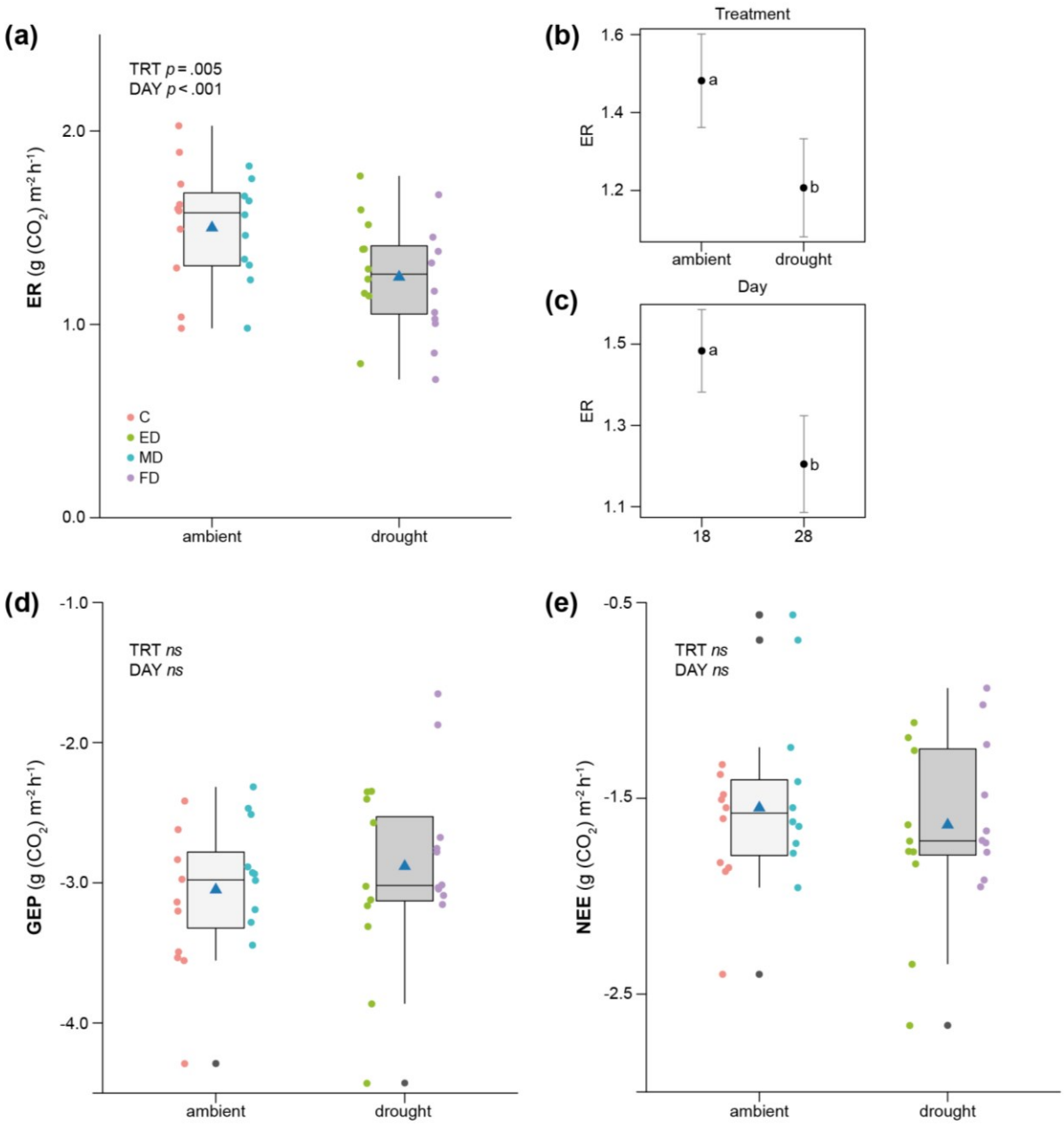


Fig. 7.9: (a) Ecosystem respiration (ER), **(d)** gross ecosystem production (GEP) and **(e)** net ecosystem exchange (NEE) measured during the early season in two treatments: "ambient" [C and MD, no shelters] and "drought" [ED and FD, with shelters]. Coloured dots refer to single values for each of the 4 treatments, while the blue triangle indicates the mean. For ER the LMM output (estimated means and 95% confidence interval) of significant factors, **(b)** treatment and **(c)** day, is reported.

Regarding the mid-season period, treatments significantly affected ER (**Table 7.6, Fig. 7.10a-c**) and GEP (**Table 7.6, Fig. 7.10d-f**), whereas there was no influence on NEE (**Table 7.6**), despite a tendency towards higher NEE mean values being detected under shelters (**Fig. 7.10g**). With regard to ER, post-hoc contrasts revealed a significant reduction (-21%) in FD compared to C, while MD, although exhibiting a lower mean value, did not statistically differ from C (**Fig. 7.10a, b**). In ED, on the other hand, ER was statistically equivalent to that detected in C (p>0.05) (**Fig. 7.10b**).

Gross CO_2 assimilation in FD was significantly lower (-26%) than C, while values recorded in ED and MD were statistically equivalent ($p>0.05$) to those recorded in both FD and C (**Fig. 7.10d, e**).

Both CO_2 emissions and gross assimilation significantly differed between the two mid-season dates (**Table 7.6**), with fluxes recorded on the second date (15 August) being 34% (ER) and 19% (GEP) lower than those measured on the previous date (5 August) (**Fig. 7.10c, f**). There was no significant interaction between treatment and day for either ER or GEP ($p>0.05$). Finally, total vascular plant biomass significantly influenced ER [+6.61 mg (CO_2) m^{-2} h^{-1} per g of biomass], GEP [-17.25 mg (CO_2) m^{-2} h^{-1} per g of biomass] and NEE [-8.10 mg (CO_2) m^{-2} h^{-1} per g of biomass] (**Table 7.6**).

7.4.5 Resistance and recovery

Resistance of ER – i.e. sensitivity during drought – exhibited significantly negative mean values (i.e. reduced fluxes under drought compared to controls) only in MD [$t(4)=-4.110$, $p=0.015$] and in FD [$t(4)= -4.832$, $p=0.008$]. Resistance of GEP was significantly negative only in FD [$t(4)=-3.949$, $p=0.017$] (**Fig. 7.11a**). No significant difference between treatments within the same process was found ($p>0.05$), although a tendency towards greater resistance – i.e. a less negative mean value – of ED compared to MD and FD could be seen (**Fig. 7.11a**). Recovery – i.e. sensitivity following drought – was not significantly different from zero ($p>0.05$) for either ER or GEP (**Fig. 7.11b**). In addition, plots undergoing different drought timing and duration displayed statistically equivalent mean recovery values ≥ 10 days after the end of the dry period (**Fig. 7.11b**). Finally, when testing for differences in resistance and recovery between ER and GEP within the same treatment, no significant difference was detected ($p>0.05$) (**Fig. 7.11a, b**).

