Plant-fungus interactions in the alpine environment subjected to future climatic conditions

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1 INTRODUCTION

1.1 GLOBAL CHANGE

Ecosystems around the world are being altered by global change (Scheffer et al. 2001; Pielke Sr 2005; Parmesan 2006). Land use change due to a rapid population expansion and globalisation led to changes in ecosystems worldwide during the last two centuries (Durand 1974; Goldewijk 2001; Parmesan and Yohe 2003): Forests disappear to be replaced by farmland, waterways are altered, urban and industrial areas have expanded and biological invasions increased (Scheffer et al. 2001; Foley et al. 2005; Hulme 2009). Severe consequences to all these changes are for example biodiversity loss and soil degradation (Foley et al. 2005). Increased emissions of nitrogen (N) compounds, and accumulation of phosphorous (P) compounds, lead to their increased availability in ecosystems and eutrophication. Strong impacts to ecosystems due to increased nutrient availability are reported worldwide (Foley et al. 2005). Soil and water nutrient availability is changed and their acidification increased (Matson et al. 2002; Körner 2003; Foley et al. 2005; IPCC 2013; Tipping et al. 2014), even in remote mountain ecosystems (Bobbink et al. 2010; Bowman et al. 2012; Schroeder et al. 2014; Zong et al. 2015). Furthermore, land use change has also proven to strongly affect the climate and contribute to climate change (Pielke Sr 2005; Heald and Spracklen 2015).

Climate change is mainly caused by increased anthropogenic emissions of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), which have led to a warming of the entire globe by an average of about 0.8 K during the last 100 years (IPCC 2013). The Earth's surface temperatures during the last 30 years were recorded the warmest since the middle of the 19th century and more heavy rainfall events and longer drought periods are observed and predicted for all terrestrial ecosystems (IPCC 2013). Visible results of climate change are sea level rise (0.19 m over the last 100 years; IPCC 2013), higher frequencies of extreme weather events, advances of spring events, a shift of species range pole-wards and upwards in altitude as well as a global decline of ice and snow cover (Parmesan and Yohe 2003; Parmesan 2006; IPCC 2013). The IPCC's different climate change scenarios are based on research on socioeconomic, environmental, and technological trends. They project six different plausible outcomes of continued emissions of greenhouse gases and

predict possible future warming ranging from a rise of 1.2 to 1.6 K for the middle of the 21st century and temperature rise of 2.7 to 3.8 K by the end of the 21st (IPCC 2013).

However, it is a big challenge for ecologist to predict the impact of global change on ecosystems (Wildbanks and Kates 1999), because some global change drivers act on global scale while others act on local scale and even micro-environmental processes (Wildbanks and Kates 1999). Interrelationships are seen between all temporal and spatial scales (Wildbanks and Kates 1999; Bonan 2002). For example, warming is known to increase global plant productivity (Parmesan 2007; Kullman 2010; Lin et al. 2010). However, at a certain latitude, warming might have negative effects on the productivity of one plant species in a grassland community, while it might not affect its neighbouring plant species with different plant functional traits (Lin et al. 2010), nor the same plant species growing at other latitudes (Parmesan 2007). Acknowledging the importance of local area studies and integrating so far neglected local processes into climate change models will make them more reliable. However, better understanding of the contribution of each local process and their interrelationship is necessary beforehand (Wildbanks and Kates 1999; Bonan 2002).

1.2 MOUNTAIN GRASSLAND

Mountain ecosystems cover about one quarter of the earth's surface (UNEP World Conservation Monitoring Centre 2002; Körner 2007). They provide a high number of services to mankind: mountain pastures and meadows are for example central to agricultural productivity in mountain regions, and are important landscape elements the European Alps (Lamarque et al. 2011). They also reduce the risk of soil erosion (Tasser et al. 2003), serve as carbon sink (Follett and Reed 2010), are home to a diversity of medicinal plants (Grabherr 2009) and contribute the landscape's attractiveness through their high biodiversity (Väre et al. 2003), thus increasing the touristic value (Körner 2003). These ecosystem services provided by mountain grasslands depend on the protection of their biodiversity and integrity (Tilman et al. 1997; Fitter and Gilligan 2005), which in turn are dependent on land use type and intensity as well as other on environmental factors such as climate (Dirnböck et al. 2003).

Extreme environmental conditions, micro-climatic heterogeneity, habitat patchiness and ecological gradients, such as temperature or soil moisture gradients are characteristic for mountain grassland and offer therefore unique possibilities for studies along environmental gradients (Körner 2007). Like many other ecosystems, they currently suffer from divers pressures related to global change, such as an increase of about 2 K in the minimum

temperatures and altered precipitation patterns (Körner 2003; IPCC 2013), what leads to a mass loss of glaciers, shorter periods of snow cover and higher nutrient availability (Körner 2003; Bobbink et al. 2010; Bowman et al. 2012; IPCC 2013; Schroeder et al. 2014; Zong et al. 2015). Land use changes such as abandonment, intensification of agriculture and tourism, mining, hydroelectric or wind turbine installation and the construction other infrastructures present a further threat to mountain ecosystems (Allan 1986; UNEP World Conservation Monitoring Centre 2002; Körner 2003; Tappeiner et al. 2006; Niedrist et al. 2009).

Mountain vegetation is generally distinguished in five different vegetation zones defined by different vegetation characteristics due to the varying climatic and environmental conditions from lowland to summit: foothill, montane, subalpine, alpine and nival (von Humboldt and Bonpland 1807; Körner 2007). The altitudinal location of each zone varies among mountain ranges and even within a mountain range according to its geographical position and allows for the comparative studies of climate change effects across mountain ranges and even continents (von Humboldt and Bonpland 1807; Körner 2003). Even though mountain plants have evolved under extreme conditions over time, and have developed a wide diversity of adaptions to changing environmental conditions, they currently face overall long-term environmental changes bigger and more significant compared to any alteration during history due to environmental changes triggered by global change (Guisan and Theurillat 2000; Sala et al. 2000; Beniston 2003; Körner 2003). Considerable attention is therefore given to determine and predict climate change for mountain vegetation.

A variety of responses to climate change is reported for mountain vegetation. Increasing temperatures and concomitant altered precipitation induce an upward move of plant species resulting in richer but unbalanced vegetation communities: initially dominant species are submitted to suboptimal growing conditions while at the same time facing increased competition for important resources (Kullman 2010; Grabherr et al. 2010; Pauli et al. 2012). Increased seed production and better viability of most tree species leads to an upward move of the treeline and therefore a shrinking alpine life zone situated above (Kullman 2010; Gottfried et al. 2012). Higher up the altitudinal gradient, a retreat and a transformation of snow bed communities to rich grasslands can be observed (Kullman 2010; Gottfried et al. 2012; Pauli et al. 2012). Enhancing the low CO_2 availability in alpine ecosystems lead to the expected extend to positive effects on photosynthesis and some plant species specific effects on plant tissue quality or growing rate (Körner 2003). N and P enrichment in cool ecosystems such as mountain grasslands leads to an accumulation of N

respectively P and chlorophyll in plant tissue, but doesn't necessarily increase plant biomass. Mainly grass species react with increased growth and specific leaf area. This leads to a change in plant functional groups: while the grass proportion and especially the sedge proportion increased, the proportions of legumes and herbaceous species decreased (Michelsen et al. 1996; Bassin et al. 2007; Bassin et al. 2009; Liu et al. 2012).

1.3 PLANT-SOIL INTERACTIONS

A lot of research has been done to identify the drivers of vegetation dynamics and plant community structures and it is known that abiotic interactions alone cannot explain the underlying mechanisms for vegetation community processes (e.g. Ellenberg 1952). It becomes more and more clear that soil microbes influence plant communities and vice versa (Xiong et al. 2003; Wardle et al. 2004; Bardgett et al. 2005; van der Heijden et al. 2008). Plants provide dead plant material for decomposition and root exudates for soil microorganisms (Dakora and Philipps 2002; Wardle et al. 2004). Soil microbes which act as decomposers affect vegetation indirectly because they regulate nutrient availability in the soil (Petersen and Luxton 1982). Other soil microbes, such as root pathogens, nitrogen-fixing bacteria and mycorrhizal fungi have a more direct effect on plants and their community composition (van der Heijden et al. 1998; van der Heijden et al. 2006; van der Heijden et al. 2008; Ossler et al. 2015; Bever et al. 2015). However, comprehensive knowledge on the importance of belowground biota that drive plant community composition and plant-plant interactions is still uncompleted (Wardle et al. 2004). This lack of knowledge might partially be due to the fact that processes which take place in the soil are difficult to study and have therefore been neglected (Bever et al. 1997). Among the three main groups of soil microorganisms – bacteria, fungi and algae – mainly those groups which are beneficial to plants have been intensively studied (Denison and Kiers 2011) and mycorrhizal fungi for example are currently in the centre of interest of research on plant-soil interaction and mutualistic associations (van der Heijden et al. 2015). The impact of the other groups of soil organisms is less understood, especially under climate change conditions (Singh et al. 2010). All in all, this calls for new frameworks of plant community ecology which include soil microorganisms (Schenk 2006; Dickie et al. 2015; Hacquard and Schadt 2015).

Plant ecological niches, the realized space in the ecosystem assigned to each species according to its environmental requirements and interactions with neighbour species (Hutchinson 1957; Colwell and Rangel 2009) can be affected by soil microbes (Bever et al.

1997; Silvertown 2004; Reynolds et al. 2009). Other ecological theories as for example Grime's (1977) C-S-R-framework (Competitor, Stress tolerator, Ruderal) regroup plants according to their functional traits (Grime 1977; Kattge et al. 2011). Incorporating beneficial microbes such as arbuscular mycorrhizal fungi (AMF) into Grime's C-S-R-framework (1977), opens new possibilities for the understanding of AMF-plant interactions and plant biodiversity patterns (Chagnon et al. 2013). While research on competition and negative interactions has a long history, research on positive plant-plant and plant-soil interactions has been restricted, until some time ago, to the particular context of extreme environments and succession (Brooker and Callaghan 1998; Bruno et al. 2003; Brooker et al. 2008). Meanwhile, new ecological concepts which include plant-plant facilitation (e.g. Bertness and Callaway 1994; Brooker and Callaghan 1998; Bruno et al. 2003; Michalet et al. 2006) and plant-soil facilitation (van der Heijden and Horton 2009; Montesinos-Navarro et al. 2012; Ossler et al. 2015) into ecological theory have been developed during the last 20 years and are aggregated under the term "stress gradient hypothesis". Exploring facilitative interactions has greatly advanced the understanding of plant community dynamics. Even though awareness for facilitative interactions is raised and research interest high, advances in research are not far enough to understand the role of facilitation processes in mediating climate change impact yet (Brooker et al. 2008; Powell et al. 2013; van der Heijden et al. 2015).

1.4 THESIS OUTLINE

This thesis aims to contribute to a better understanding of interactions between AMF and plants in mountain grassland ecosystems, concerning plant productivity and AMF abundance, in the context of climate change. I address the existing literature on AMF in mountain grassland in detail and present a new working hypothesis on the AMF-plant relationship along altitudinal gradients. This dissertation further explores AMF colonisation rate in plant roots along a dry altitudinal gradient from the foothill to the subalpine vegetation zone in the Italian Alps in the second part. In addition, the ecological implications for the AMF-plant relationship is investigated at an in situ experiment set at the same altitudinal gradient. The climate change simulation is based on the A1B IPCC climate change scenario, which is a scenario of moderate (2K warming) climatic changes (Moss et al. 2010; IPCC 2013). Temperature rise and increased evapotranspiration were simulated by downward transplantation of soil monoliths.

To complement the findings of the field experiment, to assess the direct AMF effect and to disentangle the different climate change effects on plant productivity an experiment was conducted under controlled conditions in a growth chamber, using local seeds and soil inoculum and by simulating the local climatic conditions from the field site at 1600 m above sea level in the French Alps.

The main questions addressed in this thesis are:

1 What is the state of the art of research about AMF in mountain ecosystems and what is their role for climate change? (Chapter 1)
2 Does AMF abundance in plant root and soil change along an altitudinal gradient? (Chapter 1 and 2)
3 Does the plant-AMF relationship shift along a parasitism-mutualism continuum with increasing altitude? (Chapter 2)
4 What is the effect of climate change (drought/ increased temperature) on AMF abundance in plant roots and soil along an altitudinal gradient? (Chapter 3)
5 Does the plant-AMF relationship in mountain ecosystems change with climate change (drought/ increased temperature)? (Chapter 4 and 5)
6 What are the effects of climate change, AMF presence in the soil and plant diversity on plant productivity? (Chapter 5)

Although AMF play an important role in almost all terrestrial ecosystems and research on their functioning in mountain ecosystems has visibly increased during the last years, many questions concerning their function in mountain ecosystems remain momentarily unanswered. The state of the art on this subject is presented in Chapter 2.

In Chapter 3 I investigate the abundance of AMF in plant roots and soil in dry calcareous meadows along an altitudinal gradient in the Italian Central Alps. I discuss the altitudinal effects in context with land use plant identity and water and nutrient availability.

In Chapter 4, the results of three years of climate change simulation on the microbial community composition and AMF colonisation rate. A field approach is used along the very same altitudinal gradient that was investigated for Chapter 3. Water conditions were levelled at all altitudes and I discuss the effects of warming and increased evapotranspiration on different plants species.

In Chapter 5, I discuss how above- and belowground plant productivity are influenced by high or low presence of AMF, warming, drought and plant diversity by disentangling the different factors using a climate chamber approach which was designed to be close to natural conditions. Together with the results of Chapter 4 it sheds light on processes that influence the plant-AMF relationship in mountain grassland under climate change.

All in all, my thesis wants to advance the knowledge about AMF in mountain grassland under current and future climate conditions by assessing their distribution and abundance as well as their relationship to plants and suggesting a new hypothesis. I discuss the main research questions for AMF in changing mountain grassland ecosystems in the final chapter of this dissertation.

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2 ARBUSCULAR MYCORRHIZAL FUNGI IN CHANGING MOUNTAIN GRASSLAND ECOSYSTEMS – A CHALLENGE FOR RESEARCH

In the following I present a review about AMF in mountain ecosystems under current and future global change conditions. This article is in print for a special issue on 'Mycorrhizas and Global Change' to be published in the Journal "Botany" in June 2016. It is available from the "Just-In" section at the journals website.

Arbuscular mycorrhizal fungi in changing mountain grassland ecosystems – a challenge for research

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2.1 ABSTRACT

Even though arbuscular mycorrhizal fungi (AMF) are present from foothills to all alpine habitats, research on their role in mountain ecosystems remains incomplete. Here we provide a literature review of the ecology and functioning of AMF in mountain ecosystems as well as their response to global change. We investigated how AMF abundance, community composition and fungal traits are studied under field conditions and affected by altitude, habitat patchiness, succession, host identity, seasonality and interaction with other living organisms. The effects of climate change, nutrient enrichment, land use change and their interactions are also addressed in this review, as well as possible applications of AMF for grassland restoration. We show that altitudinal effects on AMF are blurred by other environmental gradients and by host plant identity. The benefits to plants and possible facilitation effects by AMF in mountain ecosystems have not yet been identified. Based on the stress gradient hypothesis, the symbiosis between plants and AMF should become more

mutualistic with increasing environmental stress. We propose a working hypothesis for the functioning of the AMF–plant interaction along altitudinal gradients by grading it along the mutualism–parasitism continuum considering current and global change conditions. We conclude by suggesting further research directions.

KEY WORDS: Arbuscular mycorrhizal fungi, mountain ecosystems, global change, AMF-plant relationship, stress gradient hypothesis

2.2 RESUME

Bien que les champignons mycorhiziens arbusculaires (AMF) soient présents des habitats collinaires aux habitats alpins, les recherches sur leur rôle dans l'écosystème montagnard sont encore incomplètes. Nous présentons ici une analyse des publications concernant l'écologie et le fonctionnement des AMF ainsi que leur réponse au changement global dans les écosystèmes montagnards. Nous traitons séparément l'altitude, la fragmentation de l'habitat, la succession, l'identité de la plante hôte, la saisonnalité et les interactions avec d'autres organismes. Les facteurs climatiques, la fertilisation et le changement d'utilisation des terres et leurs interactions sont discutés ainsi que l'intérêt possible des AMF pour la restauration des communautés végétales. Nous présentons les principaux enjeux scientifiques concernant le rôle des AMF dans l'écosystème montagnard et tentons de répondre à des questions de recherche suivantes : quels sont les effets de l'altitude sur les AMF indépendamment des effets des autres gradients et de la relation AMF-plante hôte ? Quelles relations de facilitation se développent dans les écosystèmes montagnards et quels bénéfices les plantes tirent-elles des AMF ? Avec l'augmentation d'un stress environnemental la symbiose devrait théoriquement devenir plus mutualiste. Nous émettons l'hypothèse d'une modification du fonctionnement des interactions AMF-plante selon un gradient altitudinale dans les conditions environnementales actuelles et puis dans les conditions futures. Nous décrivons ces modifications le long d'un continuum mutualisme-parasitisme. Il est particulièrement important de comprendre et de qualifier ces processus en zone montagnarde pour prévoir leur évolution possible dans un contexte de changement global. Nous suggérons donc de nouvelles orientations de recherche.