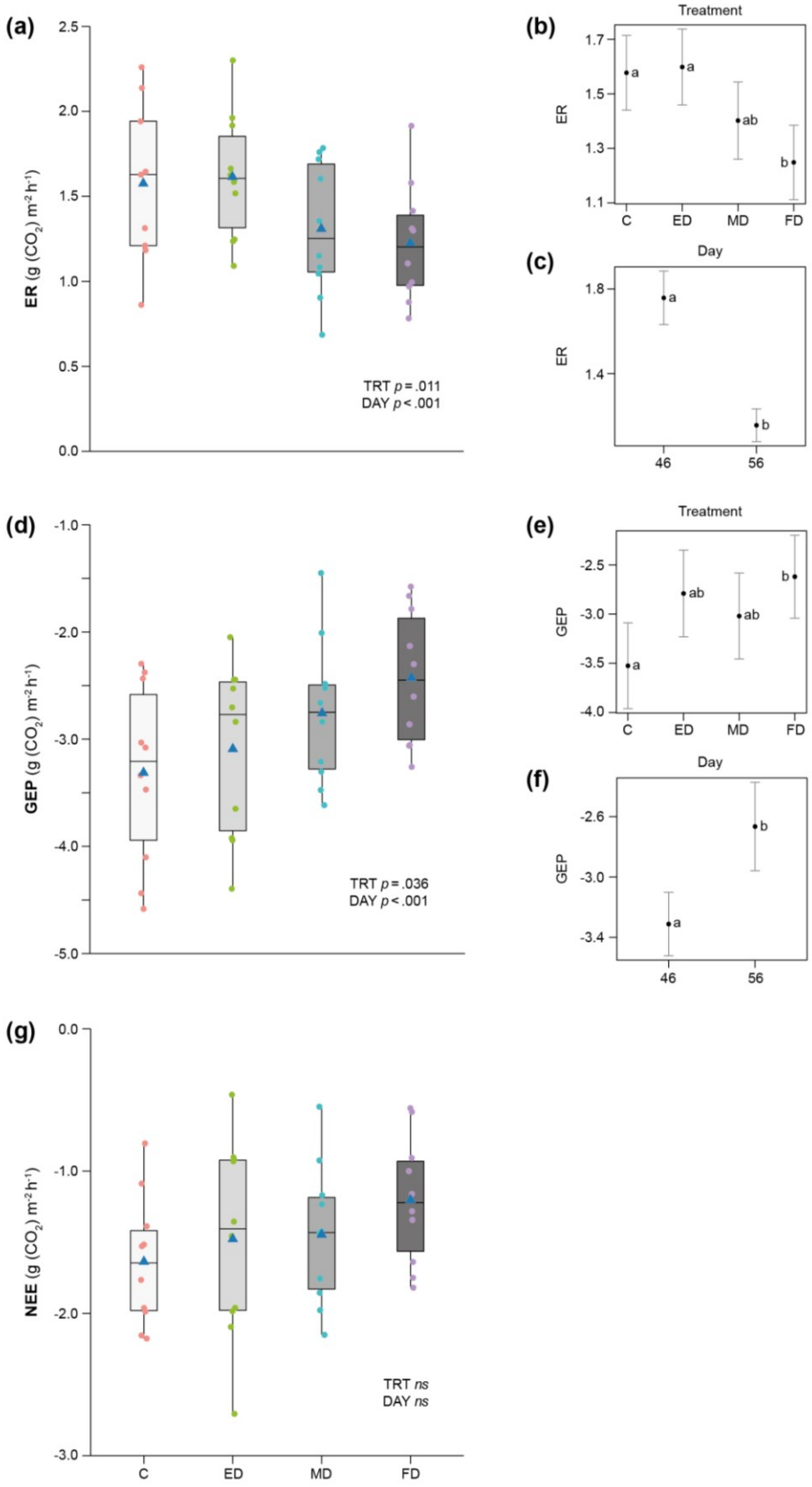


Fig. 7.10: (a) Ecosystem respiration (ER), **(d)** gross ecosystem production (GEP) and **(e)** net ecosystem exchange (NEE) measured during the mid-season period in the 4 treatments [control (C), early-season drought (ED); mid-season drought (MD); full-season drought (FD)]. For each boxplot, dots refer to single values for each of the 4 treatments, while the blue triangle indicates the mean. For ER and GEP the LMM output (estimated means and 95% confidence interval) of significant factors is reported: **(b, e)** treatment (TRT) and **(c, f)** day (DAY). Different letters indicate significant differences between both treatments and days (post-hoc Tukey contrast, $p<0.05$).

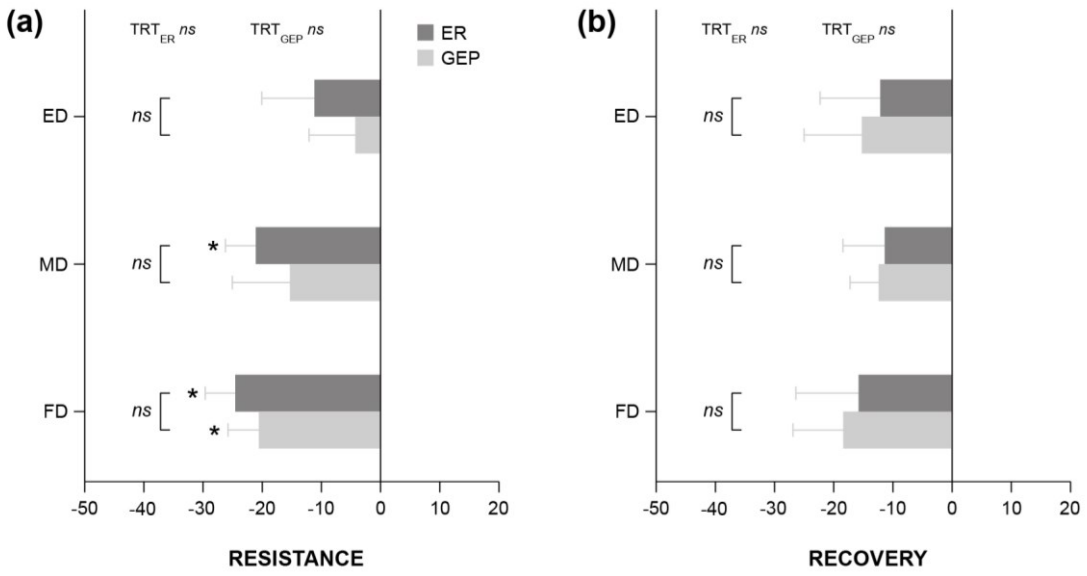


Fig. 7.11: **(a)** Average resistance – i.e. sensitivity during the drought period – and **(b)** recovery – i.e. sensitivity following drought – of ecosystem respiration (ER) and gross ecosystem production (GEP) within the different drought treatments TRT [ED – early season drought; MD – mid-season drought; FD, full summer drought]. Sensitivity represents the % of change in drought plots against controls. Bars indicate the standard error (n=5); * represent mean values of resistance or recovery significantly different from zero (one-sample *t-test*, p<0.05).

7.5 DISCUSSION

The timing of drought during the growing season represents a crucial aspect in determining the degree to which ecosystem responses may be impacted by water scarcity. Results from a mesocosm experiment performed on the same alpine mesic grassland revealed that both plant growth and CO_2 exchange were diversely affected by early and mid-season drought (see Chapter 6), thus confirming results from other studies comparing natural dry spells occurring at different times during the growing season (Craine et al., 2012; Lund et al., 2012; Liu et al., 2019) and from manipulation experiments testing drought timing in the field (Denton et al., 2017; Hao et al., 2018; Zhang et al., 2019b; Yang et al., 2020b). It was necessary, however, to perform a complementary field experiment on the same plant community in order to upscale responses derived from a highly controlled environment to one experiencing natural conditions, where the complexity of the ecosystem – a factor inevitably absent or altered in lab experiments – could be adequately addressed (Kreyling et al., 2014). In the current experiment, the same mesocosm drought treatments were applied in an alpine *Carex curvula* grassland in the field, with hypotheses on drought-timing effects on plant growth and CO_2 fluxes formulated on the assumption that precipitation exclusion would negatively impact these processes. Results from the first year revealed that ecosystem functions remained stable in the face of drought, with

drought duration, rather than timing, producing the greatest impact whenever significant responses to manipulations were detected; it was only for ER that significantly lower values were also found in the ED treatment which, in all likelihood, was the result of reduced microbial activity in the drier topsoil layer. Analyses on drought resistance, on the other hand, revealed no difference between GEP and ER, highlighting equal sensitivity on the part of both processes to different drought treatments. These results were probably due to the high soil moisture levels characterising the site, which did not exceed drought stress thresholds identified for ER and GEP in the mesocosm experiment.

7.5.1 Drought effects during the entire study period

The first aim of the present study was to evaluate plant growth responses and CO_2 fluxes to different drought treatments over the entire study period. Results revealed that, whenever present, the greatest effects of drought were found in the longer drought treatment (FD), in line with the first hypothesis based on results from the mesocosm experiment (see Chapter 6). In particular, long-term drought negatively affected ER and the photosynthetic efficiency of the dominant species; in contrast with what had been predicted, however, neither plant growth nor GEP and NEE were affected by any of the drought treatments imposed when the whole study period was considered.

Effects on ANPP and species-specific morphometric traits

With regard to plant growth, in contrast to what had been predicted, no influence of drought was found in the field either in the case of ANPP (**Fig. 7.7**) or in that of morphometric traits for the most abundant grassland species (**Table 7.4**). This outcome is not in line with findings from other drought timing experiments (Denton et al., 2017; Meng et al., 2019), but is consistent with results from a similar manipulation experiment carried out in a semi-arid steppe, which reported stable ANPP responses to early-, mid- and late-season drought (Zhang et al., 2019b). In the current study, it was surprising to find that no effects were produced by even the most extreme drought treatment, in view on the fact that outcomes from other studies on similar communities have reported a negative impact of seasonal rainfall exclusion on ANPP (Gilgen and Buchmann, 2009; De Boeck et al., 2016; Schmid, 2017). Several reasons could explain the current results: (a) the high levels of soil moisture within the site; (b) the number of days available between snowmelt and the experiment set up for plant growth; (c) the relatively dry early-season period naturally experienced by plants; (d) the shelter-induced increase in soil temperature; and (e) the method used to estimate ANPP.

With regard to the first point, the high levels of soil moisture characterising the site were revealed by data on soil WP and VWC. The high spatial heterogeneity of soil conditions in the field

is known to make comparison between treatments difficult (see Chapter 3, Section 3.3.2); raw WP values such as those presented in the study, however, are indicative of the high water potential recorded at -5 cm, which exhibited average values greater than -20 kPa in all treatments (**Fig. 7.5b**). These values were well above those found to induce drought stress responses in plants in the mesocosm experiment, where grassland monoliths exhibited reduced ANPP in both early- and full-drought treatments. In fact, in the 0-5 cm soil layer of monoliths, measurements as low as 12% of VWC (approx. -50 kPa) were made during the first period in ED and FD. In all likelihood, soil conditions such as these have never been experienced by plants in the field, even when VWC measurements in the drier near-surface soil layer, at 0-3.8 cm, were considered (**Fig. 7.6a**). Analysis revealed an average VWC of 25% under shelters at the end of the early-season drought period (**Fig. 7.6b**). According to the WP-VWC relationship obtained in the lab (Chapter 6, Section 6.4.6), this value corresponded to a WP of approx. -30 kPa, still too high, therefore, to result in detectable plant growth stress. These considerations, together with the hypothesis suggesting a water-acquisitive strategy on the part of the plant community (see Chapter 6, Section 6.5.4), could underlie the lack of a detectable drought-induced impact on ANPP.

A second explanation for the lack of drought effects on ANPP could be found in the 17-day long period between snowmelt and the experiment set up, sufficient to have allowed plants to effect a considerable portion of their growth under ambient conditions. The hypothesis that most plant growth takes place prior to water stress was put forward by Carroll et al. (2021) to explain the lack of ANPP responses of C_3 grassland species to a 4-year drought. Results on ANPP measured in the mesocosm experiment support this explanation, by illustrating the high plant growth rate exhibited by plant species in the grassland under investigation (Chapter 6, Section 6.4.2, 6.5.1). The important part played by the first days after snowmelt was also evident in the current study when estimated correction values applied to biomass during the season were considered: as can be seen in **Table 7.3**, the community had already reached almost 80% of its final aboveground mass on the first flux measurement date in the middle of the early season. It should be noted that the values obtained are characterised by a certain amount of error, including the assumption that biomass estimates in mid-August correspond to peak biomass, the point intercept method results in an indirect biomass estimate, and the correction percentage of biomass was calculated on plants collected and measured during another growing season (2018). Despite this, results provided in the literature on the growth and phenology of high-elevation plant communities (Bowman and Fisk, 2001; Körner, 2003; Jonas et al., 2008), together with ANPP records under controlled experimental conditions, both support the hypothesis that the investigated plant community was able to carry out an important part of its growth in the time interval between snowmelt and the beginning of drought treatments. Having said this, it should

be noted that De Boeck et al. (2016) reported negative effects of drought on ANPP even when grassland monoliths in the field were subjected to treatments 2-3 weeks after snowmelt.