MOTS-CLES: champignons mycorhiziens arbusculaires, écosystèmes montagnards, changement global, relations AMF-plante hôte, stress gradient hypothesis

2.3 INTRODUCTION

Mountain ecosystems cover about a quarter of the earth's surface (UNEP World Conservation Monitoring Centre 2002; Körner 2007). They are characterised by habitat patchiness and different ecological gradients, such as temperature and soil moisture gradients. They therefore offer unique possibilities for studies along these gradients as well as useful space-for-time substitutions to examine the effects of climate change (Körner 2007).

Currently, mountain ecosystems suffer from diverse pressures related to global change, for example a 2-K increase in the minimum temperatures and altered precipitation patterns, which lead to glacier mass loss, shorter periods of snow cover and higher nutrient availability (Körner 2003; Bobbink et al. 2010; Bowman et al. 2012; IPCC 2013; Schroeder et al. 2014; Zong et al. 2015). Increased emissions of nitrogen (N) compounds, and accumulation of phosphorous (P) compounds, lead to their increased availability in ecosystems and severe impacts to ecosystems are reported worldwide (Foley et al. 2005), even from remote mountain ecosystems (Bobbink et al. 2010; Bowman et al. 2012; Schroeder et al. 2014; Zong et al. 2015). Soil and water nutrient availability is changed and their acidification and eutrophication increased (Matson et al. 2002; Körner 2003; Foley et al. 2005; IPCC 2013; Tipping et al. 2014). Moreover, land use changes such as abandonment, intensification of agriculture and tourism, mining, hydroelectric and wind turbine installation and construction of other infrastructures present a further threat to mountain ecosystems (UNEP World Conservation Monitoring Centre 2002; Körner 2003; Tappeiner et al. 2006; Niedrist et al. 2009; Harris 2010; Zubek et al. 2014).

2.3.1 MOUNTAIN VEGETATION UNDER GLOBAL CHANGE

Considerable attention is hence given to determine and predict climate change effects for mountain vegetation. For the time being, it can be considered that increasing temperatures and concomitant altered precipitation induce an upward movement of plant species resulting in richer but unbalanced vegetation communities: initially dominant species are submitted to suboptimal growing conditions while facing increased competition for important resources (Kullman 2010; Grabherr et al. 2010; Pauli et al. 2012). Increased seed production and better viability of most tree species leads to an upward movement of the treeline and therefore a shrinking alpine life zone situated above (Kullman 2010; Gottfried et al. 2012). Higher up the altitudinal gradient, a retreat and a transformation of snow bed communities to rich grasslands

can be observed (Kullman 2010; Gottfried et al. 2012; Pauli et al. 2012). Enhancing the low CO_2 availability in alpine ecosystems can lead to positive effects on photosynthesis and to some plant species' specific effects on plant tissue quality or growth rate, but not to the expected extent (Körner 2003). Nitrogen (N) and phosphorus (P) enrichment in cool ecosystems such as mountain grasslands leads to an accumulation of N (respectively P) and chlorophyll in plant tissue, but does not necessarily increase plant biomass in all plant species. Graminoid species in particular tend to react to increased nutrient availability with increased growth and increased specific leaf areas. In addition to that, a general increase of graminoid proportion in grassland communities is observed, especially of sedges. The proportion of legumes and herbaceous species is reported to decline with increased nutrient availability, which results in a general change in the dominant plant functional groups (Michelsen et al. 1996; Bassin et al. 2007; Bassin et al. 2009; Liu et al. 2012).

Plant community composition is structured by the competition for resources in the majority of ecosystems, and community composition in grassland ecosystems by belowground competition for nutrients in particular (Tilman 1985; Casper and Jackson 1997). A change of plant community composition is expected whenever the availability of limiting resources is changed. Specialised root functional traits as well as symbiotic relationships with mutualistic soil microbes such as arbuscular mycorrhizal fungi (AMF) to enhance nutrient acquisition become therefore a crucial factor for the persistence of a plant species in a grassland ecosystem and also play an important role when environmental parameters are changed (Tilman 1985; Casper and Jackson 1997).

2.3.2 AMF AND THEIR RELATIONSHIP TO PLANTS

AMF from the fungal phylum Glomeromycota colonise the roots of about 70% of the world's vascular plant species and thus provide the plant with important nutrients, mainly N and P, as well as water and protect it from pathogens (Smith and Read 2008). Thereby they can also alter plant responses to different global change parameters (Kivlin et al. 2013). In exchange, AMF receive photosynthetic carbon compounds which they invest in intra- and extra-radical fungal structures such as hyphae, spores and vesicles (Smith and Read 2008). Via their extra-radical hyphae AMF also positively affect soil aggregation and structure (Piotrowski et al. 2004; Wilson et al. 2009; Leifheit et al. 2014). While AMF are obligate symbionts, plant species can be either dependent on AMF, optional in a relationship with AMF, or completely non-mycorrhizal (Klironomos 2003; Bidartondo 2005; Smith and Read 2008). The AMF–

plant relationship is generally defined as mutualistic. However, in about 25% of studies it is described as parasitic (Johnson et al. 1997; Klironomos 2003; van der Heijden and Horton 2009; Johnson and Graham 2013). The functioning of the plant–AMF relationship has been conceptualised in different ways over the last few years (Smith and Smith 2015), for example as a mutualism–parasitism continuum (Johnson et al. 1997; Johnson and Graham 2013).

Individual plant species or groups of plant species display a varying responsiveness to different AMF species (Werner and Kiers 2015). The growth of subordinate plant species is for example promoted by AMF and the competitiveness of dominant species reduced (Urcelay and Diaz 2003; Mariotte et al. 2013a). In that way, AMF community composition seems to determine plant community composition (van der Heijden 2002). Altogether, AMF biodiversity and plant biodiversity are correlated (van der Heijden et al. 1998). Two possibilities to explain the drivers of interactions between AMF and plant communities are presented in the Driver/Passenger hypothesis by Hart et al. (2001): if AMF were Drivers, they would orchestrate the changes in the plant community. If they were Passengers, changes in their community composition would be a by-product of changes within the plant community. Zobel and Öpik (2014) add the Habitat hypothesis as a third possibility to explain concurrent changes in plant and AMF communities by abiotic conditions. First and last, the role of abiotic drivers and which community drives which remains to be determined (Hart et al. 2001; Zobel and Öpik 2014).

The advanced use of molecular tools in AMF research during the last few decades has illustrated that individual AMF species or whole species communities differ in their characteristics and needs. Knowledge on different traits of AMF species and their specific interaction with plants contributes to the development of new trait-based frameworks to understand symbiotic partner selection, specific adaptations to particular environmental conditions and eventually whole-ecosystem processes (van der Heijden and Scheublin 2007; Reinhart et al. 2012; Chagnon et al. 2013; Behm and Kiers 2014; Mccormack et al. 2014; Aguilar-Trigueros et al. 2015; Chagnon et al. 2015; Werner and Kiers 2015). Advances in research on their traits and functioning also serve to quantify and eventually predict soil aggregation and ecosystem productivity (van der Heijden and Scheublin 2007; Aguilar-Trigueros et al. 2015; Rillig et al. 2015).

2.3.3 AMF RESPONSE TO GLOBAL CHANGE

The effects of global change can affect AMF both directly and indirectly. For example, warming induces mineralisation of N compounds, which leads to an increase of N and P availability in the soil (Sierra et al. 2015), which in turn affects AMF community composition and properties as well as their relationship to plants (e.g. Lesica and Antibus 1986; Mullen and Schmidt 1993; Kivlin et al. 2013; Ren et al. 2013). Warming-induced shifts of host–plant community composition additionally affect AMF via the identity of their individual host plant and its (new) neighbours (van der Heijden et al. 2003; Hausmann and Hawkes 2009; Compant et al. 2010; Mohan et al. 2014). Warming also affects water availability: drought stress can have negative, positive or no effect at all on AMF. This variety of results might be due to AMF species-dependent reactions (Fitter et al. 2004; Compant et al. 2010; Sun et al. 2013; Mohan et al. 2014). Plants under drought stress are known to benefit from AMF symbiosis in many different aspects, such as greater leaf water content or an increased photosynthetic rate (Augé 2001; Mohan et al. 2014).

Direct effects of warming on AMF are mainly demonstrated by increased root colonization rates, but opposite or neutral effects of warming on AMF or their relationship to plants can also be found. Like other soil micro-organisms, AMF normally achieve their optimal performance at higher average temperatures than are found in mountain ecosystems. They might therefore increase their performance and biomass with increasing temperatures in previously cooler ecosystems (Tibbett and Cairney 2007; Margesin et al. 2009).

Furthermore, warming enhances carbon allocation to AMF (Compant et al. 2010; Kivlin et al. 2013; Mohan et al. 2014), an aspect that becomes even more interesting with regard to globally rising CO_2 levels. These can increase plant photosynthesis and thus the availability of carbohydrates for allocation to AMF. A positive effect of increased CO_2 on both AMF abundance and plant performance has been shown by several studies. In return, however, AMF showed no direct beneficial effects on plant performance under elevated CO_2 (Treseder 2004; Alberton et al. 2005; Kivlin et al. 2013). N and P enrichment in ecosystems is also known to directly affect AMF community composition, AMF abundance, the AMF colonisation rate and allocation depending on the degree of nutrient limitation of host plants in the ecosystem concerned (Treseder and Allen 2002; Johnson et al. 2003; Treseder 2004; Egerton-Warburton et al. 2007; Johnson 2010). The beneficial effect of AMF on plant performance commonly decreases with nutrient enrichment (Kivlin et al. 2013). Changes in

air chemistry - mainly increased presence of nitrogen oxides, sulphur dioxide and carbon dioxide - lead to acidification of soils. However, the effects of changes in air chemistry on soil organisms like AMF can be delayed to a certain extent because most soils feature a certain buffering capacity (Bellgard and Williams 2011). As a result, AMF performance and community composition in mountain ecosystems may at first be indirectly impacted by altered air chemistry through the changes in plant life forms and functional groups (Davison et al. 2011; López-García et al. 2014) induced by warming and increased nutrient availability. Different land use practices alter nutrient conditions in the soil as well. Besides that, they also affect AMF in a direct way through soil disturbance: AMF sporulation cycles, root colonisation rate, abundance and diversity differ between organic and conventional farming practices, between arable land and grassland (Oehl et al. 2009; Oehl et al. 2010) as well as between meadows and pastures (Morris et al. 2013). AMF abundance, root colonisation rate and diversity generally decrease as land use intensity increases (Oehl et al. 2009; Oehl et al. 2010; Morris et al. 2013).

2.3.4 TIME TO REASSESS

Due to their profound long-term effects on both vegetation and soil, research on AMF community composition, their traits and ecology under the conditions of future global change in mountain grasslands is important for predicting shifts in vegetation composition, carbon sequestration and soil stability in mountain ecosystems (Entry et al. 2002; Rillig 2004; Treseder and Turner 2007; Hawkes et al. 2008; Wilson et al. 2009). Twenty years ago, a review on mycorrhizal fungi in arctic and alpine tundra (Gardes and Dahlberg 1996) was published, followed several years later by another paper assessing the functioning of the symbiosis in cooler ecosystems including mountain ecosystems (Tibbett and Cairney 2007). Since then research on AMF in mountain ecosystems has progressed, also due to new laboratory methods and techniques. Here we summarise research on AMF in grasslands along altitudinal gradients. We present a working hypothesis on the functioning of the AMF–plant interaction along altitudinal gradients: we expect this interaction to change along the mutualism–parasitism continuum following changes in environmental stress. Based on this hypothesis we propose future research directions. In the second part we present the state of the art of the research on AMF in mountain grasslands under global change, attempt to predict the functioning of the AMF–plant interaction along the mutualism–parasitism continuum at

different altitudes and highlight the considerable potential for future research on mountain AMF and global change.

2.4 AMF IN MOUNTAIN ECOSYSTEMS

2.4.1 AMF PERFORMANCE AND DISTRIBUTION ALONG ALTITUDINAL GRADIENTS

Research on AMF distribution and root colonisation along altitudinal gradients has gained increased attention over the last 10 years (App. 1). We note a concentration of studies in the European Alps, the Rocky Mountains and the Chinese Tibetan Plateau, but no studies from an African mountain range or mountains in Australia were found (Fig. 2.1).

Fig. 2.1: Worldwide distribution of studies on AMF in mountain ecosystems: studies focus on A – altitudinal gradients, C – climate change, F – fertilisation and nutrient enrichment, H – habitat patchiness and environmental gradients, I – host identity, L – land use change, S – succession and V – season

Published studies show that AMF commonly colonise plant roots from mountain foothill zones up to as high as 5391 m above sea level (asl) in the Peruvian Andes (Haselwandter and Read 1980; Read and Haselwandter 1981; Cripps and Eddington 2005; Schmidt et al. 2008;

Ranelli et al. 2015). The colonisation rate varies widely between plant hosts and among studies from an approximately 13% average colonisation rate (e.g. Lugo et al. 2012; Rudgers et al. 2014) to more than 70% (e.g. Ruotsalainen et al. 2004; Kagawa et al. 2006) of root lengths colonised by AMF. Inter-annual AMF colonisation rates are consistent (Ranelli et al. 2015) but can vary seasonally (Lugo et al. 2003; Sun et al. 2013). Spore density also displays seasonal patterns (Lugo and Cabello 2002).

The intensity of AMF root colonisation, spore density as well as the abundance of phospholipid fatty acid (PLFA) biomarkers for AMF in the soil microbial community generally declines with increasing altitude (Read and Haselwandter 1981; Haselwandter 1987; Väre et al. 1997; Ruotsalainen et al. 2004; Cripps and Eddington 2005; Lugo et al. 2008; Budge et al. 2011; Gai et al. 2012; Li et al. 2015). This is in line with an observed vegetation cover increase of non-mycorrhizal plants as well as the increase in the proportion of plants in symbiosis with other mycorrhiza than AMF with increasing altitude (Väre et al. 1997). The percentage of total root length colonised by vesicles or arbuscules is not affected and does not decrease with rising altitude in most studies (Schmidt et al. 2008; Lugo et al. 2012; Li et al. 2014). Vesicles are regarded as AMF storage organs (Smith and Read 2008), and therefore their proportion of intra-radical colonisation is expected to be higher in extreme environments where storage organs are necessary to survive times of adverse conditions. Oehl and Körner (2014), for example, report the abundance of multiple vesicles in plant roots from an ice-free crest at 4545m asl in the Swiss Alps, the most extreme place showing evidence of a well-established AMF community, which is comparable to AMF communities in alpine and mountain grasslands (Oehl and Körner 2014).

Different colonisation rates and abundance levels of AMF structures in soil and root can also be the expression of different AM fungal traits in a changing AMF community along the altitudinal gradient, depending on host plant species and on changing environmental conditions according to different seasons (e.g. Bever et al. 1996; Egerton-Warburton and Allen 2000; Oehl et al. 2009). The AMF community composition along altitudinal gradients is documented in many studies by identification of colonisation type and spores (Crush 1973; Haselwandter and Read 1980; Lugo et al. 2008; Gai et al. 2009; Zubek et al. 2009; Gai et al. 2012; Shi et al. 2014; Coutinho et al. 2015) as well as PCR-based genetic analysis (Li et al. 2014; Li et al. 2015). It differs between root and soil (Liu et al. 2012; Yang et al. 2013), in accordance with other observations (Hempel et al. 2007; Varela-Cervero et al. 2015).

Different AMF species are active or inactive (dormant as spores) under varying environmental conditions. Some AMF species are known for example to be active under cooler or wetter environmental conditions (seasons) and dormant during warmer conditions (Pringle and Bever 2002; Oehl et al. 2006; Oehl et al. 2012b), while other are active under exactly opposite conditions and still others do not display seasonal patterns at all (Oehl et al. 2009). It is no surprise therefore that AMF diversity in mountain ecosystems also changes with varying conditions of temperature and drought depending on the season of the year (Lugo and Cabello 2002), which is displayed in a seasonal variation of different mycorrhizal traits such as sporulation or root colonisation (Lugo et al. 2003; Sun et al. 2013). However, diversity indices are not always affected by altitude. While AMF species richness decreases with increasing altitude (Lugo et al. 2008; Gai et al. 2012; Shi et al. 2014) other diversity indices do not show consistent patterns across studies and mountain ranges. α-diversity calculated by Shannon-Wiener index either decreases with increasing altitude (Shi et al. 2014) or is not affected by it (Gai et al. 2012) and β-diversity assessed by Whittaker's index indicates that the heterogeneity is increased with increasing altitude (Shi et al. 2014).

All in all, up to more than 60 different AMF species are found in mountain ecosystems (Oehl and Sieverding 2004; Shi et al. 2014). Acaulosporaceae and above all Glomeraceae are the dominant AMF families reported from altitudinal studies (Sýkorová et al. 2007; Gai et al. 2012; Li et al. 2014; Zheng et al. 2014; Coutinho et al. 2015). The abundance of Glomeraceae decreases with altitude, whereas species of other genera tend to increase (Li et al. 2015). Altitude favours the intra-radical colonisation of fine root endophytes (Crush 1973; Haselwandter and Read 1980; Read and Haselwandter 1981; Ruotsalainen et al. 2004; Zubek et al. 2009), also described as *Glomus tenuis* (Hall 1977), which again shows that different fungal traits are displayed at higher altitudes what could also be caused by a change of AMF community composition.