Regarding the third possible reason for plant growth not being affected by drought, climate data revealed that shelters reduced precipitation by only 30% during the first period (**Fig. 7.4**), producing an effect which might not have been sufficient to induce a detectable difference in primary production. In fact, as a result of the co-varying nature of controls and treatments characterising field manipulation experiments, drought effects on ANPP are expected to be minimal when the considered study period is either very dry or very wet (Henry et al., 2018; Hoover et al., 2018).

A further explanation could be the shelter-induced soil temperature increase (+1 K) recorded at -5 cm (**Fig. 7.5a**), which may have stimulated plant growth until water was fully available (Winkler et al., 2016), counteracting negative effects of drought occurring later in the season (Wolf et al., 2016). It should be noted, however, that air temperature at the canopy level exhibited similar values both under and outside the shelter, indicating that air circulation was not affected by the infrastructure: although the soil was warmer, the air produced a potentially cooling effect. Additionally, the soil temperature increase may have been dependent on soil drying (Al-Kayssi et al., 1990).

Finally, the indirect estimation of biomass using the point intercept method may not have been able to capture either anticipated drought-induced leaf senescence (Munné-Bosch and Alegre, 2004), which might have occurred under the most extreme drought treatment (De Boeck et al., 2016; Cremonese et al., 2017; Stampfli et al., 2018), or small differences in shoot and leaf length, leaf area or leaf number (Jung et al., 2014; Rosbakh et al., 2017; Wellstein et al., 2017). When analysing leaf length of the six most abundant grassland species, the majority of *taxa* exhibited a tendency towards lower means (**Table 7.4**), particularly evident for *Leontodon helveticus* (p=0.058). Furthermore, the grass *Poa alpina* exhibited significantly lower reproductive height values, which could be further evidence for drought influence. In fact, drought has been reported to affect plant reproduction in mountain grasslands (Zeiter et al., 2016; Rosbakh et al., 2017). In particular, a study analysing the effects of a natural drought event in alpine grasslands in Australia found that growth of *Poa* species were negatively impacted (Griffin and Hoffmann, 2012). However, there is still a lot of uncertainty regarding the responses of *P. alpina*, since it cannot be excluded that the current result was due to the uneven, independent distribution of flowering individuals across plots, which may have generated a bias in the interpretation of treatment effects (**Table 7.1**).

Effects on chlorophyll fluorescence

Measurements of the PSII maximum quantum efficiency, yielding information on changes in the efficiency in photochemistry and heat dissipation (Maxwell and Johnson, 2000), have been carried out by a number of studies to estimate if, and to what extent, the photosynthetic apparatus is damaged by drought stress (e.g. Marchand et al., 2006; Bollig and Feller, 2014; Walter et al., 2011; Jentsch et al., 2011; De Boeck et al., 2016). In the present study, F_v/F_m measures taken on dark-adapted leaves at the end of each drought period revealed a significant reduction of PSII maximum quantum efficiency in *Carex cuvula* individuals in FD compared to those in C, whereas no significant difference was found in F_v/F_m when values were compared at the end of early-season drought (**Table 7.5**). In contrast, on both measurement dates, F_v/F_m of *Alchemilla pentaphyllea* under drought did not statistically differ from values in C, thus revealing no influence of either drought timing or length on the photosynthetic efficiency of this species.

Different F_v/F_m responses across species have been reported in other drought studies (Bollig and Feller, 2014; Zhang et al., 2019b). With regard to results for the dominant sedge in the current research, findings are in line with those reported by Thorn et al. (1997) for the same species in unfertilized, ambient conditions; the occurrence of photo-inhibition in *C. curvula* only during hot, dry summers, and not in cold, rainy conditions, was also reported by Thorn et al. (1997). On the other hand, values of PSII maximum quantum efficiency of *A. pentaphyllea* corresponded to the average optimal F_v/F_m – i.e. approx. 0.8 – identified for a variety of plant species and ecotypes by Björkman and Demmig (1987). It appeared, therefore, that no drought stress occurred in the most abundant forb of the studied plant community.

Different responses to drought between the two most abundant species may have depended on rooting depth (Stampfli et al., 2018): in mountain and alpine grasslands, graminoids have often been reported to exhibit shallower roots than forbs (Zeiter et al., 2016; Liu et al., 2018). The first few centimetres of soil below the surface are mostly occupied by *C. curvula* roots, which may have suffered progressive drying in the first layer during the experimental period, whereas *A. pentaphyllea* might have been able to access deeper water resources still available in FD (**Fig. 7.5b**). Furthermore, by receiving direct radiation under drier conditions, *C. curvula* may have undergone greater stress than *A. pentaphyllea*. The latter species may have benefitted from the protection of the dense *C. curvula* mat which might have kept it from excessive light exposure. Photo-oxidative stress has, in fact, been reported to occur under drought, resulting in a reduced use of absorbed light energy for CO_2 assimilation and a consequent reduction in the effective quantum efficiency of PSII (Bolling and Feller, 2014 and references therein).

Overall, chlorophyll fluorescence results highlighted that duration, rather than timing, was the aspect of drought which determined drought stress in the dominant grassland species. The lack of significant differences in F_v/F_m values among *C. curvula* individuals in drought plots and ambient

conditions at the end of the first drought period leads to the conclusion that drought stress had taken place at least one-month after precipitation exclusion, so that the dominant sedge was not affected by early-season drought. The lack of measurements in MD at the end of the treatment period, however, means that a definitive conclusion on whether damage occurred under mid-season drought cannot be reached, although the absence of significant GEP responses in this treatment suggests that no damage occurred.

Effects on CO_2 fluxes

When analysing CO_2 flux responses during the entire season – including measurements taken after the end of treatments – ANPP was found to strongly influence photosynthetic and respiration processes, reflecting results from the mesocosm experiment and other studies which indicate that ecosystem-level respiration and photosynthetic rates are often tightly coupled (Parton et al., 2012). The inclusion of ANPP as a covariate in CO_2 flux analyses, after it was found not to significantly differ across treatments, also allowed for spatial variability to be taken into account for a more accurate evaluation of treatment effects on CO_2 exchange processes.

In contrast to what was observed in the mesocosm experiment, where all CO_2 fluxes were negatively impacted by drought, in the current study only ER was significantly affected. In particular, it was only in FD that the reduction was significant (-21% compared to C), whereas CO_2 emissions were not influenced by different drought timings (**Fig. 7.8a**). A general ER reduction, such as the one observed in the present study, is potentially the result of a decrease in microbial activity in the top soil layer occurring in both periods (see Section 7.5.2) and is in line with recent findings by Yang et al. (2020b) indicating reduced soil respiration in all drought timings artificially imposed on a semiarid grassland. On the other hand, the absence in the present study of drought effects on GEP during the entire growing season could probably be due to a reduction in GEP which only occurred during the second part of the full-season drought treatment. The mechanisms explaining seasonal outcomes are discussed in the section below (Section 7.5.2).

Therefore, as was observed for plant growth, these results did not concur with the initial assumption on which the hypotheses were based – i.e. that all CO_2 exchange processes would be impacted by drought. The assumption was only true in the case of ER and then only for the 2-month long drought treatment, even though there was a tendency towards reduced values in the FD treatment for GEP (**Fig. 7.8b**) and NEE (**Fig. 7.8c**).With regard to NEE, in particular, in the field experiment the grassland maintained its role as a C-sink under all drought manipulations, whereas in the mesocosm experiment grassland monoliths were a C-source in FD and shifted towards positive NEE values by the end of the two drought timing treatments. Such a contrast is likely to be the result of the following factors: absence of drought effects on ANPP; non-reaching of thresholds for GEP; and the concurrent ER reduction. All these maintained the GEP-ER balance at

higher GEP values throughout the season, irrespective of drought treatments. NEE field results are also confirmed by the absence of significant differences in sensitivity between GEP and ER (**Fig. 7.11a, b**): studies reporting reduced strength of the ecosystem as a carbon sink highlight different sensitivity to low soil moisture on the part of GEP and ER, with GEP being generally more sensitive to drought than ER (Hoover and Rogers, 2016; Liu et al., 2019; Zhang et al., 2019a).

Furthermore, average flux values measured in the field were higher compared to those recorded in the mesocosm experiment, where a 22% lower ER was recorded compared to values measured in controls, together with a -34% and -45% GEP and NEE, respectively. Such a difference in the magnitude of fluxes is probably the result of the limited amount of soil in the mesocosm pots – i.e. reduced nutrients, microorganisms, soil invertebrates and roots (Poorter et al., 2012); 12 cm-diameter pots, with a depth of only 15 cm, do not fully replicate the much more extensive, unconfined belowground environment experienced by the grassland in the field.

When outcomes from each drought period were analysed in more detail, it appeared that the ER reduction could have resulted from a decrease in microbial activity in the top soil layer in both periods (see Section 7.5.2). Such a general ER reduction is in line with recent findings by Yang et al. (2020b) indicating a reduced soil respiration in all drought timings artificially imposed on a semiarid grassland. On the other hand, the absence in the present study of drought effects on GEP during the entire growing season was probably due to a reduction in GEP which only occurred during the second part of the full-season drought treatment. The mechanisms explaining seasonal outcomes are discussed in the section below.

7.5.2 Drought effects within each drought period

CO_2 fluxes within each drought period were investigated to address the second aim – i.e. achieve a better understanding of the mechanisms underpinning seasonal patterns. As for the lab experiment, CO_2 flux dynamics within each period were differently affected by drought depending on whether it occurred in early or mid season; as was previously noted, however, the magnitude of responses differed from those in the mesocosm experiment and not all processes were significantly affected by drought. In detail, during early-season drought, only ER was found to be significantly reduced by precipitation exclusion (-18% compared to ambient conditions, **Fig. 7.9b**). On the other hand, during mid-season drought, respiration processes were found be significantly lower only in FD (-21%), whereas in MD fluxes were equal to C, even though a tendency towards reduced values was detected – i.e. MD statistically equivalent to FD (**Fig. 7.10b**). As for GEP, this was found to be significantly affected by drought only during the second period, with the response driven by the significant reduction in FD (-26%, **Fig. 7.10e**). However, GEP responses also showed a trend towards reduced photosynthesis in MD and ED, both statistically equivalent to FD, suggesting possible drought carry-over effects during the early period and a greater response

of plants to drought during the second period. Although changes occurred in both ER and GEP, NEE remained stable irrespective of drought timing and duration (**Fig. 7.9ec, Fig. 7.10g**). Therefore, in contrast to what was hypothesised on the basis of the mesocosm experiment, mid-season drought did not produce a greater impact on CO_2 fluxes compared to a dry spell occurring during the early season, although the tendency exhibited by GEP seemed to mirror findings from the lab experiment.

With regard to ER, the absence of significant drought effects on ANPP and chlorophyll fluorescence suggested that the main driver of early-season reduction was the heterotrophic component of ER. In support of this hypothesis, no significant effect of ANPP was, in fact, found on ER during the early season (**Table 7.6**). Consequently, the decrease in ER under shelters was probably caused by a decline in microbial activity in the topsoil layer (Borken and Matzner, 2009; Parton et al., 2012; Schimel, 2018), which underwent greater drying compared to the deeper soil layer (**Fig. 7.6a**). Furthermore, mid-season ER values recorded in the ED treatment after re-wetting exhibited a complete recovery 18 days after the end of drought, with values being statistically equivalent to those in C (**Fig. 7.10b, Fig 7.11b**). Several studies have reported a fast recovery, or even pulses, in microbial respiration processes after post-drought re-wetting – i.e. the "Birch effect" (Bloor and Bardgett, 2012; Parton et al., 2012; Frank et al., 2015 and references therein; Karlowski et al., 2018). This occurs as a result of increased soil N availability after dought-induced reduction in N uptake by plants and/or increased microorganism mortality (Borken and Matzner, 2009). In monoliths monitored in the mesocosm experiment, re-watering following an early drought event did not produce a respiration pulse, or an ER recovery, which was probably a result of reduced plant growth during early-season drought (Chapter 6, Section 6.5.1). In the field experiment, on the other hand, the apparent lack of drought influence on plant growth at the community level, together with a total ER recovery after the first drought period, suggests that the early-season reduction was mainly driven by heterotrophs. No ER pulses were detected, however, after re-wetting (**Fig. 7.8a**), which could have been due to the low measurement frequency of CO_2 fluxes (Hagedoorn and Joos, 2014) which did not permit very rapid, short-term processes, such as the re-wetting mineralization flush, to be recorded (Borken and Matzner, 2009). As for mid-season respiration processes, these were significantly affected by drought only in the FD treatment, potentially a result of both lower heterotrophic and autotrophic respiration.