Taxonomic studies lead to the conclusion that both generalist AMF and unique specialised AMF species are common in mountain ecosystems (Liu et al. 2011; Oehl and Körner 2014; Liu et al. 2015a) and several new AMF species from mountain ecosystems are described (Oehl and Sieverding 2004; Oehl et al. 2006; Spain et al. 2006; Oehl et al. 2011a; Oehl et al. 2012a; Oehl et al. 2012b; Palenzuela et al. 2013) and distinct AMF communities reported (Liu et al. 2015a). Species such as *Acaulospora alpina*, *Acaulospora nivalis* and *Ambispora reticula*, which have been extracted exclusively from mountain grasslands, might be

characteristic of these ecosystems and may have as yet unidentified adapted mycorrhizal traits (Oehl et al. 2006; Oehl et al. 2012a; Oehl et al. 2012b). So far, research cannot account for the underlying mechanisms of species diversity and distribution along altitudinal gradients, as different studies and methods are difficult to compare. When considering growth-limiting factors, plant-derived carbon is the main factor for AMF (Chagnon et al. 2013). This is reflected in a model for optimal AMF colonisation and advantage for the plant along altitudinal gradients, which suggests that the host plant's photosynthetic nutrient use efficiency determines AMF colonisation along altitudinal gradients when nutrient concentration remains constant (Ruotsalainen et al. 2002). More research evaluating the Driver/Passenger and Habitat hypotheses (Hart et al. 2001; Zobel and Öpik 2014) in mountain ecosystems will provide answers.

As shown, altitude is only a weak predictor for AMF distribution and colonisation (Ranelli et al. 2015). AMF distribution does not necessarily have an altitudinal limit but is determined by multiple other co-occurring variables such as land use intensity, water availability, soil structure and composition and AMF propagule availability with decreasing vegetation cover (Haselwandter and Read 1980; Lesica and Antibus 1986; Cázares et al. 2005; Schmidt et al. 2008; Gai et al. 2009; Casanova-Katny et al. 2011; Oehl et al. 2011b; Coutinho et al. 2015; Liu et al. 2015a), which can blur the altitudinal effect. In the following we discuss factors other than altitude that determine AMF presence and performance in mountain ecosystems.

2.4.2 THE ROLE OF HABITAT PATCHINESS AND ENVIRONMENTAL GRADIENTS

Mountain ecosystems with their environmental changes over short distances lead to a visible mosaic of plant communities. Both abiotic and biotic factors are known to condition AMF distribution and performance (Entry et al. 2002; Kivlin et al. 2011; Soudzilovskaia et al. 2015). Research on whether the belowground AMF mosaic mirrors the aboveground patchiness helps to disentangle environmental factors from the host plant impact (Gardes and Dahlberg 1996; Barnola and Montilla 1997; Cripps and Eddington 2005; Becklin and Galen 2009; Ranelli et al. 2015) and eventually understand if AMF are Drivers and assist plants in defining their ecological niche (Hart et al. 2001; de Carvalho et al. 2012; Zobel and Öpik 2014; Li et al. 2015). We found few studies that directly address habitat patchiness and localised spatial environmental gradients in mountain ecosystems (App. 1).

Water availability is found to be a determining factor for the colonisation rate in several studies conducted in mountain grassland settings: wetter patches display lower AMF root colonisation (Barnola and Montilla 1997; Rudgers et al. 2014) and higher abundance of extra-radical hyphae (Rudgers et al. 2014) than dryer patches. Soil texture, a property directly linked to retaining and soaking water as well as drainage, also determines AMF richness (De Carvalho et al. 2012).

In another study, the variation of light, nutrient availability and host-specific life strategies leads to a mosaic of mycorrhizal patterns that mirrors plant patterns above (Becklin and Galen 2009). Eventually, all of these studies partly confirm the Habitat hypothesis (Zobel and Öpik 2014) and suggest that numerous environmental parameters determine AMF community composition and AMF performance (Barnola and Montilla 1997; Becklin and Galen 2009; de Carvalho et al. 2012; Rudgers et al. 2014; Zhang et al. 2014; Ranelli et al. 2015). Changing AMF parameters along different environmental gradients can also be interpreted as expression of different fungal traits, which could also indicate a change in AMF community composition along these gradients. The development of AMF community composition, their dispersal capacities as well as the drivers of plant–soil–AMF interactions can be studied especially well along successional sequences.

2.4.3 AMF ALONG SUCCESSION GRADIENTS

Successional sequences ranging from 0 to 135 years in the forefront of glaciers, after volcanic eruptions and mechanical anthropogenic disturbances have allowed research along developmental gradients in mountain ecosystems in the USA, the European Alps, the Brazilian Altiplano and Japan (Allen et al. 1984; Allen et al. 1987; Allen et al. 1992; Titus et al. 1998; Titus and Del Moral 1998; Titus and Tsuyuzaki 2002; Wu et al. 2004; Fujiyoshi et al. 2005; Cázares et al. 2005; Wu et al. 2007; Liu et al. 2011; Oehl et al. 2011b; Duchicela et al. 2013; Welc et al. 2014).

Plants in their initial successional stages, emerging after glacier retreat or on volcanic substrates, are not in relationship with AMF (Allen et al. 1984; Allen et al. 1992; Titus and Tsuyuzaki 2002; Fujiyoshi et al. 2005; Cázares et al. 2005; Oehl et al. 2011b). Heat sterilisation and subsequent gas emissions after volcanic eruptions can lead to an inhibited and slow reestablishment of fungal propagules. Moreover, soil-borne AMF spores together with extra-radical hyphae comprise low-mobility fungal propagules. This is illustrated on the

pumice plain of Mount St. Helens where AMF symbiosis was established only after the arrival of ants and rodents (Allen et al. 1984; Allen et al. 1992). Distribution by birds, as described by other studies from lowland ecosystems (Nielsen et al. 2016), is also conceivable but has not yet been reported from mountain ecosystems. Spores of some smaller species are also transported by wind, but bigger spores and extra-radical hyphae might arrive in bare soils mainly through animals, erosion or events such as landslides (Allen et al. 1984; Allen et al. 1987; Allen et al. 1992; Cázares et al. 2005; Oehl et al. 2011b; Oehl and Körner 2014). Moreover, since AMF are obligate symbionts, it may be concluded that, as stated in the Passenger hypothesis (Hart et al. 2001), AMF follow the development of the vegetation community rather than precede it during primary succession (Titus et al. 1998; Hart et al. 2001; Cázares et al. 2005; Oehl et al. 2011b; Zobel and Öpik 2014). The development of AMF–plant interaction is, however, not only dependent on propagule availability or successional age. Microhabitat factors such as soil moisture, nutrient availability, plant seed trapping ability and light conditions affect AMF distribution across different successional sequences in mountain ecosystems as well (Allen et al. 1987; Titus et al. 1998; Titus and Tsuyuzaki 2002). A linear improvement of soil aggregation due to the fungal community and in particular mycorrhiza during secondary succession (Duchicela et al. 2013) is not always observed (Liu et al. 2011).

Changing AMF parameters along successional sequences in mountain ecosystems with advancing soil successional stage, namely increased AMF propagule abundance and increased species richness in the soil as well as a decreased intra-radical colonisation rate (Allen et al. 1987; Fujiyoshi et al. 2005; Cázares et al. 2005; Liu et al. 2011; Oehl et al. 2011b; Welc et al. 2014), suggest a concurrent underlying change in AMF community composition with progressing succession. At first view this seems in accordance with a trait-based C-S-R (competitor, stress tolerator, ruderal) framework (Chagnon et al. 2013), which suggests that early successional stages are dominated by ruderal AMF, fast-growing and early-sporulating species, followed by a dominance of competitor AMF, species investing mainly in resource acquisition by growing extra-radical mycelium, which are finally replaced by stress-tolerant AMF, species that cope well with low carbon input. Whether AMF species distribution along successional sequences indeed follows the proposed C-S-R framework remains to be examined. Additionally a transition of the dominance in mycorrhizal types from AMF to other mycorrhizal types such as ecto-mycorrhizae has been observed and fits the shift of the

vegetation community to a dominance of woody plants with increasing successional stages (Welc et al. 2014).

Based on studies on habitat patchiness and succession in mountain ecosystems, we conclude that abiotic soil properties determine AMF community composition and performance only to a certain extent and that other factors such as plant species identity, vegetation cover and neighbouring plants have to be taken into account (Cázares et al. 2005; Liu et al. 2011; Oehl et al. 2011b; Welc et al. 2014).

2.4.4 THE ROLE OF HOST IDENTITY AND ITS NEIGHBOURING PLANTS

Several studies in mountain ecosystems suggest that host identity is more important for the colonisation rate and AMF community than environmental parameters (Lugo et al. 2008; Becklin et al. 2012; Rudgers et al. 2014; Zubek et al. 2014; Welc et al. 2014; Ranelli et al. 2015). In some studies, the intra-radical colonisation rate, for example, is strongly determined by host plant identity and its evolutionary history in mountain ecosystems (Rudgers et al. 2014; Ranelli et al. 2015). However, it was not possible to regroup specific AMF colonisation patterns by plant functional groups or metabolic type in the available studies set in mountain ecosystems (Lugo et al. 2008; Ranelli et al. 2015). Different plants growing in the same habitat patch can host different AMF communities. This is visibly expressed in different colonisation rates and clearly suggests host specificity of AMF communities in mountain ecosystems (Sýkorová et al. 2007; Becklin et al. 2012; Li et al. 2014). In the study reported by Sýkorová et al. (2007), a strong host preference of AMF species was visible. Only *Glomus intraradices* sequences are present in the closely related target species *Gentiana verna* and *Gentiana acaulis* at 1800 and 2000 m asl in the Swiss Alps. However, not all studies support this (Li et al. 2015).

Alien plant species or weeds in mountain ecosystems were less dependent on AMF symbiosis (Becklin and Galen 2009; Casanova-Katny et al. 2011). However, the use of local AMF inoculum promotes the native plant community after the removal of alien plant species (Rowe et al. 2007; Rowe and Brown 2008), indicating that a specific local AMF community might nevertheless provide a certain barrier to plant invasion. The interaction of invasive plant species with AMF in mountain ecosystems was considered in only a few studies and leaves room for more research. All in all, the role of abiotic drivers (Zobel and Öpik 2014) and

whether AMF are Drivers or Passengers (Hart et al. 2001) in mountain ecosystems remains to be determined.

2.4.5 AMF INTERACTION WITH OTHER SOIL MICROBES AND ABOVE-GROUND SYMBIONTS IN MOUNTAIN ECOSYSTEMS

AMF are also studied in context with other soil organisms such as bacteria or other fungi (e.g. Haselwandter and Read 1980; Väre et al. 1997; Cripps and Eddington 2005; Budge et al. 2011; Zubek et al. 2011; Vanesa et al. 2013; Ranelli et al. 2015) as well as above-ground endophytes (Ranelli et al. 2015) in mountain ecosystems. Factors such as host plant species (Haselwandter and Read 1980; Cripps and Eddington 2005; Ranelli et al. 2015), successional stage (Welc et al. 2014) and pH (Ruotsalainen and Eskelinen 2011) appear to have more direct influence on other soil microbes and above-ground endophytes than their interaction with AMF and vice versa. However, not a lot of evidence exists because all but few studies in mountain ecosystems ignore that multiple symbiotic interactions can have different effects on the host plant than pair-wise interactions alone. In multiple symbiotic interactions, as they exist for example when both foliar endophytes and root endophytes colonise a plant, it is likely that those symbionts also affect each other, especially when they receive similar benefits from the host plant (Mack and Rudgers 2008). Ranelli et al. (2015), to our knowledge the only study that considered the interaction between above-ground plant endophytes, DSE and AMF in mountain grassland, see no interaction of above-ground and below-ground endophytes and report that DSE and AMF were positively correlated. Including other endophytes and their effects into future studies will help to get a clearer picture of the actual effect mechanisms driving AMF presence in mountain ecosystems. Interaction of AMF with non-fungal soil organisms is rarely studied, because it is necessary to manipulate both underground microbes and AMF. This is difficult in the field, hence the lack of realistic field studies (van der Heijden et al. 2003).

Many studies along altitudinal gradients report additional root colonisation by dark septate endophytes (DSE; e.g. Haselwandter and Read 1980; Read and Haselwandter 1981; Ruotsalainen et al. 2004; Cripps and Eddington 2005; Zubek et al. 2009; Vanesa et al. 2013; Oehl and Körner 2014; Ranelli et al. 2015). Their increased abundance with increasing altitude and other stress factors, e.g. high salinity (Vanesa et al. 2013), leads to the assumption that DSE and other septic fungi take over the mutualistic part for AMF in high-altitude ecosystems (Haselwandter and Read 1980; Read and Haselwandter 1981; Urcelay et al. 2011;

Vanesa et al. 2013), or at least have complementary functions (Ranelli et al. 2015), which is debated by other studies (Ruotsalainen et al. 2002; Ruotsalainen et al. 2004; Cripps and Eddington 2005; Zubek et al. 2009). Vanesa et al. (2013)

DSE are described as fungi with darkly pigmented, septate hyphae (Jumpponen and Trappe 1998; Jumpponen 2001; Wu and Guo 2008), which colonise plant roots from many families. They are particularly common in cold ecosystems such as the arctic (Jumpponen and Trappe 1998) or mountain areas (Haselwandter and Read 1980; Read and Haselwandter 1981) and are also present at the coldest place known for angiosperm plant life (Oehl and Körner 2014). DSE can be beneficial to the plant, but their functioning and importance for plants and ecosystems remains unclear (Jumpponen and Trappe 1998; Jumpponen 2001). They are reported to affect biomass and growing height in alpine plants (Wu and Guo 2008). DSE systematics is still under development and spore, conidiophore and DNA sequences have not yet been identified in different DSE species (Oehl and Körner 2014).

2.4.6 APPLYING THE PRESENT KNOWLEDGE

Knowledge on AMF–plant–soil interactions is important for the conservation and reintroduction of endangered plant species, especially where highly specialised endemic species are concerned (Schmid et al. 2008; Zubek et al. 2011; Binet et al. 2011; de Carvalho et al. 2012). Binet et al.'s study (2011) provides useful information on the application of AMF inoculum that is native to alpine areas of the endangered aromatic plant species Artemisia umbelliformis when they are grown in field culture. Local field inoculum was also the best choice to reduce cheat grass (Bromus tectorum) invasion and promote the reestablishment of the late-successional native vegetation community for a high-altitude grassland in the Rocky Mountains (Rowe et al. 2007; Rowe and Brown 2008). Advances in knowledge on AMF in mountain ecosystems will help to professionalise the application of AMF inoculum for restoration and revegetation.

2.4.7 OUTLINING THE FUNCTIONING OF AMF–PLANT INTERACTION ALONG AN ALTITUDINAL GRADIENT

The variation of ecosystems studied and methods used makes it difficult to formulate reliable general conclusions about the underlying mechanisms of AMF species diversity, distribution, performance and, finally, their interactions with plants along altitudinal gradients. In general the assembly of many different ecosystems abide by the mechanisms of negative interactions

(competition) and positive interactions (facilitation) (Brooker and Callaghan 1998; Bruno et al. 2003; Kawai and Tokeshi 2007). The stress gradient hypothesis (SGH) conceptualises the existing balance between positive and negative interactions along environmental stress gradients (Menge and Sutherland 1987; Daleo and Iribarne 2009). The theory that competition among interacting organisms under benign environmental conditions turns into facilitation as adverse environmental conditions increase in severity is based on the SGH. But interactions turn back into competition when environmental stress is extreme (Bertness and Callaway 1994; Brooker and Callaghan 1998; Kawai and Tokeshi 2007), following a humped-back model first described by Grime (1973) and recently reassessed by Michalet et al. (2006). Plant–plant interactions along altitudinal gradients, for example, change from competitive interactions to facilitation with increasing altitude (Choler et al. 2001; Callaway et al. 2002; Michalet et al. 2014). Evidence for the SGH was also presented for plant-microbe interactions along an altitudinal gradient, when fertile soil at low altitudes was observed to hold a microbial community with an overall negative effect on pine seedling growth while soil at higher altitudes was less fertile and hold a microbial community that promoted pine seedling growth (Wagg et al. 2011).

While Wagg et al. (2011) focused on ecotmycorrhizal fungi and DSE, the role of the AMF–plant interaction has not been considered along altitudinal gradients so far, even though AMF are known to have a facilitating impact on plant–plant interactions (van der Heijden and Horton 2009; Casanova-Katny et al. 2011; Montesinos-Navarro et al. 2012) in addition to their direct effect on plants and plant communities. From the available studies set in mountain ecosystems, we can see that most of the AMF parameters decrease with increasing altitude. This either suggests that the plant–AMF relationship becomes less important for plant performance and/or that a change occurs in AMF community composition to more stress-tolerant AMF species with increasing temperature stress. For further investigation of this matter, we propose the working hypothesis that, just like plant–plant interaction shifts from competition to facilitation, the functioning of the AMF–plant relationship shifts towards the more mutualistic end of the parasitism–mutualism continuum along altitudinal gradients. Based on the SGH and considering the main environmental stress factors (nutrients, land use changes, temperature and water availability), we propose that plant–AMF interaction follows a humped-back model and becomes more mutualistic with increasing altitude and simultaneously increasing temperature stress. We presume that stress due to land use changes and lower water availability are not severe enough in the foothill zone to induce a shift

towards a more mutualistic AMF–plant relationship. The best mutualistic state is expected in the subalpine zone. At higher altitudes (alpine to the nival zone) a renewed shift towards the parasitic end of the continuum is to be expected when temperature and nutrient stress become severe (Fig. 2.2). However, the currently available data make it impossible to grade the observed change of AMF traits with increasing altitude along a parasitism–mutualism continuum. Future studies should consider the AMF effects on plants and plant–plant interactions as an important factor for the vegetation assembly and distribution along altitudinal gradients.