Significantly lower GEP was, in fact, recorded in FD during the mid-season period (-26% compared to C), whereas no significant drought-induced was found during the early period. Several factors could have accounted for these results, including: (a) the lower maximum quantum efficiency of PSII exhibited by the dominant sedge (**Table 7.5**, see Section 7.5.1.2), possibly leading to reduced GEP; (b) the earlier drought-induced leaf senescence (De Boeck et al., 2016; Cremonese et al., 2017; Stampfli et al., 2018), which could have led to lower stomatal

conductance and assimilation capacity (Zhang et al., 2020b); and (c) the potentially smaller leaf area exhibited at the community level, as suggested by results from species' morphometric traits (**Table 7.4**, see Section 7.5.1), which might have led to reduced GEP under drought (Rogiers et al., 2005; Suttle et al., 2007; Noormets et al., 2008; van der Molen, 2011). The last two factors could go some way to explain GEP responses in ED plots during the second period, where gross assimilation, although statistically equivalent to C, exhibited a tendency towards lower values, being also statistically equal to FD. The smaller leaf area available for photosynthesis, due reduced leaf dimension and/or to early senescence, together with a reduction in heterotrophic respiration, might explain the further mid-season ER reduction in FD (Darenova et al., 2017; Zhang et al., 2019a).

In addition, it was notable that, during the mid season, the MD treatment showed a tendency towards reduced ER and GEP (**Fig. 7.10b, e**), exhibiting values statistically equivalent to both C and FD. This trend, partially supported by GEP resistance values measured for each treatment, could indicate a potentially greater impact of drought during the second period of the growing season, in line with findings from the mesocosm experiment. Unfortunately, continuous WP measurements were hindered by technical issues concerning the isolation of drought plots from external water input (**Fig. 7.5b**). Although decreased soil moisture levels under the shelters were detected during the mid season, values also revealed that re-wetting occurred in drought treatments (MD and FD) when there were particularly abundant rainfall events; this was probably the result of upward, capillary water movement from the deeper soil layers and/or episodic water inflow from the saturated trench around the plots. Topsoil re-wetting, in particular, may have influenced ER and GEP, reducing drought treatment effects. Difficulties in controlling soil moisture levels, together with other microclimate variables which can interfere with drought manipulation treatments – e.g. high atmospheric humidity and strong wind – represent a common issue in manipulation experiments (Beier et al., 2012; Kreyling et al., 2017; Schmid, 2017) and, therefore, require the setting up of parallel studies under controlled conditions to better comprehend the mechanisms underlying ecological responses in the field. Additionally, more frequent measurements of CO_2 fluxes would enable the influence of seasonal abiotic factors over that of treatments to be detected.

Finally, neither the early-season ER reduction nor the mid-season reduction of both GEP and ER appeared to significantly affect NEE, which exhibited statistically equal values to ambient conditions – neither diverse drought timing nor drought duration were found to affect net CO_2 assimilation. Similar outcomes have been reported for mountain grasslands under natural droughts, attributed to wilting thresholds not being exceeded in the field (Brilli et al., 2011). It has also been reported that when drought is not accompanied by heat waves, impact on NEE is limited as a result of equally reduced assimilation and respiration processes (von Buttlar et al.,

2018). Impact on NEE is only evident when its two components exhibit different sensitivities to drought (Hoover and Rogers, 2016; Liu et al., 2019; Zhang et al., 2019a). In the present study, ER and GEP exhibited equal resistance to drought, therefore explaining the lack of significant NEE responses to drought.

7.5.3 ER and GEP: drought resistance and recovery

The final aim of the study was to assess whether GEP and ER showed diverse sensitivity to drought and whether differences occurred between the two processes and across the three drought treatments. For resistance and recovery results revealed no significant difference either between the GEP and ER under different drought treatments or when considering for each process differences between early-, mid- or full-season drought (**Fig. 7.11a, b**). Values did, however, show a negative tendency: resistance was significantly negative – i.e. low resistance – for GEP under the mid-season treatment and for ER and GEP under the full-drought treatment, partially confirming the hypothesis based on lab findings. Nevertheless, it cannot be excluded that the outcome for MD was due to the greater contrast between ambient and drought conditions experienced during the second half of July, where there was more rain than the early period – i.e. 70% of the rainfall recorded during the entire study fell during the mid-season drought period: on the first measurement date, ER and GEP were particularly high in C (**Fig. 7.8a, b**), probably as a consequence of the preceding intense water input (**Fig. 7.4**). Similarly, apparently high ER and GEP recovery for MD and FD treatments could be attributed to low CO_2 fluxes in C plots 9 days after the end of the experiment (**Fig. 7.6c**) as a result of the scarce rainfall during the "re-wetting" period (**Fig. 7.4**).

Not surprisingly, analyses for both drought-periods revealed the strong temporal fluctuations in CO_2 fluxes (**Table 7.6, Fig. 7.9c, Fig. 7.10c, e**), underlying the dependence of these processes on the microclimate conditions pertaining on each measurement date – i.e. air and soil temperature, solar radiation and humidity (Harper et al., 2005; Wohlfhart et al., 2008; Virkkala et al., 2018). Intra-seasonal climate variability is recognised as determining the extent of the drought treatment in the field (Kreyling et al., 2017; Hoover et al., 2018), making results based on a single year of manipulation difficult to generalise. Multiple-year measurements will have to be carried out to capture inter-annual variability and assess whether soil moisture thresholds could actually be reached in the field.

7.6 CONCLUSION

The present experiment consisted of a complementary field study carried out in order to upscale findings for drought-timing effects on ecosystem functions obtained under controlled, lab

conditions. Results from the first year of manipulations revealed that, in the field, key ecosystem functions, such as ANPP and net ecosystem exchange, were stable under drought conditions. Over the entire first-year season, the 2-month drought treatment produced the most pronounced effects. In contrast to what had been predicted, apart from ER, drought timing did not affect CO_2 fluxes. Ecosystem respiration was the only process to be significantly affected by a 1-month drought period during the early season which was probably the result of soil moisture thresholds experienced by heterotrophs being reached in the topsoil layer. It is clear, therefore, that thresholds identified in the lab were not triggered in the field.

Overall, the investigated alpine ecosystem was found to be particularly resistant to pulse drought events in the short-term, but *in situ* conditions, characterised by a complex multiplicity of factors, make it difficult to accurately assess ecosystem responses to drought, especially short-term ones. These impediments include: the difficulty of controlling the high-levels of soil moisture; plant species' potential for accessing water supplies from deeper soil layers; the high intra-seasonal and inter-annual climate variability; and logistical constraints on rapid experiment set up and high frequency of measurements.

Results obtained in the mesocosm did help to quantify soil moisture status in the field from a drought-stress perspective and prepare the ground for a smoother interpretation of field results – the latter being necessary to evaluate the extent to which mechanisms identified in controlled studies actually occur in natural, multifaceted contexts. Further CO_2 measurements, however, will have to be gathered to capture effects of inter-annual variability on drought treatments and the evaluation of responses to natural dry events will also have to be undertaken to obtain a deeper insight into CO_2 dynamics in high-elevation ecosystems under future climate change scenarios.