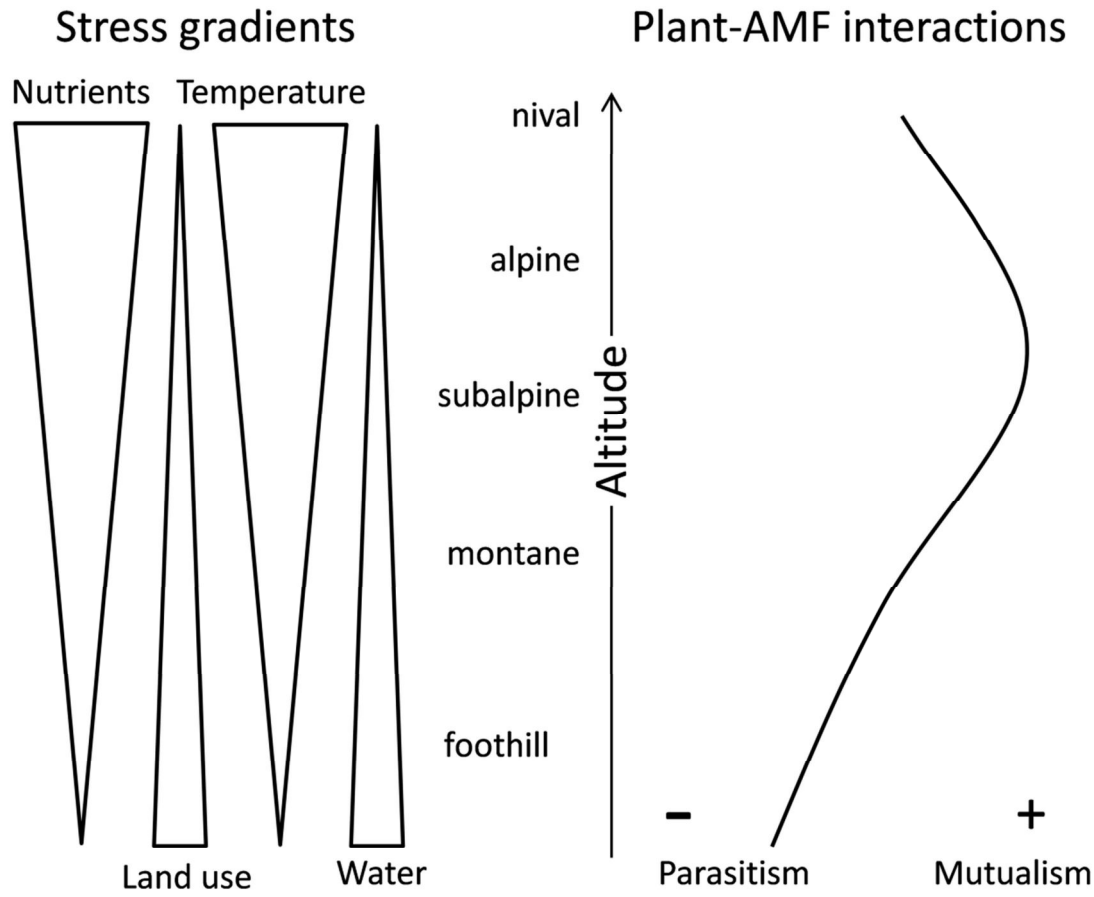

Fig. 2.2: Plant–AMF interactions along the parasitism–mutualism continuum depending on four main environmental stress factors: nutrients, land use, temperature and water availability. The relationship becomes more mutualistic with increasing altitude and simultaneously increasing temperature and nutrient stress. The highest benefit is expected in the subalpine zone. Higher up from the alpine to the nival zone, a renewed shift towards the parasitic end of the continuum is to be expected when temperature stress becomes more extreme.

2.4.8 FUTURE RESEARCH ON AMF ALONG ALTITUDINAL GRADIENTS

To test our working hypothesis and assess the cost and benefit of AMF symbiosis for plants along altitudinal gradients, the presence of AMF needs to be manipulated in the field. Fungicides such as benomyl for the removal of AMF in the field are simple to apply and can be used for a larger area (Kahiluoto and Vestberg 2000). However, benomyl also affects other fungi and organisms in the microbial community (Edgington et al. 1971), which, with varying persistence and effectiveness in plant roots and soil, leads to undesirable side effects (Pedersen and Sylvia 1997). Purely AMF-related effects are not achieved with the application of fungicides. In contrast, in-growth cores (Johnson et al. 2001) and other constructions (e. g. Allison et al. 2013) made out of nylon material of low mesh size (0.45 - 35 μm) allow the manipulation of AMF origin and AMF colonisation at the individual plant level. The low small pores allow water, nutrients and smaller soil microbes like bacteria to pass through and, depending on the chosen mesh size, retaining either only plant roots or both plant roots and AMF extra-radical hyphae and spores (Johnson et al. 2001; Allison et al. 2013). It seems that the potential for their use in the field has not yet been fully exploited. We suggest that manipulation techniques like these or modified versions are, for now, the key to obtaining information on the functioning of AMF symbiosis along the parasitism–mutualism continuum and altitudinal gradients.

In general, we encourage a coordinated continuation of the current research efforts, ideally in intercontinental long-term studies across all altitudes, different ecosystem types and all longitudes with standardised methods. These studies should additionally characterise seasonal patterns and different host plant traits. We suggest the repeated sampling of root and soil samples on several sampling dates per year over several years. Analysing these samples using the newest molecular techniques provides a refined determination of AMF species and their specific traits. This will primarily contribute to answer questions concerning the drivers of AMF community composition and distribution, but also provide important information on AMF effects on soil aggregation and ultimately give some idea about their contribution to carbon stock in mountain grasslands.

We further emphasise the importance of studies on AMF effects on plant–plant interaction under both simplified greenhouse conditions and more realistic but also more complex field conditions. In most studies, target species were plant species that dominate the local vegetation community. Consequently, mainly grass species were studied (see e.g. Lugo et al.

2012; Rudgers et al. 2014; Li et al. 2015; Ranelli et al. 2015). However, AMF are known to decrease the competitiveness between dominant and subordinate plant species (Mariotte et al. 2013a) and the presence or absence of subordinate plants can in turn affect soil processes and the microbial community (Mariotte et al. 2013b).

2.5 AMF IN CHANGING MOUNTAIN ECOSYSTEMS

2.5.1 CLIMATE CHANGE: THE EFFECTS OF WARMING AND ALTERED PRECIPITATION ON AMF IN MOUNTAIN ECOSYSTEMS

Field studies on climate change and AMF in mountain ecosystems are set at different altitudes ranging from 1324 m asl (Sun et al. 2013) up to 4659 m asl (Zhang et al. 2014) with mean annual temperatures between −3.8°C (Zhang et al. 2014) and 2°C (Sun et al. 2013; Kim et al. 2014). Mean annual precipitation is generally lower at the Asian sites (380–500 mm), but up to 2000 mm at other sites, e.g. in the Swiss Alps (Budge et al. 2011). Methods used to simulate warming differ between studies. Active artificial heating (Yang et al. 2013; Sun et al. 2013; Kim et al. 2014; Rudgers et al. 2014), passive heating using open top chambers (Zhang et al. 2014) or downward transplantation along the altitudinal gradient (Budge et al. 2011) were used to simulate a temperature increase from 1 to 3 K over 2–20 years. All available studies but one (Sun et al. 2013) do not actively simulate altered precipitation but observe dryer conditions as a side effect of the warming treatment.

Short-term studies (2 – 3 years of treatment) found no warming effect on the intra-radical colonisation rate (Yang et al. 2013; Sun et al. 2013). In the long term however, a plant species-dependent shift of AMF investment into extra-radical hyphae towards intra-radical hyphae was observed with warming and decreasing water availability after 20 years (Rudgers et al. 2014), which may be interpreted as a change in the dominant AMF species in the community displaying different life history traits. While there is no visible warming effect on the AMF colonisation rate of sedge roots and *Poa pratensis* roots, a soil moisture-dependent increase of the intra-radical colonisation rate is visible in the dominant grasses *Achnatherum lettmannii* and *Festuca thuberi* as well as in the herb *Artemisia tridentate*. It seems impossible to group the varying reactions to warming according to plant families or functional traits, and no correlation was found between graminoid diversity and the AMF root colonisation rate in this study (Rudgers et al. 2014).

In contrast to lowland and greenhouse experiments (Heinemeyer and Fitter 2004; Hawkes et al. 2008), no warming effect on extra-radical mycelium was observed in mountain ecosystems after 1 °C warming for up to 5 years (Yang et al. 2013; Kim et al. 2014). This suggests that other factors than warming affect the production of extra-radical hyphae under natural conditions. Twenty years of 2 °C warming in a subalpine meadow revealed that spatial water availability patterns were stronger than warming effects as the abundance of extra-radical hyphae decreased about 40% when environmental conditions in the soil were drier from the outset (Rudgers et al. 2014).

Warming significantly increases AMF spore density and the proportion of AMF biomarkers in the soil in semiarid grassland and in most of the wetter alpine grasslands studied (Budge et al. 2011; Yang et al. 2013; Kim et al. 2014; Zhang et al. 2014). This fits the results reported by Sun et al. (2013), who found that warming reduced the abundance of species sporulating little or rarely. It shows that the expression of AMF traits changes together with the varying dominant AMF species following changing environmental conditions. Lower soil microbial communities and AMF (10–20 cm) seem solely controlled by abiotic soil factors (Zhang et al. 2014), and warming effects were even more pronounced at this depth after 11 years of warming in Swiss mountain grasslands (Budge et al. 2011). In contrast, warming effects on the soil's microbial composition and AMF in the upper 10 cm are also dependent on vegetation parameters such as root abundance and photosynthesis efficiency (Kim et al. 2014; Zhang et al. 2014). In summary, it appears that spore but not extra-radical hyphae contribute to the increased proportion of AMF in the soil microbial community in mountain grasslands after experimental warming. We conclude that a shift of AMF species composition to AMF communities which are dominated by species investing more in spores than extra-radical hyphae explains this.

Several studies report that changed abiotic soil conditions due to warming influence AMF community richness (Rillig et al. 2002; Budge et al. 2011; Kim et al. 2014). Even though night-time warming only does not affect host plant photosynthesis, for example, it affects AMF species community composition via changes in soil organic carbon, N and respiration (Kim et al. 2014).

A warming-induced decrease of AMF community richness in general appears simultaneously with a decrease of plant community richness at the cost of grass and herb species and promoting sedge species, soil organic C content and total N levels (Yang et al. 2013; Rudgers

et al. 2014; Zhang et al. 2014). The varying AMF communities in root and soil react differently to warming (Yang et al. 2013; Sun et al. 2013; Kim et al. 2014), which changes the competitive balance between different AMF species (Sun et al. 2013). The abundance of Glomeraceae in soil, for example, increases with warming while the abundance of Gigasporaceae in soil decreases (Yang et al. 2013). Taking into account that species from the Gigasporaceae were shown to be less effective in stimulating soil aggregation than species of other AMF families (Piotrowski et al. 2004), a shift in species composition towards less Gigasporaceae and more Glomeraceae could enhance soil aggregation with warming in mountain ecosystems. Information on effects of single AMF species on soil aggregation, especially in mountain ecosystems, are scarce however (Li et al. 2015).

Considering the apparent influence of soil moisture content on warming effects, predicted shifts in precipitation patterns towards longer drought periods and heavier rainfall events might be as important for AMF in mountain ecosystems as warmer temperatures. However, we found only one study on AMF in mountain ecosystems that manipulated precipitation actively as a climate change factor in mountain grasslands (Sun et al. 2013). This reveals that a 30% precipitation increase over 1.5 years had no effect on the intra-radical root colonisation rate but on the AMF spore community composition in the soil. With its increased presence in the spore community, *Ambispora gerdemannii* can be identified as an indicator species for increased precipitation. The effects of precipitation, however, seemed pronounced only in the first half of the growing season, indicating that precipitation changes in spring might have stronger effects on AMF community composition in the soil than later in the year (Sun et al. 2013). AMF follow seasonal patterns (Sun et al. 2013), and so does the severity of the impact of climate change (IPCC 2013). Earlier snowmelt due to warmer spring temperatures, for example, negatively affects dry meadows by reducing water availability. On the other hand, it can have positive effects on wet meadows and tundra by extending their growing season (Rudgers et al. 2014). In particular, these wetter grassland ecosystems have a potential as carbon sink in presence of warmer temperatures (Zhang et al. 2014), another reason to grant them greater research interest.

From the few available studies on AMF and climate change in mountain grasslands, it appears that AMF respond rather slowly to warming and that soil moisture as well as soil organic content determine the warming effect on AMF independent of the host plant. It is difficult,

however, to draw sound conclusions because of the studies' different vegetation communities, simulation approaches, durations and AMF variables studied.

2.5.2 MOUNTAIN AMF AND ALTERED ATMOSPHERIC CHEMISTRY AND NUTRIENT ENRICHMENT

While studies on the effect of CO_2 enrichment on plants in mountain grasslands are common, research on how CO_2 affects AMF in the same environment is nearly inexistent. One explication could be that temperature effects are greater for AMF performance than CO_2 enrichment under cooler conditions (Gavito et al. 2003).

N and P are limiting elements in mountain ecosystems. Their increase affects plants from individual to community levels as well as the soil microbial community. Nevertheless, only a few recent field studies have assessed effects of N and P enrichment on AMF in mountain environments. One study was set in a tropical mountain pasture system in the Ecuadorian Andes with a mean annual temperatures of 15°C and 2176 mm mean annual precipitation (Tischer et al. 2015). All other available studies were conducted under alpine or arctic climatic conditions (mean annual temperatures between −2.6°C (Ruotsalainen and Eskelinen 2011) and 1.2°C (Liu et al. 2012; Yang et al. 2012; Liu et al. 2015b; Liu et al. 2015c), mean annual precipitation from 420 mm (Ruotsalainen and Eskelinen 2011) up to 1300 mm (Blanke et al. 2012) per year) on the Tibetan Plateau (Liu et al. 2012; Zheng et al. 2014; Liu et al. 2015b; Liu et al. 2015c), the Swiss Alps (Blanke et al. 2012) and north-western Finland (Ruotsalainen and Eskelinen 2011) at altitudes ranging from 700 m asl (Ruotsalainen and Eskelinen 2011) up to 3500 m asl (Liu et al. 2012; Yang et al. 2012; Liu et al. 2015b; Liu et al. 2015c). The duration of the varying N and/or P fertilising treatments stretches from one vegetation period (Blanke et al. 2012) over 4 years (Tischer et al. 2015; Liu et al. 2015c) to 8 years (Liu et al. 2012). The addition of P compounds ranged from 10 to 282 kg/ha/yr, while N addition ranged from 15 to 254 kg/ha/yr. Critical loads for N deposition in montane and alpine grasslands are 10–20 kg/ha/yr and 5–10 kg/ha/yr, respectively (Bobbink et al. 2010). So most of the studies reviewed herein simulate N additions, which are equal to or exceed critical loads by a factor ten to 20.

No effects of fertilisation on extra-radical hyphal length density are reported when separate N or P enrichment is 50 kg/ha/yr or lower (Zheng et al. 2014; Tischer et al. 2015). At higher fertiliser rates, addition of combined N and P compounds has no effect on extra-radical

hyphae after 4 years (Liu et al. 2015c) but is negatively correlated with a fertilisation intensity gradient from 64 N kg /ha/yr and 70 P kg/ha/yr up to 254 N kg/ha/yr and 282 P kg/ha/yr and concomitant decline of pH after 8 years of fertilisation treatment (Liu et al. 2012). Extra-radical hyphal length density also depends on the season and form of N application: ammonium enrichment up to 15 kg/ha/yr, for example, reduces the extra-radical hyphal length density in May but not in August (Zheng et al. 2014). This underlines once more the importance of taking seasonal patterns of nutrient availability into account when studying AMF. Pure ammonium seems to be the most suitable form of plant-available N to which plants have the best direct access without mycorrhizal assistance. Neither nitrate nor the combination of both nitrate and ammonium have a significant effect (Zheng et al. 2014) on extra-radical hyphae.

Like extra-radical hyphae, the spore density is not affected by NP fertilisation after 4 years (Tischer et al. 2015; Liu et al. 2015c). After 6 years of nitrate enrichment, spore density decreases marginally at application rates of 15 kg/ha/yr (Zheng et al. 2014). Eight years of fertilisation treatment reveal that spore density peaks at the lowest fertiliser rate and then decreased with increasing fertilisation (Liu et al. 2012). This might indicate a limitation of N and P that restricts AMF growth (Liu et al. 2012). Sporulation can also depend on different stages of soil fertility relative to the physiological traits of different AMF species (Treseder and Allen 2002). From the results of the available studies, it seems possible that the effect of fertilisation on sporulation or the shift towards AMF species that sporulate less is dependent on the duration of the fertilisation treatment. It is difficult, however, to draw satisfying conclusions given that original soil conditions and therefore nutrient limitations are quite different across study sites.

Fertilisation effects on the intra-radical colonisation rate vary widely among the different studies and depend on host plant identity: while 60 kg/ha/yr of additional P compounds significantly reduce the colonisation rate in one study (Blanke et al. 2012), P fertilisation of 10 kg/ha/yr leads to a higher colonisation rate in another (Tischer et al. 2015). The dominant grass species also react quite differently to 50 kg/ha/yr fertiliser treatment: the intra-radical colonisation rate decreases, for example, with increasing N+P addition in *Elymus nutans* roots after 8 years of fertilisation (Liu et al. 2012). In contrast, the colonisation rate increases in *Festuca violacea* roots after only one vegetation period of fertiliser treatment (Blanke et al.

2012) but does not affect the colonisation rate of the dominant ruderal grass species *Setaria sphacelata* (Tischer et al. 2015).

A decline of arbuscules, which are the main interface for plant–fungus nutrient exchange (Garbaye 2013; Gutjahr and Parniske 2013), at higher fertilisation levels (Liu et al. 2012) and combined shading (Liu et al. 2015c) suggests less reliance of plants on AMF for nutrient acquisition with increasing fertilisation. The dominant grass species seem to directly benefit from N fertilisation with increased biomass and a decrease in associated AMF species and root colonisation (Liu et al. 2012; Blanke et al. 2012). This fact is corroborated seeing that N addition induces a decrease of plant diversity and a shift of vegetation to ruderal grass species domination (Liu et al. 2012; Blanke et al. 2012). Resource competition seems to change from belowground competition for nutrients to aboveground competition for light (Liu et al. 2015c).

The alpine microbial community is often rather N-limited compared to microbial communities at lower altitudes (Körner 2003). Some studies suggest that AMF and plants in mountain grasslands compete for nutrients and that AMF only provide N to the host when their own demand is satisfied (Treseder and Allen 2002; Liu et al. 2012; Blanke et al. 2012; Tischer et al. 2015). Liu et al. (2012) observe that both spore density and the root colonization rate peak at intermediate application levels of 64 kg N and 70 kg P /ha/yr. Competition for nutrients exists, however, not only between plants and AMF, but also between different AMF species. A distinct decrease in the number of AMF phylotypes in the plant roots as well as spore species richness is observed with an increasing amount of fertilisation, while plant species diversity decreases simultaneously. Different AMF species prefer soils of different fertility levels (Treseder and Allen 2002; Liu et al. 2012). Their different life strategies can lead to the variation observed in AMF structures in soil and roots as is reported for example from a nutrient manipulation experiments in alpine grassland (Liu et al. 2012).

It is possible that different AMF species depend mainly on abiotic soil conditions (habitat hypothesis; Zobel and Öpik 2014) occur mainly under specific nutrient conditions. For example, *Archaeospora trappei* phylotype is specific for the roots from the highest fertiliser treatment (256 kg N and 282 kg P /ha/yr) in mountain grasslands, while certain phylotypes that are associated with Glomus were mainly observed in roots from the control plots (Liu et al. 2012). Species specific effects of AMF phylotypes to different nutrient levels is also reported from nutrient manipulation experiments in other mountain ecosystems, for example

from a tropical montane forest understorey (Camenzind et al. 2014; Camenzind et al. 2016). On the other hand, if we assume that AMF are merely passengers in the plant community (Hart et al. 2001; Zobel and Öpik 2014), it is also possible that the nutrient induced change of plant species or plant functional groups is the driver of the AMF community. Supporting evidence for this possiblity exists from mountain ecosystems where, following fertilisation treatment, a reduction of AMF species richness in grass roots by half and a reduction of root colonisation of grass roots by a quarter was observed, while other mixed or forb root where not affected (Ruotsalainen and Eskelinen 2011; Liu et al. 2012; Blanke et al. 2012). A nutrient-induced loss of plant diversity which promotes graminoid or sedge dominance is therefore expected to have a significant impact on the community composition of AMF in mountain grasslands (Haselwandter and Read 1982; Muthukumar et al. 2004; Li et al. 2015).

The manipulation of AMF presence in one study (Blanke et al. 2012) suggests that AMF had little influence on seedling establishment under both future and current nutrient conditions in an alpine meadow. Only the non-mycorrhizal Carex sempervirens benefits with higher biomass from P fertilisation in the absence of an intact AMF-hyphal network in this study, probably because neighbouring mycorrhizal species could not access the additional P without AMF (Blanke et al. 2012). This underlines the importance of AMF effects on plant–plant interaction.

In summary, we can say that the negative effects of fertilisation on AMF structures and richness in mountain soil and plant roots are mainly related to fertilisation intensity and soil nutrient conditions at the outset and that the role of host plant identity as well as plant–plant interactions needs to be closely reviewed.

2.5.3 LAND USE CHANGE

Studies on AMF and land use change in mountain grasslands focus on changes in grassland management practices (Frank et al. 2003; Lugo et al. 2003; Börstler et al. 2006; Su and Guo 2007; Ruotsalainen and Eskelinen 2011; Yang et al. 2013) as well as abandonment (Duchicela et al. 2013) and disturbance by infrastructure or mining (Allen et al. 1987; Liu et al. 2011). Abandonment of fields and the cessation of mining or road work activities can also be seen as the introduction of a succession, as discussed above.

Although understanding the grazing impact on AMF seems to be important for the management and conservation of grassland ecosystems, only a few studies with contradictory

results concern mountain ecosystems. Here, the grazing effects on sites with cattle fenced in (Su and Guo 2007; Yang et al. 2013) are compared to sites where herbivores are excluded (Lugo and Cabello 2002; Frank et al. 2003; Lugo et al. 2003; Su and Guo 2007; Ruotsalainen and Eskelinen 2011). Grazing duration in the different studies varies from short, with only two to three vegetation periods (Ruotsalainen and Eskelinen 2011; Yang et al. 2013), intermediate, about 20 years (Lugo and Cabello 2002; Lugo et al. 2003; Su and Guo 2007), up to 40 years (Frank et al. 2003).

The responses of AMF structures in root and soil are as diverse as there are different studies. Herbivore types, grazing intensities and timing, target plant species, soil parameters and soil microbial community compositions vary widely in the different studies.

Moderately grazed AMF communities display higher spore abundance in one study (Frank et al. 2003), while over-grazing and its resulting strong reduction of plant diversity and vegetation cover from 80 to 20% in the Mongolian steppe leads to lower spore density in another (Su and Guo 2007). Furthermore, others report that grazing has no effect on spore density, extra-radical mycelium (Yang et al. 2013) or the root colonisation rate (Lugo et al. 2003), apart from interaction effects with seasonally changing water availability (Lugo and Cabello 2002) or depending on soil biotic conditions from the outset (Ruotsalainen and Eskelinen 2011). For example, overall spore density is not modified by grazing in the Argentinian pampa, but *Acaulospora laevis* shows higher sporulation with grazing and Scutellospora without grazing in the dry winter season, while the sporulation of some Glomales species are affected by grazing in the wetter spring season (Lugo and Cabello 2002). This corresponds to the findings of some of the climate change experiments described above (Sun et al. 2013; Kim et al. 2014; Zhang et al. 2014) and underlines the AMF species-specific preference of different soil conditions. Exclusion of mammalian herbivores has a negative impact on the intra-radical colonisation rate in fertile graminoid- and forb-dominated Dryas heaths, while it affects the colonisation rate positively in infertile ericoid dwarf shrub-dominated Empetrum heath in Finland. Whether grazing effects on AMF colonisation rate are positive or negative seems therefore related to the habitat dependent plant community composition rather than nutrient availability as such (Ruotsalainen and Eskelinen 2011).

Since grazing changes the allocation of C compounds to roots, it may result in a change of specific AMF receivers in the plant root (Yang et al. 2013). Soil mycorrhizal communities differ in grazed and ungrazed mountain grasslands (Frank et al. 2003) and both, direction and

extent of grazing effects on AMF richness and diversity in soil (Lugo and Cabello 2002; Frank et al. 2003; Su and Guo 2007; Yang et al. 2013) and roots (Yang et al. 2013) were dependent on AMF species community composition, grazing intensity and other soil factors. Combined grazing and warming, however, increase AMF richness in roots (Yang et al. 2013). Non-Glomus species are more affected by over-grazing than most of the Glomus species (Su and Guo 2007). Gigasporaceae decrease in soil due to grazing (Yang et al. 2013). This shows that different AMF species deal differently with a reduced carbon supply, and that the research on AMF traits is key to understand environmental effects on AMF. An experiment on mowing – altogether a more unselective removal of plant biomass than grazing – supports this with the finding that AMF species richness in the soil and roots is not affected by either extensive or intensive mowing, while AMF species community composition is changed (Börstler et al. 2006).

Frank et al. (2003) found that dominant grass species grew better with microbial community from grazed soil. This could be useful for restoration and conservation schemes. Special seed mixtures with integrated AMF inoculum for restoration purposes following disturbance by winter sport activities on ski slopes, for example, are already offered by commercial suppliers in the European Alps. Several studies show that AMF inoculum can be used to improve the restoration of mountain grassland (Rowe et al. 2007; Rowe and Brown 2008; Schmid et al. 2008), which is necessary due to different land use practices.

2.5.4 INTERACTION OF GLOBAL CHANGE FACTORS

Global environmental change has many different aspects and a multitude of interfaces. Four main categories can be distinguished – atmospheric gases, climate change, land use change, and biotic exchange – but most of the studies addressing global change and AMF in mountain grasslands only focus on one aspect. Studies combining several global change factors reveal that interaction effects are changing the general picture of the AMF response to global change (Ruotsalainen and Eskelinen 2011; Yang et al. 2013; Liu et al. 2015c). A combination of warming and grazing in an alpine meadow, for example, strongly increases the abundance of certain AMF species, while single treatments have a strong opposite effect (Yang et al. 2013).

2.5.5 OUTLINING THE FUNCTIONING OF THE AMF–PLANT RELATIONSHIP ALONG AN ALTITUDINAL GRADIENT UNDER FUTURE GLOBAL CHANGE CONDITIONS

Contrasting results of a single global change factor and interaction of several global change factors clearly demonstrate that it is urgent to address their interaction effects. Only when we assess the larger picture for AMF in changing mountain ecosystems with all its dynamic complexity can we understand their current effects on ecosystem processes and make assumptions about future consequences. In general we expect a changed functioning of the AMF–plant relationship along the altitudinal gradient, just as multiple stress factors along one gradient can affect the shift from competition to facilitation differently than a single factor (Kawai and Tokeshi 2007). Future global change conditions will affect the main stress factors along altitudinal gradients: 1) nutrient deposition is increasing even in remote mountain ecosystems (Foley et al. 2005; Bobbink et al. 2010; Bowman et al. 2012; Zong et al. 2015); 2) pressure on mountain ecosystems due to land use change is growing worldwide (UNEP World Conservation Monitoring Centre 2002). 3) with climate change, the temperature stress due to extreme cold at high altitudes is alleviated but increases again towards the lower altitudes; 4) water stress increases especially at the lower altitudes, where not only changed precipitation patterns, but also increased evapotranspiration due to warmer temperatures affects water availability in the soil (Della Chiesa et al. 2014). The studies presented above show that different global change factors and their interactions affect AMF performance and community composition in mountain ecosystems. We therefore think that the above-proposed humped-back model describing the relationship between AMF and plants along altitudinal gradients is not valid for future environmental conditions. Consequently, we propose a second working hypothesis for the functioning of the AMF–plant relationship under future environmental conditions (Fig. 2.3). Stress due to high land use intensity, warm temperatures, which increase evapotranspiration and further reduce the already low water availability in the lowlands, might be so severe that the AMF symbiosis is situated at the parasitic end of the continuum. With declining stress, the relationship becomes more mutualistic towards the foothill zone.

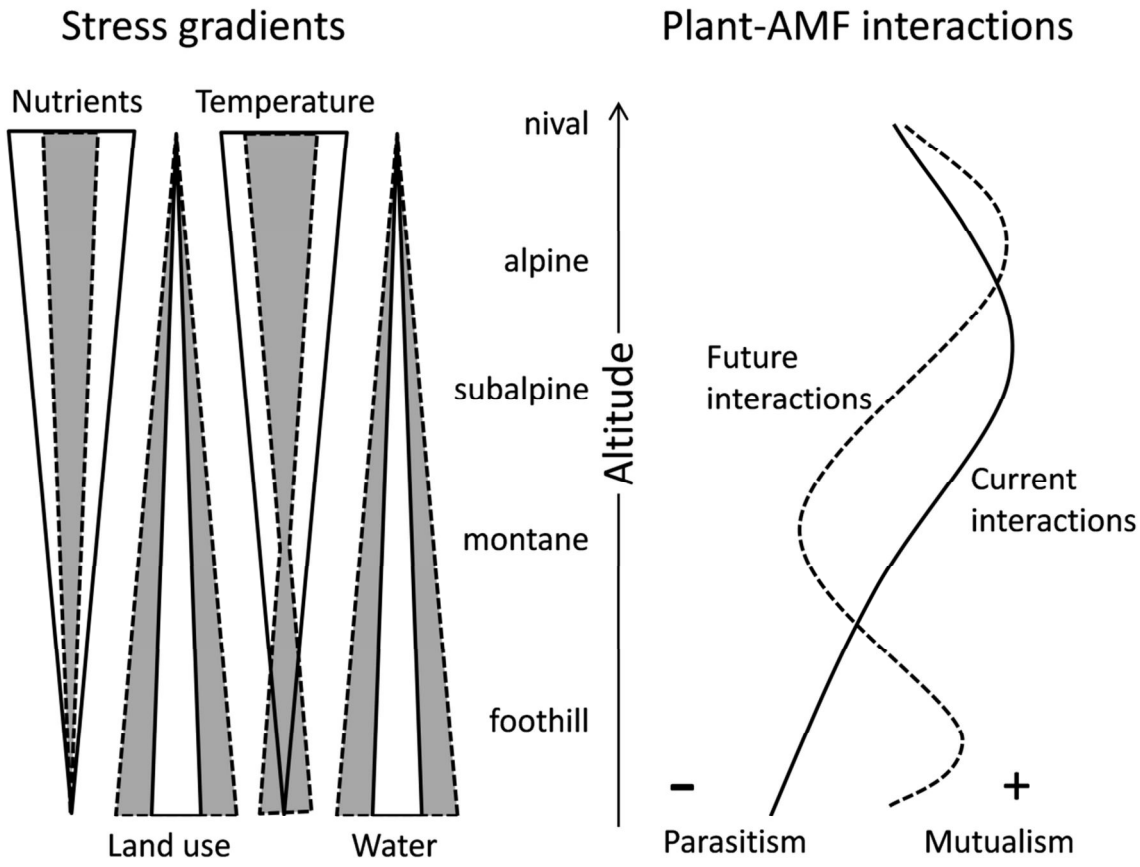

Fig. 2.3: Plant–AMF interactions under current and future climatic conditions along the parasitism–mutualism continuum depending on four main environmental stress factors: nutrients, land use, temperature and water availability. The current stress gradient situation and interactions are displayed with a solid line and white fill. The future stress gradient situation and interactions are displayed with a dashed line and grey fill. The relationship becomes more mutualistic with increasing environmental stress and more parasitic under more benign conditions.

With further decreasing environmental stress towards the montane zone, we expect a return to more parasitic interactions, which might peak in the range of the montane zone. With newly increasing temperature and nutrient stress due to dropping temperatures and decreasing nutrient availability with altitude, the AMF–plant relationship is moving towards the mutualistic end of the continuum, describing a humped-back distribution for the upper part of the altitudinal gradient from the montane to the nival zone, peaking at the alpine zone before temperature and nutrient stress is too severe at the nival zone, which leads to a shift towards a more parasitic relationship (Fig. 2.3).

2.5.6 FUTURE RESEARCH ON AMF IN CHANGING MOUNTAIN ECOSYSTEMS

Only one study on mountain grasslands has manipulated AMF presence in the field using in-growth cores (Johnson et al. 2001; Blanke et al. 2012). To assess for the role of AMF in mountain grasslands under future global change conditions and test our second hypothesis, we need more studies that manipulate AMF presence in the field in combination with laboratory studies that use adapted genotypes of plant species and corresponding field inoculum from mountain grasslands. Only the use of realistic combinations of soil, AMF and plants in laboratory studies can produce results that are transferable to natural conditions.

Considering the predicted shift in plant community competition towards graminoid and sedge domination with global change, and the uncompleted research on the importance of their mycorrhizal status (Miller et al. 1999; Muthukumar et al. 2004) in mountain ecosystems (Haselwandter and Read 1982; Li et al. 2015), we see research on the functioning of AMF-sedge relationship another interesting research direction.

2.6 CONCLUSION AND PERSPECTIVES

The interest in AMF in mountain ecosystems has visibly increased since Gardes and Dahlberg (1996) published their review. Fundamental steps towards a better understanding of the ecology and functioning of AMF in mountain ecosystems and altitudinal gradients have been taken. First, descriptions of AMF community composition using molecular or morphotyping approaches in mountain regions throughout the world are available. We also observe an increased interest in the effects of global change on AMF in mountain grasslands. The available information is mainly regionally confined with about two-thirds of all studies conducted at a few research hot spots in the European Alps, the North-West of the USA and the Chinese Quinghai-Tibet Plateau. In most of the available studies, the altitudinal effects are blurred by other environmental gradients and habitat patchiness. Also, methodological features such as sampling dates may lead to different results, given that all studies with several sampling dates across seasons confirmed the seasonality of AMF patterns. The results are not easy to synthesise because different vegetation types, soils, altitudes and climatic regions are studied. Contradictory results for global change studies might also stem from different simulation periods and approaches. We suspect that the stress gradient hypothesis can be applied to the AMF–plant relationship along the altitudinal gradients and propose two working hypotheses to test in further research: 1) the AMF-relationship shifts along the

mutualism–parasitism continuum following changing environmental stress along the altitudinal gradient; 2) this shift differs under global change conditions. To investigate this, a detailed analysis of AMF and plant species performance under various (future) environmental conditions is required to understand the general principles of biotic interactions and their role in plant community composition and distribution as well as ecosystem processes. Both gradient analysis and manipulative experiments will provide important elements to confirm or reject these hypotheses by unravelling ecosystem processes and the functioning of the AMF–plant relationship.