7.7 CONTRIBUTIONS

Alessandro Petraglia, Michele Carbognani and T'ai G. W. Forte initiated the project, designed and supervised the experiment. T'ai G. W. Forte, AP and MC planned the research and led the field sampling. Giorgio Chiari, Francesca Arici and Vincenzo De Cancellis also participated in the experimental set-up, field sampling and lab work. TGWF and MC performed the statistical analyses. TGWF wrote the manuscript, which was subsequently critically revised by AP and MC.

CHAPTER 8. GENERAL CONCLUSION

Various mountain regions around the world are already facing warmer, drier climate conditions, with some areas, such as the Alps, predicted to experience reduced summer precipitation by the end of this century. The effects of increased temperatures on alpine ecosystems have been addressed in a number of studies, but the impact of drought still remains poorly investigated; only recently has research begun to assess the responses of high-elevation ecosystems to water shortage and analyse the underlying mechanisms.

This research project investigated how key ecosystem functions of a typical alpine grassland respond to summer warming and reduced precipitation. In particular, drought was considered in terms of duration, magnitude and timing. The first two studies – Study I and Study II – consisted of a manipulation experiment to investigate the effects of a chronic, seasonal (press) rainfall reduction, in which summer rain was reduced by a percentage matching the site-specific extreme derived from historical climate records. This treatment was combined with summer warming in a factorial experiment evaluating net primary production (NPP) and litter decomposition responses. A further two studies consisted of a mesocosm experiment (Study III) and a complementary field experiment (Study IV) focusing on the mechanisms driving plant growth and CO_2 flux responses to acute, pulse dry spells occurring in two different periods of the growing season (early- and mid-season drought); a full-season drought treatment was also incorporated in both experiments to test drought duration and to detect soil moisture and temporal thresholds triggering different rates of CO_2 gross assimilation and emission.

Taken as a whole, findings from the present research project reveal that key ecosystem processes in the target high-elevation grassland are particularly resistant to short-term press drought, but can be vulnerable to pulse drought events. Impacts on ecosystem functions are found to differ according to the time when such events occur, potentially producing a greater detrimental effect on photosynthetic and respiration processes when occurring in the middle of the growing season. However, findings on pulse drought timing and duration in the field also reveal that *in situ* key ecosystem functions, such as aboveground productivity (ANPP) and net ecosystem exchange are particularly stable in the face of drought, with the major effects over the entire season being produced by the longer dry spell. Some of the mechanisms driving the investigated ecosystem processes have been identified in the preceding chapters, when different levels of organisation (Study I), time-scales (Study II), and experimental conditions (Study III and Study IV) were considered.

Turning to Study I, the outcomes of this experiment support the conclusion that it is at lower levels – i.e. individual, population and community – that processes driving stability in plant community composition and functioning often operate with respect to climate change and

extremes (Felton and Smith, 2017). Negligible impacts on key functions at the community level were revealed in this study, but this does not necessarily imply that there are no responses at lower organization levels to changing environmental conditions. In fact, the major *taxa* exhibited different responses to precipitation reduction when plant cover variation was analysed, a result partially supported by trends observed in ANPP which, however, did not appear to be significantly influenced by manipulations. The study did not uncover the extent to which results on diverse species derive from direct effects of drought on species' physiological functioning and/or from an indirect consequence of modified plant-plant interactions – aspects which need further investigation. In fact, a trait-based approach would enable functional traits at the basis of species-specific responses to be more easily identified.

Such trait-based research would not only have to include aboveground plant portions, but also roots, since belowground responses in the present study were found to exhibit a different sensitivity to reduced precipitation compared to ANPP. Belowground production was, in fact, reduced by lower rainfall to a sufficient degree to determine marginally lower root:shoot ratios under shelters; root responses also differed between soil layers, with a tendency towards lower values in the near-surface layer and no responses being detected below this level. Drought-induced root dynamics, therefore, needs further analysis using alternative approaches in addition to the in-growth core method used in this study; using mini-rhizotrons, for instance, would allow non-destructive, time-efficient root measurements and also enable root distribution dynamics along the vertical soil profile to be accurately measured, alongside fine-root turnover rates.

Even though data gathered in the first study (Study I) represents only an initial, short-term response to altered summer conditions, the assessment of plant community composition dynamics and biomass production under climate change is vital for a full understanding of the future dynamics of carbon and nutrient cycling. This underlines a further conclusion, revealed by the litter decomposition experiment (Study II), pointing to the possibility that long-term shifts in biomass allocation, species abundance and/or identity will be the main drivers behind major changes in decomposition processes in the studied grassland. In support of this conclusion, litter quality and the micro-environment in which decomposition takes place were found to be the major drivers of annual and seasonal decay processes.

The long snow-covered period makes an important contribution to the early stage of decomposition as revealed by the experiment of Study II, with the state of alteration of litter approaching the growing season being the main factor constraining decomposition rates under what would potentially be more favourable summer conditions. This outcome, together with those deriving from Study I, reveal that under altered climate conditions during the growing season, the investigated ecosystem has the capacity to resist these changes in the short-term.

Such a conclusion, however, only refers to warming and press drought and does not take into account other inter-related aspects of climate change.

In fact, one of the main conclusions reached by this research project is that pulse drought events and, especially, their timing will be of crucial importance in plant growth and CO_2 exchange under climate change. Findings from Study III, the mesocosm experiment, reveal that the impact on ecosystem functions differs according to the time when such events occur: a more detrimental effect on gross assimilation and ecosystem respiration is produced by drought occurring in the middle of the growing season, when the plant community has reached peak biomass. It was surprising to find that, for this period, it took only 13 days in the lab for the plant community to exhibit almost null GEP and faster declining rates of ER. When this is considered in the context of climate data from the last two decades for the study area, together with the general increase in drought and heat-waves episodes recorded in recent years, the results lead to the conclusion that thresholds for CO_2 exchage processes could potentially be reached under natural conditions. In the field experiment, however, the detection of high levels of soil moisture naturally present within the site suggests that these could act as a buffer in drier climate conditions.

The findings from the last two studies underline the importance of identifying ecological thresholds for key ecosystem processes under drought in order to accurately interpret the relationship between water availability and ecosystem responses. The timing of dry spells is a fundamental aspect which should be factored in when ecological thresholds are analysed. Moreover, results from the current research evidence, once more, the need for a combination of lab- and field-based experiments, which not only allows for the identification of the mechanisms underlying responses to drought and the uncovering of the actual dynamics operating under natural conditions, but also helps to pinpoint the limitations of manipulation experiments: *in situ* conditions in alpine sites are characterised by multiple factors, making it difficult to accurately assess ecosystem responses to drought, especially in the short-term. Among these, for instance, there is the lateral and upward capillary water movement within the soil, the ability of plants to uptake water from deeper soil layers and high intra-seasonal and inter-annual climate variability.