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3 ARBUSCULAR MYCORRHIZAL FUNGI ALONG AN ALTITUDINAL GRADIENT IN THE CENTRAL EUROPEAN ALPS

While sampling a climate change simulation experiment in the Italian Central Alps (Chapter 4) I had the possibility to take additional root samples from the donor sites to assess AMF root colonisation rate and AMF abundance via their phospholipid fatty acid markers (PLFA) in the soil. The results presented in this Chapter are in preparation to be submitted together with more data on the PLFA composition of the soil microbial community, a molecular approach for a subset of plant roots and the results of Chapter 4.

Abundance of arbuscular mycorrhizal fungi along an altitudinal gradient in an inner alpine dry Valley

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3.1 ABSTRACT

The colonisation rate by arbuscular mycorrhizal fungi (AMF) was quantified for plant roots of 4 herb and 1 grass species originating from grasslands along an altitudinal gradient ranging from the foothill to the subalpine zone in the Mazia Valley, an inner-alpine dry valley in the Italian Central Alps. In addition, the abundance of these fungi was assessed via the presence of their phospholipid fatty acids markers in the soil microbial community. The mean colonisation rate in the roots of all target species was high. However, no pattern that links colonisation rate or abundance to altitude could be discerned. The abundance of AMF vesicles

in the plant roots increased with altitude, while the overall presence of arbuscules seemed to decrease when analysing all plant species together, but was in fact host species dependent. The results presented by other studies located at wetter gradients could not be applied to the investigated study site, which is located in one of the driest valleys in the European Alps, where water availability is the limiting resource for plant growth. The biotic and abiotic drivers of AMF abundance in soil and roots for these dry grasslands remain unclear.

3.2 INTRODUCTION

The natural distribution of plants is limited by their environmental requirements, especially under extreme environmental conditions, by their capacity to deal with disturbance as well as by the interaction with neighbouring plants (Grime 1977). While plant-plant interactions are described to be competitive under benign environmental conditions they turn into facilitation as environmental stress increases in severity and change back to competition when environmental stress is too severe (Bertness and Callaway 1994; Brooker and Callaghan 1998; Bruno et al. 2003). Plant–plant interactions along altitudinal gradients, for example, change from competitive interactions to facilitation with increasing altitude (Choler et al. 2001; Callaway et al. 2002), a shift that is driven by environmental stress and biotic plant interactions (Michalet et al. 2014). As they link plants with their belowground fungal network, arbuscular mycorrhizal fungi (AMF) in particular can be an important feature for plant interactions and community composition, (Bender 2014; Dickie et al. 2015). They are in relationship with a majority of all terrestrial plant species from which they receive carbon compounds and provide nutrients, water and protection against pathogenic fungi in return (Smith and Read 2008). AMF are found in ecosystems from the equatorial tropics to the Arctic and Antarctic and from sea level to altitudes higher than 5250 m above sea level (a. s. l.; Ruotsalainen et al. 2004, Frenot et al. 2005, Schmidt et al. 2008, Camenzind and Rillig 2013, da Silva et al. 2015).

New information about AMF community composition, colonisation rates and distribution patterns along altitudinal gradients around the world is available thanks to increased research efforts during the last 10 years (Wahl and Spiegelberger 2016). Many studies report a decrease of AMF colonisation rate and abundance as well as a shift in AMF community composition along the gradient with increasing altitude. However, different results have been obtained from drier mountain ranges (Schmidt et al. 2008). It is clear that even with the visibly increased interest on AMF ecology along altitudinal gradients, the

determining factors for natural occurrence of AMF along an altitudinal gradient as well as their function for plants and plant community composition in mountain ecosystem need further investigation under varying climatic conditions across mountain ranges (Wahl and Spiegelberger 2016).

A change of the AMF effect on plants long altitudinal gradients is expected, as AMF-plant relationships are not always mutually positive. They are rather described to be ranging from positive to neutral and even negative along a parasitism-mutualism continuum (Johnson et al. 1997; Johnson and Graham 2013; Smith and Smith 2015). Whether the symbiosis acts more on the parasitic or mutualistic end of this continuum can be determined by assessing the cost and benefit for the plant (Johnson et al. 1997). The functioning of the AMF-plant relationship along environmental stress gradients might follow the same patterns as described by the stress gradient hypothesis for plants (Wagg et al. 2011a; Wahl and Spiegelberger 2016).

It can be hypothesised that with increasing elevation along the altitudinal gradient and its concomitant decrease in temperature 1) the AMF root colonisation rate decreases as a result of lower fungal performance but that 2) the abundance of vesicles, fungal storage organs increases. Finally, according to the first working hypothesis stated in Chapter 2, 3) the cost-benefit ratio of the AMF-plant relationship is believed to improve for the plant with increasing altitude, indicating a shift towards a more mutualistic plant-fungus relationship.

3.3 STUDY SITE

Samples were taken from the sites of the climate change simulation experiment at the LTER-site Matsch/Mazia-Valley (46°41'08"N 10°34'48"E; Autonomous Province of Bozen/Bolzano; Italy). Three south-west inclined hay meadows situated about 1950 m, 1450 m, and 950 m a. s. l. in the subalpine, montane and foothill vegetation belt respectively were sampled. Management of those meadows followed traditional land use at the respective elevation: two cuts at the subalpine site, three at the montane and four cuts at the foothill site. All three sites were fertilized once a year with 3 kg m^{-2} cattle farmyard manure. The vegetation was assigned to the class Molinio-Arrhenatheretea at all sites, with a slight variation of the dominant plant species. Mean annual air temperature in the period 2010 to 2013 ranged from 3.4 °C at the subalpine site to 9.0 °C at the foothill site. Mean air temperature during the growing season (April to October) reached from 8.3 °C at the

subalpine to 13.9 °C at the foothill site. The temperature difference between the three sites dropped 2.8 K along the increasing altitudinal gradient during growing season. The Matsch/Mazia-Valley is part of the Vinschgau/Venosta Valley, which is one of the driest areas in the European Alps due to the sheltering effect of the surrounding mountains (Bertoldi et al. 2010; Della Chiesa et al. 2014). Mean annual precipitation (2010-2013) reached 708 mm at the subalpine site and decreased with about 12 mm per 100 m elevation to 655 mm at the montane site and 586 mm at the foothill site. Especially at the foothill site, natural precipitation during summer would not be enough for grassland production and therefore artificial irrigation is common in this region at all altitudes. The irrigation system used by land users for each site leads to a homogenisation between all three sites to about 830 mm of total water received per year and site. In summary, the subalpine site received 828 mm (708 mm + 4 x 30 mm) water per year, the montane site 835 mm (655 mm + 6 x 30 mm), and the foothill site 826 mm (586 mm rain + 8 x 30 mm irrigation).

3.4 MATERIAL AND METHODS

Soils were classified as Skeletic Humic Cambisols on siliceous bedrock at all three sites with a sandy clay loam texture in the fine earth (WRB 2014). A slight increase in the sand fraction was noticed with increasing altitude. Three soil samples per altitude were cut out with a knife from 0 - 5 cm soil depth (length x width = 10 cm x 2 cm) and mixed together in the field. Soil samples were air-dried, sieved < 2 mm and stored for subsequent analysis in the laboratories. Soil pH was determined potentiometrically in a 0.01-M CaCl2 solution at a mass/volume ratio of 1 : 2.5 (Soil Survey Stuff 2004). Total organic carbon (C org) and total nitrogen (Nt) of samples were measured by dry combustion (Tabatabai and Bremner 1991) in an elemental analyser (Carlo Erba Nitrogen Analyser 1500, Milano, Italy). As carbonate C was absent in the soil, no acid pre-treatment was included. Plant available potassium and phosphorus were estimated with Calcium-Acetate-Lactate (CAL) extraction (Schüller 1969). Extracted phosphate was measured spectrophotometrically using the molybdenum blue method according to (Murphy and Riley 1962) and extracted potassium was measured with flame atomic absorption spectroscopy.

The abundance of AMF in the soils' **microbial community composition** was measured by the abundance of phospholipid fatty acids (PLFA) in the soil. The PLFAs were extracted according to Bligh and Dyer (1959), following the description by Frostegård et al.

(1991). In 2011 and 2013, 1.5 g of moist field-soil was extracted with 2-4ml (depending on soil water content) of chloroform: methanol: citrate buffer (1:2:0.8) and 3-5 ml of chloroform: methanol (1:2) at each altitude. After phase separation, the non-polar phase (extracted lipids) was collected. Neutral lipids, glycolipids and phospholipids were fractionated using chloroform (10 ml), acetone (20 ml), and methanol (5 ml) solutions, respectively, over silica solid phase extraction columns (500 mg, ISOLUTE, Separtis). The PLFAs were methylated with methanol-toluol (1:1) solution and 0.2 M methanolic KOH to produce fatty acid methyl esters (FAMEs) (Sundh et al. 1997; Chowdhury and Dick 2012). Samples were then analysed on a gas chromatograph with a flame ionization detector following the procedure in Djukic et al. (2010). The fatty acid nomenclature described by Frostegård et al. (1991) was applied. Indicator of AMF fungal biomass was the PLFA 16:1ω5c (Kroppenstedt 1985; Olsson 1999).

Root samples were taken at different dates to account for a different phenology at the three different altitudes (12th June: foothill site; 25th June: montane site; 12th July: subalpine site). Each sampling date corresponded to the flowering of *Agrostis capillaris* (Common Bent) at the given altitude, which is also the period where the majority of the sampled plants were at the state of early seed, just after flower withering. Plant species were identified using an Austrian Flora (Fischer et al. 2008) and chosen according to their presence and abundance along the gradient. A minimum of three and a maximum of five individuals were sampled for four herb species (*Achillea millefolium, Carum carvi, Rumex acetosa, Taraxacum officinale agg.*) and one grass species (*Poa pratensis*). Samples were pooled per altitude and species. Samples were stored in cooling boxes and taken to the laboratory the following day, where they were stored at 4 °C until roots were cleaned and dried for 36 h at 60 °C.

To assess **mycorrhizal colonisation rate** of dried root samples, all samples were rehydrated in water for one hour, bleached for 15 min in 10% potassium hydroxide at 90 °C, rinsed twice with tap water and acidified for 10 min in 3.7% hydrochloric acid. Afterwards roots were stained for 5 min in a 5% ink-vinegar solution (5 ml Waterman Serenity Blue Ink in 95 ml 5% acetic acid) at 90 °C. After 30 min of decolourisation of plant cell structures in pure vinegar, mycorrhizal structures were visible in the roots (Vierheilig et al. 1998).

For each sample, 300 fields of view were checked for the presence or absence of mycorrhizal structures inside the root at 300fold magnification with a Nikon Optiphot microscope, using the line-intersect method (McGonigle and Miller 1990) in a modified way.

Only intact root segments with a diameter smaller than 1 mm, and presenting more than 10 fields of view were examined. Percentage of root-length colonised (%RLC) by intraradical mycelium, arbuscules and vesicles was calculated by dividing the number of observations for each mycorrhizal structure by the number of observed intersections. Weighted mean colonisation rate was calculated by multiplying the root colonisation rate of individual plant species by its abundance for each plot. A cost-benefit ratio (Johnson et al. 1997; Dekkers and van der Werff 2001) was calculated by dividing %RLC by arbuscules by %RLC by intraradical mycelium (A/M), where A can be considered to represent AMF benefit to plants and M is an indicator of the plants costs (Dekkers and van der Werff 2001).

For **statistical analysis** to test for differences between %RLC by intraradical mycelium, arbuscules or vesicles along the natural altitudinal gradient (LAs), as well as for differences concerning soil properties (pH, C org, N_t, C/N, CAL-P and CAL-K) and AMF PLFA marker abundance in the soil along the gradient, one-way ANOVAs were calculated with altitude as a fixed factor for each species and also for weighted means of all species, according to their relative abundance in the vegetation cover. Normality was ascertained before assessing equal variance of variables by using non-parametric Kruskal-Wallis-tests or parametric ANOVA. When necessary, variables were transformed to respect statistical requirement (square root transformation: %RLC by vesicles for *A. millefolium*; log transformation: %RLC by intraradical mycelium for *P. pratensis*, %RLC by arbuscules for *P. pratensis* and *R. acetosa*; cube root transformation: %RLC by vesicles for *R. acetosa* and all colonisation percentages for the weighted means). Tukey's HSD tests were applied post-hoc to ANOVAs and pairwise Wilcoxon rank sum tests succeeding Kruskal-Wallis-tests. As A/M and %RLC by arbuscules were highly correlated (R^2=0.93; $p < 0.001$), further statistical analysis considers only %RLC by arbuscules.

3.5 RESULTS

3.5.1 SOIL PROPERTIES

Soils at all altitudes were generally acidic. However, pH decreased significantly with elevation with pH ranging from 6.2 to 4.2 (Fig. 3.1a). The amount of plant available phosphorus showed a significant increase with elevation (Fig. 3.1b) while concentration of plant available potassium was highest at the foothill site and lowest at the montane site (Fig.

3.1c). Soil analysis further revealed a strong impact of altitude on C org and Nt storage, which represents the net balance of organic matter input and losses. C org and Nt both increased with increasing altitude with more than twice the amounts in the subalpine zone compared to the montane zone (Fig. 3.1d, e). The C/N ratio (Fig. 3.1f) was significantly different at all sites, high at the foothill and subalpine site (12.4-12.7), reflecting a similar possibility of microbial activity and soil organic matter decomposition and lower at the montane site (11.3). Measured soil moisture content at 5 cm soil depth during the four vegetation periods from 2010 to 2013 was 27% for the foothill site, 32% for the montane site and 35% for the subalpine site on average. For a detailed report on soil hydrology at the specific sites see Della Chiesa et al. 2014.

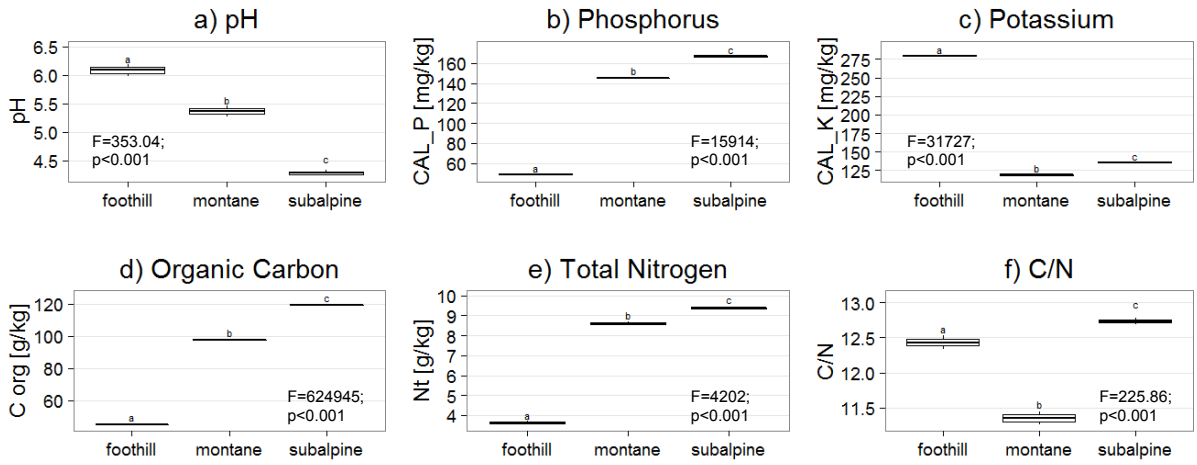

Fig. 3.1: Abiotic soil conditions for different sites: **a)** pH, **b)** potassium CAL-K (mg / kg), **c)** phosphorus CAL-P (mg / kg), **d)** organic carbon OC (g / kg), **e)** total nitrogen Nt (g / kg) and **f)** carbon to nitrogen ratio (C/N). Letters indicate significant differences between sites resulting, like indicated F- and p values, from analysis of variance (ANOVA).

3.5.2 AMF ABUNDANCE IN THE SOIL

Neither the relative abundance of AMF PLFA marker in the soil microbial community ($F_{1,9}=2.2451$); $p= 0.162$) nor their total abundance in the soil assessed by PLFA ($F_{1,6}=0.713$; $p= 0.527$) differed along the studied altitudinal gradient.

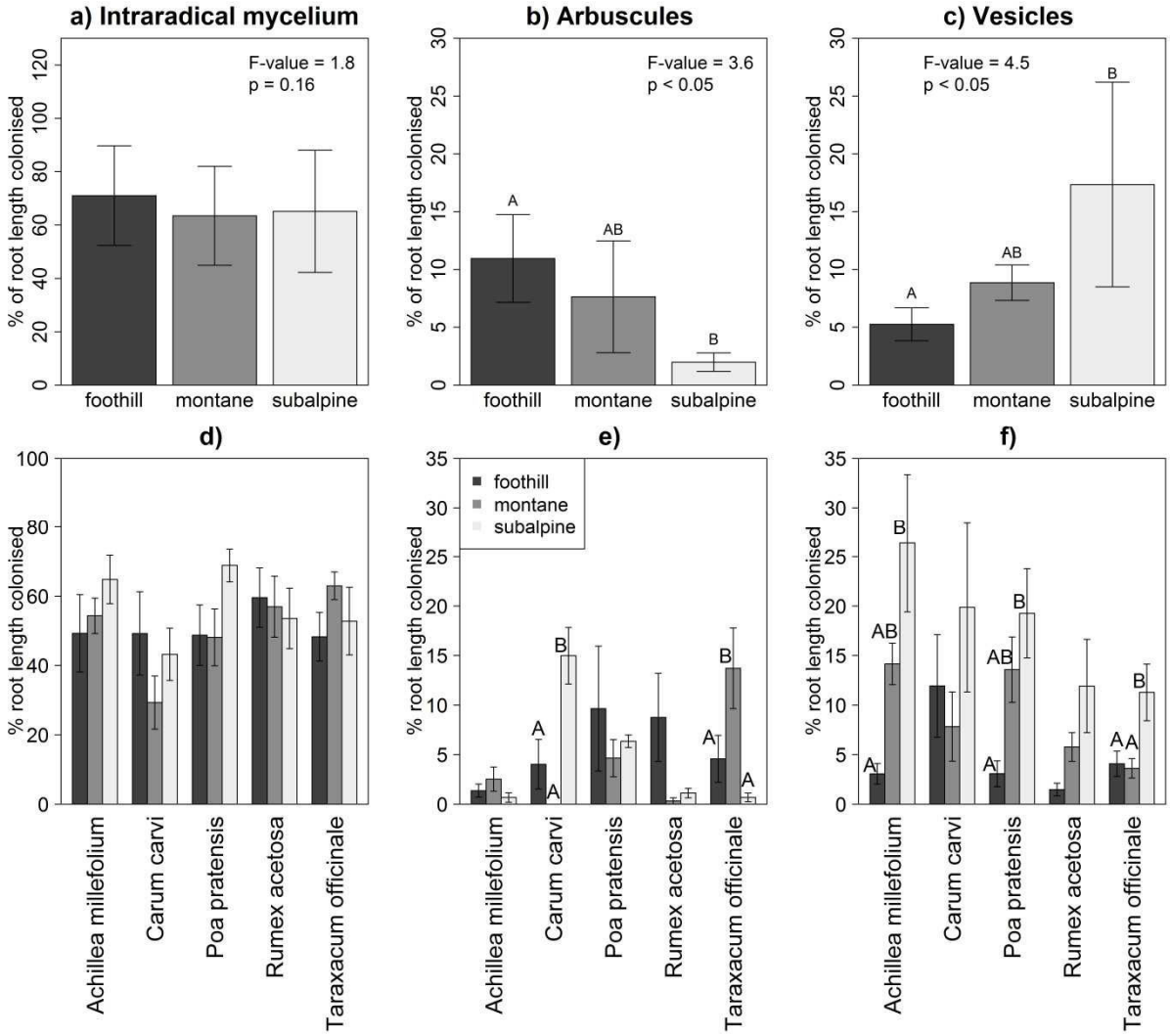

Fig. 3.2: Weighted mean values of % of root length colonised under current climate conditions (local areas, Las) by **a)** intraradical mycelium, **b**) arbuscules or **c)** vesicles and values for examined plant species of % of root length colonised by **d)** intraradical mycelium, **e**) arbuscules or **f)** vesicles at different altitudes along the altitudinal gradient are displayed. Letters indicate significant differences between altitudes resulting, like indicated F-and p-values, from analysis of variance (ANOVA). Error bars show standard errors ±1.

3.5.3 AMF COLONISATION RATE

All five species present were colonised by all mycorrhizal structures. However, no significant change was observed neither when comparing species specific data nor weighted means of %RLC by intraradical mycelium between different altitudes (Fig. 3.2a). Only *P. pratensis*

showed a marginal significant trend (p = 0.052) towards a higher %RLC by intraradical mycelium at the subalpine zone compared to the two lower zones (Fig. 3.2d).

However, when comparing weighted means of %RLC by arbuscules, it proved to be significantly lower at the highest altitude than at the lowest altitude, with an intermediate colonisation rate at medium altitude (Fig. 3.2b). On species level *C. carvi* showed a significantly higher %RLC by arbuscules at subalpine site, while *T. officinale* showed a significant peak at the montane site and a trend was observed for *R. acetosa* to have highest arbuscule abundance at the foothill site (Fig. 3.2e). Weighted means of %RLC by vesicles showed an inverse pattern compared to arbuscules. They were significantly higher at the subalpine zone (Fig. 3.2c). In contrast to arbuscules, the different species did all show similar trends for % RLC by vesicules, which was significantly higher for *A. millefolium*, *P. pratensis* and *T. officinale* at the subalpine site (Fig. 3.2f). Colonisation rate of *R. acetosa* was not significantly affected by altitude.

3.6 DISCUSSION

The mean colonisation rate of plant roots by AMF in this study lies within the higher range of that reported from other mountain ecosystems (e.g. Ruotsalainen et al. 2004; Kagawa et al. 2006) and also the abundance of AMF in the soil corresponds to other studies from mountain environment (Djukic et al. 2010). In general, a decline of AMF colonisation rate and abundance in the soil is expected for grassland ecosystems with increasing altitude (Haselwandter and Read 1980; Ruotsalainen et al. 2004; Schmidt et al. 2008; Zubek et al. 2009; Budge et al. 2011; Gai et al. 2012; Lugo et al. 2012), which is commonly explained by a decline in temperature and a consequently reduced AMF activity (Entry et al. 2002; Lugo et al. 2008). However, an analogous pattern for the here presented altitudinal gradient was not observed. Besides a too short altitudinal gradient – the present altitudinal gradient spans only 1000 m and has its highest point at 1950 m where environmental conditions are still suitable for fungal activity (Read and Haselwandter 1981) – soil nutrient conditions, land use and overall climatic conditions may provide further explanations for the observed absence of a continuous decrease in AMF colonisation along the here studied altitudinal gradient. Several biotic and abiotic factors could account for the lack of response to altitude.

Local soil conditions may subdue climate conditions such as decreasing temperature along an altitudinal gradient (Zubek et al. 2009; Gai et al. 2012). AMF colonisation rates are known for example to be decreased with decreasing soil pH (Covacevich and Berbara 2011; Ruotsalainen and Eskelinen 2011). In the present study, the pH differed significantly between the different sites and decreased with altitude, but no correlation with AMF abundance or root colonisation rate was observed. Also nutrient availability can influence AMF colonisation rate in mountain ecosystems to a similar extend as altitudinal climate (Lesica and Antibus 1986; Zubek et al. 2009). In some studies nutrient distribution is described as the driving factor for AMF colonisation rate and abundance in mountain ecosystems (Read and Haselwandter 1981; Ruotsalainen et al. 2004; Becklin and Galen 2009; Casanova-Katny et al. 2011). The plant-AMF relationship is generally known to be more important for plants under low nutrient availability or high competition for nutrients (Koide 1991; Johnson 2010). The lowest colonisation rate was found for example at intermediate altitudes in plant roots from a fertilised hay meadow at 1600 m a. s. l. along an altitudinal gradient in the European Alps, were plant competition for nutrients was highest compared with other sites (Read & Haselwandter 1981). However, in some studies, lower AMF colonisation rates with increasing altitude are also attributed to low plant-plant competition for soil nutrients at high altitudes, which is resulting from low vegetation density (Haselwandter and Read 1980; Read and Haselwandter 1981; Hartnett et al. 1993; Ruotsalainen et al. 2004). Being evenly fertilised hay meadows – the here presented sites displayed a uniform dense herbaceous vegetation cover at all altitudes and competition for resources is expected to be high at all sites, what could explain the observed high colonisation rate and AMF abundance. On the other hand, plant available phosphorus and total nitrogen content in the soil was discovered to increase with increasing altitude of study site, what in turn is expected to lead to decreased AMF presence (Koide 1991; Johnson 2010). It seems that other drivers than soil nutrients determine AMF presence in root and soil at the studied gradient.

Water availability is also found to be a determining factor for the colonisation rate in several studies conducted in mountain grassland settings where colonisation rate generally declines with increasing humidity (Barnola and Montilla 1997; Rudgers et al. 2014). The observed decrease of AMF colonisation rates with increasing altitude could therefore also be explained by the concomitant decrease of water stress (Wahl and Spiegelberger 2016).
The Vinschgau/Venosta Valley in South Tyrol is one of the driest valleys in the European Alps and limiting water availability and accompanying nutrient limitation drive natural plant

performance (Leibundgut 2004; Della Chiesa et al. 2014). Even though the amount of water received at all sites is artificially raised and unified among altitudes by long-term irrigation practices, it is still lower (about 830 mm / yr) than at other sites in the European Alps were AMF were studied (Read and Haselwandter 1981; Budge et al. 2011). Yearly reoccurring drought events caused by high evapotranspiration during the vegetation period are reported for the foothill site, while at the montane site, drought events occur only during dry years. No drought events are reported for the subalpine site so far (Della Chiesa et al. 2014). This increase of soil water content with increasing altitude could explain the above described increase of P and N availability as well as organic C content in the soil. In addition to plant nutrient competition, the overall dry conditions could account for the high colonisation rate at all sites.

Land use type is also known to exert a strong influence on AMF colonisation (Tchabi et al. 2008; Gavito et al. 2008; Oehl et al. 2010; Morris et al. 2013) and could therefore mask altitudinal effects on colonisation rate. The studied grasslands have been used for hay-making for at least 150 years (historic land use maps and pers. com.). As a consequence, previous differences in soil structure as well as plant species composition along the altitudinal gradient may be levelled out by anthropogenic influence (Niedrist et al. 2009). Mowing which is practised at all sites can result in a homogenous reduction of vesicles – the mycorrhizal storage organs – and an increase in spore development (Bentivenga and Hetrick 1992; Eom et al. 1999; Titus and Leps 2000; Pietikäinen and Kytöviita 2007; Morris et al. 2013). This could explain why in the present study significantly more vesicles were found in the subalpine site, where cut frequency was only half of that at the foothill site. However, this observation could also be interpreted as an adaptation of AMF's survival strategy from growth to storage, in order to cope with less favourable conditions and shorter vegetation period at higher altitudes (Hawkes et al. 2008; Wahl and Spiegelberger 2016).

Other plant related factors such root traits and mycorrhizal dependency affect the abundance and plant-AMF relationship too. Host plant identity might even be more important for the presence of AMF in soil and roots than habitat (Becklin and Galen 2009; Becklin et al. 2012). Usually, plants at high altitudes allocate more biomass to the roots. They invest it mainly into fine roots, maybe to compensate lower AMF colonisation (Brown et al. 2013). *T. officinale*, *C. carvi* and *R. acetosa* all displayed opposing patterns of colonisation rate by arbuscules, which might be a rough indicator that each species benefited more of AMF at

another altitude. In general these three plant species have coarser roots than for example grasses and might be more dependent on under dry and nutrient poor conditions. Plants with deeper root-systems generally seem to profit more from AMF colonisation than plants with shallower roots (Wang et al. 2011). With the relationship between AMF and associated plants ranking from mutualistic to parasitic (Johnson et al. 1997) it can be assumed to change also along an altitudinal gradient (Ruotsalainen et al. 2004; Zubek et al. 2009; Wahl and Spiegelberger 2016). The cost-benefit ratio in the present study was correlated with the colonisation rate by arbuscules. There seems to be an overall decline of AMF benefit to plants with increasing altitude. Broken down on plant species level it became clear that this effect is plant species and trait dependent. All in all the interspecific differences of colonisation peaks along the altitudinal could not be explained satisfyingly.

3.7 CONCLUSION

This study provided information on AMF colonisation rate and abundance in understudied dry mountain grasslands. The first hypothesis was rejected because a general decrease of AMF root colonisation rate with increasing altitude was not observed. This is in contrast to most of the current literature at first. However, the study was site situated in an inner-alpine dry valley which provides generally drier conditions than most other mountain ecosystems. Moreover, the long-lasting irrigation practise and use as hay meadows together with annual fertilisation seemed to blur the altitudinal effect on AMF root colonisation and abundance in the soil at the studied gradient. This has been reported before, also from wetter altitudinal gradients in the European Alps (Read and Haselwandter 1981). The second hypothesis concerning the increase of root colonisation by vesicles with increasing altitude could be affirmed. However, it is not clear whether the increased abundance of vesicles in the root was due to land use practices (mowing), poorer abiotic conditions or other factors. With the rough assessment of plant benefit via the ratio of arbuscules to total AMF colonisation a general decrease of plant benefit with increasing altitude was discerned at the studied gradient. However this overall effect turned out to be plant species dependent and hypothesis three could not generally be ascertained or rejected.

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4 ARBUSCULAR MYCORRHIZAL FUNGI ALONG ALTITUDINAL GRADIENTS UNDER CLIMATE CHANGE CONDITIONS

In Chapter 3 I presented the natural distribution of AMF along an altitudinal gradient in the Alps. The study I present in Chapter 4 was conducted at the same altitudinal gradient, where warming has been simulated for a number of soil monoliths by downward transplantation in an experimental set-up by the Institute for Alpine Environment from the European Academy (EURAC) Bolzano/Bozen during three years. The following article is in preparation for submission, still awaiting the sequencing results for the root samples.

Arbuscular mycorrhizal fungi in mountain grassland react altitude and host-plant dependent to future climate conditions

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4.1 ABSTRACT

- Rising temperatures affect all ecosystem compartments, yet the possible reaction of arbuscular mycorrhizal fungi (AMF) to elevated temperature is rarely studied in the field, especially in mountain regions, which are over-proportionally concerned by climate change.
- We investigated AMF colonisation rate of plant roots in grasslands along an altitudinal gradient from the foothill to the subalpine belt in the Italian Alps under current and future climate conditions, simulated by downward transplantation of soil monoliths.

Root samples of nine plant species were collected at all altitudes in summer 2013, three years after transplantation. Percentage of root length colonised by AMF mycelium, arbuscules and vesicles was determined and the plant-fungi relationship was assessed.

- Simulation of future climate conditions by transplantation from the subalpine to the montane zone (+ 2.8 K) lead to a general decrease in the mean percentage of root lengths colonised by AMF. However, no changes in average percentage of root length colonised by AMF occurred when species were transplanted from the montane to the foothill vegetation belt, thereby equally subjected to a temperature increase of 2.8 K. At this lower transplantation, individual reaction patterns were revealed by the different plant species: One third showed an increase in AMF colonisation rate, one sixth a decrease and half of the plant species showed no significant change in colonisation rates,
- Synthesis: Our study provides new insights into the development of arbuscular mycorrhizal root colonisation rate in mountain grassland under current and future climate conditions, assessed in a field experiment along an altitudinal gradient. The obtained results suggest that reaction to warmer temperatures is linked to altitude-dependent evapotranspiration and its hydrological consequences as well as to different and complex plant-fungi interactions of individual plant species. While at higher altitudes warmer temperatures seem to benefit plants and lead to a general decrease in AMF colonisation, drought stress at lower altitudes leads to plant species-specific reactions that appear to be linked to different root traits.

KEY WORDS : plant-fungi interaction, plant-climate interaction, climate change, mountain grassland, field experiment, transplantation, altitudinal gradient

4.2 INTRODUCTION

Climate change scenarios for the present century predict a rise in temperature between 1.1 and 6.4 °C as well as an increase in intensity and frequency of extreme weather events (IPCC 2013). Mountain regions are predicted to be particularly vulnerable to climate change (Beniston 2003; IPCC 2013). Warming directly affects the water budget in mountain ecosystems (Beniston 2003; Della Chiesa et al. 2014) as well as the vegetation, which has

adapted with evolution to the harsh mountain environment (Körner 2003; Kullman 2010). Observational studies of alpine and montane vegetation over the last century show that plants react to warmer climate in different ways: from advanced phenological stages, such as earlier flower set or complete fructification at higher altitudes, to enhanced productivity in some cases. In other cases an increase of warmth-adapted species or a species shift up- or northwards leads to changed or enriched vegetation assemblages (Kullman 2010; Grabherr et al. 2010; Gottfried et al. 2012; Pauli et al. 2012; Stocker et al. 2013; Frei et al. 2014).

Worldwide, more than 80% of all terrestrial plant species improve their access to limiting soil nutrients and water by forming mutualistic symbioses with arbuscular mycorrhizal fungi (AMF) from the fungal phylum Glomeromycota (Wang and Qiu 2006; Smith and Read 2008). Especially grassland vegetation is highly colonised by AMF (Treseder and Cross 2006). In addition to being beneficial by means of increased nutrient and water uptake, AMF can protect plants against pathogenic fungi and play an important role in soil cycles and soil stability, as well as for C fixation belowground (Brundrett 1991; Rillig and Mummey 2006; Daynes et al. 2013). Within the plants' roots, intra- and intercellular hyphae are fungal propagation structures and take up C compounds from the plants (Douds Jr et al. 2000). Tree-shaped fungal structures, so-called arbuscules, provide the main interface for plant-fungus nutrient exchange (Garbaye 2013; Gutjahr and Parniske 2013), and express therefore mycorrhizal benefit to the plant (Johnson et al. 1997; Dekkers and van der Werff 2001). While AMF are obligate symbionts, plant dependency on the trade of self-generated photosynthates for fungal acquired soil nutrients can range from non-mycorrhizal to facultative or obligate (Klironomos 2003; Bidartondo 2005). This host-symbiont relationship is generally mutualistic, however sometimes can become parasitic (Johnson et al. 1997; Klironomos 2003; Johnson and Graham 2013).