As Hoover et al. (2018) succinctly state in their review on precipitation experiments: “When interpreting results from experimental droughts, researchers should carefully consider the interaction between experimental drought magnitude, ambient precipitation variability, and key ecological thresholds. It is critically important that a lack of treatment effect is not misinterpreted as high drought resistance rather than a potential experimental artefact due to the co-varying nature of control and drought treatments. Knowing the connection between precipitation, soil moisture, and physiological thresholds of key or dominant species in a given ecosystem or region is essential in avoiding such pitfalls”. With this in mind, findings from the mesocosm experiment would seem to highlight the drought sensitivity of the investigated plant community. It cannot be

excluded, therefore, that outcomes in the field are related to experimental artefacts and the difficulties of artificially controlling humidity levels – i.e. soil moisture and vapour pressure deficit. Further field measurements will have to be gathered to capture the effects of inter-annual variability on drought treatments and assess soil moisture dynamics more accurately. Obtaining responses on species-specific thresholds would also allow a better understanding to be achieved regarding the way in which pulse drought events could moderate plant community composition.

It is clear that one of the main shortcomings of the results presented in the current research is that they only refer to short-term responses. It has been constantly stressed throughout the thesis that a longer time-scale is needed to verify whether short- and long-term responses differ, and whether recurrent drought weakens the resistance of plant species and the community itself to further perturbations. Short-term responses might, in fact, be transient, leading to incomplete conclusions regarding long-term dynamics under climate change. Once again, further investigations are essential in order to evaluate whether press and pulse drought, and different drought timings, produce long-term effects – i.e. carry-over and legacy – both during and beyond the dry period.

A further consideration regarding outcomes from this research project is that manipulation experiments may not provide a complete picture, as it is possible that the frequent naturally occurring combination of drought and heat waves might have a greater impact on grassland ecosystem functions in the field. This conclusion is not only supported by findings from other studies on subalpine and alpine grasslands, but could also go some way to explaining the strong inter-annual differences in vascular plant ANPP which was found after analysing the 3-year data series (Study I): the strong reduction in aboveground productivity observed after the pre-treatment year suggests that the target grassland may have been affected by naturally occurring heat waves and drought experienced in Europe in 2017. However, long-term data, species-specific physiological measurements and observations under naturally occurring climate extremes are needed if such a hypothesis is to be confirmed.

This research project, although of only limited duration, is important as an integral part of a global network of studies working on how terrestrial ecosystems will be affected by climate change in the future. By sharing common protocols regarding the type of manipulations to be applied and which variables to be measured, some of the data gathered in the present research – i.e. data from Study I and Study II – has overcome the limits imposed by different methodologies characterising climate change experiments. It will, therefore, make a contribution to an overall assessment of the degree of sensitivity exhibited by typical alpine grassland ecosystems to site-specific extreme drought and moderate warming, when compared to other terrestrial ecosystems. This factor is significant because it shows how global scientific cooperation can achieve results which will then be used to improve predictive modelling.

The use of standard methods, however, always requires the use of parallel investigations into the site-specific characteristics of the target ecosystem in order to capture the whole complexity of ecological responses. Findings from the litter experiment – the first study to employ both standard material (teabags) and local litter in a high-elevation ecosystem experiment – highlight this point. The high variability of plant material decomposing within the target site, which could not be captured by standard litter alone, illustrates the necessity for the simultaneous use of local litter alongside standard litter, also in view of the fact that the sole use of standard leaf litter may lead to belowground carbon turnover rates being overestimated as a result of the extremely slow root decomposition rates.

In conclusion, as has already been mentioned, up to now only a few studies have focused on the impacts of drought in high-elevation, alpine ecosystems, with the potential effects of drier conditions in association with warming and different timings of pulse drought receiving even less attention. In response to the main objective of the research project, this work provides new data which, hopefully, will broaden the geographic and ecological framework of drought experiments in order to gain a better understanding of how ecosystem processes in cold, non-water limited ecosystems might be affected by climate change in the future. High-elevation grasslands are stable under current climate change, but long-term changes under more frequent and intense climate extremes will require urgent, new research.

ACKNOWLEDGEMENTS

Thank you to my supervisors, Alessandro Petraglia *and* Michele Carbognani
you gave me the opportunity to carry out this research project, guided me in planning the experiment, analysing data and interpreting the results; you shared your expertise with me and taught me so much.
Thank you for your patience and your trust, for always working back to back with me and giving me the chance to collaborate on a variety of interesting projects.
"Everything has beauty, but not everyone sees it" said Confucius.
You showed me some of the beauties of this world, sharing some of the Alps' secrets and allowing me discover spectacular new places.
Thank you both for your friendship and your support throughout this PhD.

Thank you to the people who worked on the design of the shelters and the set-up of the experiments
Roberto Vacchelli, Andrea Comelli, Luca Barchi and Cristian Cavozzi
Your support also made this project possible.

A big thank you to all the researchers and the students who took part to the field and lab work,
Giorgia Andreoli, Francesca Arici, Thai Linh Bacchilega, Marco Boschetti, Giorgio Chiari, Paola Chiudinelli and Luca, Vincenzo de Cancellis, Francesca Degola, Matteo Gaudenzi, Fabio Lumiero, Mattia Malerba, Matteo Maria Maniscalco, Stefano Meroni and Federica, Letizia Pagliani, Matteo Petit Bon, Silvia Quaresmini, Nicolò Maria Sarti, Maria Timco
This thesis would not have been possible without you.

Thank you to all the people of the Botanical section of the University of Parma *for their company, especially over the last year*
thank you for making me laugh and for your help whenever I needed something.

Thank you to all my friends
the old and the new
your support during these years has been invaluable.

Thank you Stefania, Luigi, Michele and Matteo
for the company and walks and lunch and dinners together
especially in the last year, where nothing seemed normal anymore.

Thank you Giancarlo and Giovanna
for being such kind neighbours and always helping me out, especially during lockdowns.

Thank you Marco
for just being you, for your qualified help on the graphics and for giving me the strength to persevere.

Thank you Sam
I love your warm, sweet, laughing voice on the phone, you are my greatest comfort.

Thank you Alaya
you always find the right words and are so curious about the world, thank you for your unconditional love and your sisterly advice.

Thank you Bib
for the qualified help you gave me in the linguistic revision on this thesis and especially for being always by my side, guiding me patiently through life. Thank you for your love.

Ciao Sanaa, a presto!

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The stem grew thick. The nervous roots
reached to each side; the graceful head
changed its position mysteriously,
since there was neither sun nor moon
to catch its young attention.

"The Weed" by Elizabeth Bishop