Evolving alongside plants from the very appearance of the first land plants (Brundrett 2002), the AMF-plant-symbiosis has experienced climatic changes in the past. Therefore it can be assumed that predicted changes in climate are going to affect AMF-plant interactions in turn (Bellgard and Williams 2011). Knowledge on AMF-plant interaction under future climatic conditions will help to understand plant species reactions to climate change. Climate change may improve limiting abiotic conditions for plant growth in mountain grassland ecosystems, in particular at intermediate altitudes, where increasing mean annual

temperatures benefit plant performance, while changes in precipitation have not yet limiting impact (Kullman 2010; Frei et al. 2014; Wahl and Spiegelberger 2016). As a consequence, it could be assumed, that plants in those ecosystems may depend less on additional nutrient and water supply provided by AMF, what could be observed through a reduced root colonisation rate by those fungi (Johnson et al. 2013). The majority of studies on AMF colonisation rate and climate change report an increased colonisation rate as a consequence of warming and/or drought (Rillig et al. 2002; Kytöviita and Ruotsalainen 2007; Compant et al. 2010; Büscher et al. 2012; Mohan et al. 2014). However, a lot of these studies have evaluated AMF reaction to warming and drought using reductionist approaches (Staddon et al. 2003; Heinemeyer and Fitter 2004; Hawkes et al. 2008). Yet, to draw sound conclusions and to predict potential effects of climate change, it is necessary to confirm results from these studies under natural conditions. To date, field studies under natural multi-factorial circumstances assessing effects of warming and drought on AMF dominated ecosystems such as grasslands are rare (Mohan et al. 2014; Wahl and Spiegelberger 2016).

The aim of our research is to study plant-AMF interactions of plants that were subject to temperature rise and a negligible change of precipitation for three years. The study site is in the Italian Central Alps, where climate change was simulated by downward transplantation along an altitudinal gradient. We studied the reaction of AMF abundance in root and soil to the simulated climate change conditions.

(I) We first hypothesise that the presence of AMF in the soil microbial communities decreases with warming but increases with water stress.

(II) We further expect an increase in AMF colonisation rate of plant roots to occurs under these simulated climate change conditions.

(III) Finally we discuss if a AMF mitigation effect on plants is visible and whether the plant-AMF relationship is changed with climate change

To test our hypotheses, we investigated the abundance of phospholipid fatty acid markers for AMF in the soil as well as root colonisation rate of certain plant species by AMF at the long-term ecological research site ‘Mazia Valley’, Italy. We subsequently compared the effects of altitude, downward transplantation (simulation of temperature rise) and horizontal transplantation (control) of soil monoliths.

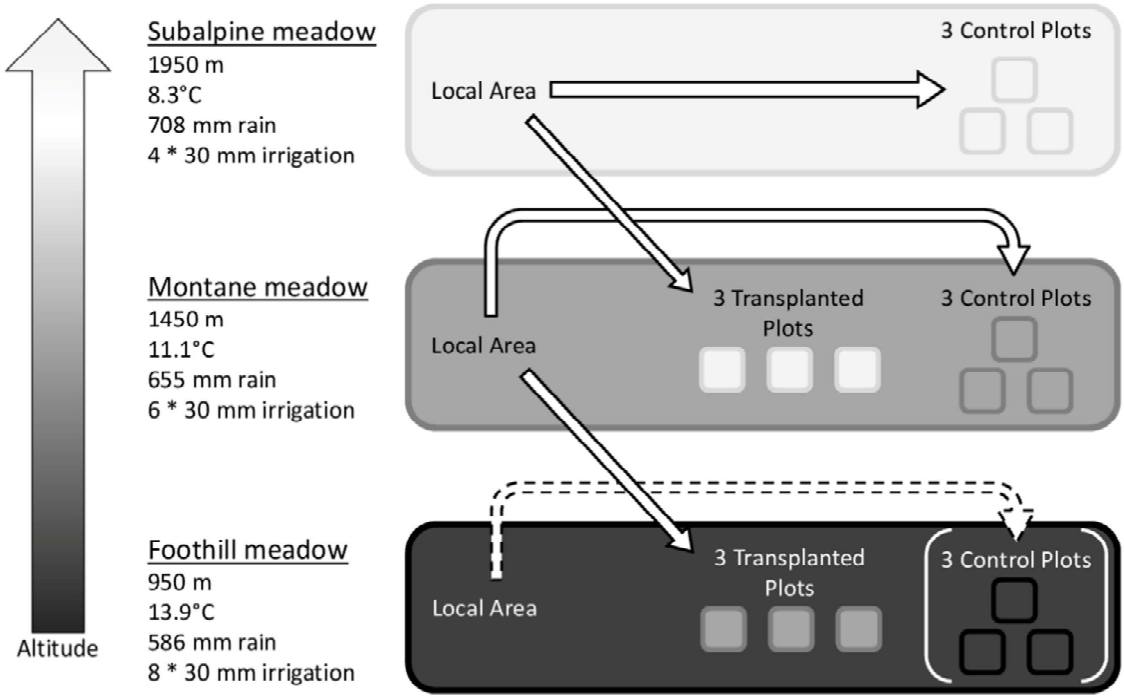

Fig. 4.1: Experimental set-up along the altitudinal gradient, displaying the different altitudinal zones (m above sea level), mean temperature as well as the amount of precipitation and irrigation during vegetation period from April to October. It comprises a "space-for-time-substitution" approach: white arrows indicate the direction of transplantation of six soil monoliths (3 per treatment) originating from Local Areas (LA) at each altitudes: either downward transplantation (Transplanted Plot; TP) to simulate warming and increased evapotranspiration or local transplantation as Control Plots (CP). No root samples were taken from the CP at the foothill zone.

4.3 MATERIAL AND METHODS

4.3.1 STUDY SITE

The study was conducted along a homogeneous elevation transect (4.2 km linear distance from the highest to the lowest site) in the LTER-site Matsch/Mazia-Valley (Autonomous Province of Bozen/Bolzano; Italy), on three south-west inclined hay meadows situated about 1950 m, 1450 m, and 950 m above sea level (a. s. l.) in the subalpine, montane and foothill vegetation belt respectively (Fig. 4.1). Management of those meadows followed traditional land use at the respective elevation: two cuts at the subalpine site, three at the montane and four cuts at the foothill site. All three sites were fertilized once a year with 3 kg m^{-2} cattle farmyard manure.

Microclimate stations were installed directly at the sites to sample each minute (averaged at quarter hourly scale) air temperature and humidity, soil moisture and temperature at 5 cm, as well as precipitation (for further hydrological site description see Della Chiesa *et al.* 2014). Mean annual air temperature in the period 2010-to 2013 ranged from 3.4 °C at the subalpine site to 9.0 °C at the foothill site. Mean air temperature during the growing season (April to October) extended from 8.3 °C at the subalpine to 13.9 °C at the foothill site. The temperature difference between the three sites dropped 2.8 K along the increasing altitudinal gradient during growing season. Soil monolith transplantation from subalpine to montane and from montane to foothill zone respectively, simulates climate change scenario A2 (IPCC 2007). Mean annual precipitation (2010-2013) reached 708 mm at the subalpine site and decreased with about 12 mm per 100 m elevation to 655 mm at the montane site and 586 mm at the foothill site. Especially at the foothill site, natural precipitation during summer would not be enough for grassland production and therefore artificial irrigation is common in this region at all altitudes. Mean annual precipitation was homogenized for all three sites at about 830 mm of total water per year by irrigation, as the study's objective was to investigate the temperature effect. In summary, the subalpine site received 828 mm (708 mm + 4 x 30 mm) water per year, the montane site 835 mm (655 mm + 6 x 30 mm), and the foothill site 826 mm (586 mm rain + 8 x 30 mm irrigation; Fig. 4.1), in accordance to the irrigation system used by land users for each altitude.

4.3.2 TRANSPLANTATION PROCESS

Six grassland monoliths (0.7 m x 0.7 m, depth ca. 0.25 m) were dug out from homogeneous grasslands (Local area = LA) in the subalpine and in the montane belt, and three monoliths from grassland at the foothill belt, resulting in 15 experimental units in total (Fig. 4.1). Digging was done manually and monoliths excavated as a whole to ensure the most cautious treatment possible and to avoid disturbances in the soil monolith as far as possible, which in turn resulted in higher working effort and lower sample size. Three monoliths were transplanted locally to serve as control plots (CP) at all altitudes, whilst the three remaining monoliths from the subalpine and montane zone were transplanted downwards (transplanted plots; TP) to the next lower altitude (Fig. 4.1).

After the insertion of the monoliths in the holes dug in their different receptor meadows, small gaps around the monoliths were filled with soil from the respective donor site. The soil monoliths were open to the sides and bottom to allow lateral and vertical soil water fluxes.

Transplantations were carried out after snowmelt (end of March - begin of April 2010). Around the plots, a 30 cm frame was continuously mown in order to prevent or leastwise decelerate plant invasion from the surrounding vegetation. CPs and TPs were mown at the same time as their respective donor sites, to keep up with the traditional harvest regime.

4.3.3 SOIL SAMPLING

Soils were classified as Skeletic Humic Cambisols on siliceous bedrock at all three study sites with a sandy clay loam texture in the fine earth (WRB 2014). A slight increase in the sand fraction was noticed with increasing altitude. From each monolith two topsoil samples were collected in April 2013. Soil samples were cut out with a knife from 0 - 5 cm soil depth (length x width = 10 cm x 2 cm) and mixed together in the field. Holes were refilled and packed to the respective bulk density with soil from the respective donor site of the monoliths to avoid disturbances and preferential water and gas flows in the soils. Soil samples were air-dried, sieved < 2 mm and stored for subsequent analysis in the laboratories.

Soil pH was determined potentiometrically in a 0.01-M $CaCl_2$ solution at a mass/volume ratio of 1 : 2.5 (Soil Survey Stuff 2004). Total organic carbon (OC) and total nitrogen (N_t) of samples were measured by dry combustion (Tabatabai and Bremner 1991) in an elemental analyser (Carlo Erba Nitrogen Analyser 1500, Milano, Italy). As carbonate C was absent in the soil, no acid pre-treatment was included. Plant available K and P were estimated with Calcium-Acetate-Lactate (CAL) extraction (Schüller 1969). Extracted phosphate was measured spectrophotometrically using the molybdenum blue method according to (Murphy and Riley 1962) and extracted potassium was measured with flame atomic absorption spectroscopy.

4.3.4 DETERMINING AMF ABUNANCE IN THE SOIL

The abundance of AMF in the soils' microbial community composition was measured by the abundance of phospholipid fatty acids (PLFA) in the soil which represent AMF. The PLFAs were extracted according to Bligh and Dyer (1959), following the description by Frostegård et al. (1991). In 2011, 2012 and 2013, 1.5 g of moist field-soil was extracted from all plots with 2-4ml (depending on soil water content) of chloroform: methanol: citrate buffer (1:2:0.8) and 3-5 ml of chloroform: methanol (1:2) at each altitude. After phase separation, the non-polar phase (extracted lipids) was collected. Neutral lipids, glycolipids and

phospholipids were fractionated using chloroform (10 ml), acetone (20 ml), and methanol (5 ml) solutions, respectively, over silica solid phase extraction columns (500 mg, ISOLUTE, Separtis). The PLFAs were methylated with methanol-toluol (1:1) solution and 0.2 M methanolic KOH to produce fatty acid methyl esters (FAMEs) (Sundh et al. 1997; Chowdhury and Dick 2012). Samples were then analysed on a gas chromatograph with a flame ionization detector following the procedure in Djukic et al. (2010). The fatty acid nomenclature described by Frostegård et al. (1991) was applied. Indicator of AMF fungal biomass was the PLFA 16:1ω5c (Kroppenstedt 1985; Olsson 1999).

4.3.5 ROOT SAMPLING

To account for a different phenology at the three different altitudes, samples were taken from the CPs and TPs 12th of June, 25th of June and 12th of July 2013, each date corresponding to the flowering of *Agrostis capillaris* (Common Bent) and to the period where the majority of the sampled plants were at the state of early seed, just after flower withering (Fig. 4.1, Tab. 4.1). No samples were taken at the foothill CPs, as those monoliths' vegetation cover has been cut previously to our sampling.

Plant species were identified using an Austrian Flora (Fischer et al. 2008) and chosen according to their presence and abundance along the gradient (Tab. 4.1). At the montane CP and foothill TP however, *Trisetum flavescens* and *Veronica serpyllifolia* were present in high relative abundance (14% and 11% at the montane CP and 9% and 15% at the foothill TP, respectively), but were not sampled due to their absence from the other sites.

A minimum of three and a maximum of five individuals were sampled for nine plant species, including four grass species (*Agrostis capillaris, Dactylis glomerata, Festuca rubra, Poa pratensis*), four herbs (*Achillea millefolium, Carum carvi, Rumex acetosa, Taraxacum officinale agg.*) and one legume (*Vicia sepium*) and pooled per plot type and species (for an overview of species available at each altitude, see Tab. 4.1). Samples were stored in cooling boxes and taken to the laboratory the following day, where they were stored at 4 °C until roots were cleaned and dried for 36 h at 60° C.

Tab. 4.1: Sample distribution by plant species relative abundance over the different plots, altitudes and corresponding sampling dates.

Root sampling date 2013	June 12th	June 25th	July 12th	June 12th	June 25th	June 25th	July 12th
Altitude	950	1450	1950	950	1450	1450	1950
Plot type	LA	LA	LA	TP	CP	TP	CP
Achillea millefolium	2,91	4,14	2,89	10,84	4,22	0,87	9,69
Agrostis capillaris	0,00	1,72	21,58	7,34	3,17	19,87	23,70
Carum carvi	1,69	2,73	1,53	1,75	1,15	2,44	0,27
Dactylis glomerata	2,18	5,15	0,00	3,15	7,58	1,22	0,00
Festuca rubra	0,00	0,00	28,91	0,00	0,00	25,62	22,62
Poa pratensis	7,02	1,21	3,47	0,35	0,67	0,52	3,23
Rumex acetosa	5,57	5,15	5,78	5,94	8,06	1,74	1,80
Taraxacum officinale	11,14	9,79	4,42	6,99	8,54	5,05	0,27
Vicia sepium	4,36	4,95	0,00	0,35	1,73	0,31	0,00
Total relative abundance	34,87	34,83	68,59	36,71	35,12	57,65	61,58

Altitude is indicated in "m above sea level". Plot types are defined as follows: LA = Local Area, TP = Transplanted Plot, CP = Control Plot. Grey colour indicates LAs, TPs or CPs where respective species were not sampled. Relative abundance of plant species (%) is displayed for sampled LAs and TPs and CPs.

4.3.6 ANALYSIS OF FUNGAL COLONISATION

To assess mycorrhizal colonisation rate of dried root samples, all samples were rehydrated in water for one hour, bleached for 15 min in 10% potassium hydroxide at 90°C, rinsed twice with tap water and acidified for 10 min in 3.7% hydrochloric acid. Afterwards roots were stained for 5 min in a 5% ink-vinegar solution (5 ml Waterman Serenity Blue Ink in 95 ml 5% acetic acid) at 90 °C. By 30 min of decolourisation of plant cell structures in pure vinegar, mycorrhizal structures were visible in the roots (Vierheilig et al. 1998).

For each sample, 300 fields of view were checked for the presence or absence of mycorrhizal structures inside the root at 300fold magnification with a Nikon Optiphot

microscope, using the line-intersect method (McGonigle and Miller 1990) in a modified way. Only intact root segments with a diameter smaller than 1 mm, and presenting more than 10 fields of view were examined. Percentage of root-length colonised (%RLC) by intraradical mycelium, arbuscules and vesicles was calculated by dividing the number of observations for each mycorrhizal structure by the number of observed intersections. A weighted mean colonisation rate was calculated by multiplying the root colonisation rate of individual plant species by its abundance for each plot. A cost-benefit ratio (Johnson et al. 1997; Dekkers and van der Werff 2001) was calculated by dividing %RLC by arbuscules by %RLC by intraradical mycelium (A/M), where A can be considered to represent AMF benefit to plants and M is an indicator of the plants costs (Dekkers and van der Werff 2001).

4.3.7 DATA ANALYSIS

To test for differences between %RLC by intraradical mycelium, arbuscules or vesicles for differences between TPs and CPs, for both, the transplantation from the subalpine to the montane and the montane to the foothill zone separately as well as concerning soil properties (pH, OC, N_t, C/N ratio, CAL-P and CAL-K) and abundance of AMF PLFA markers in the soil along the gradient, one-way ANOVAs were calculated with altitude as a fixed factor for each species and also for weighted means of all species, according to their relative abundance. ANOVAs were used in the same way to determine differences Normality was ascertained before assessing equal variance of variables by using non-parametric Kruskal-Wallis-tests or parametric ANOVA. When necessary, variables were transformed to respect statistical requirement (for a complete overview on transformation see App. 2). Tukey's HSD tests were applied post-hoc to ANOVAs and pairwise Wilcoxon rank sum tests succeeding Kruskal-Wallis-tests.

As the cost-benefit ratio A/M and %RLC by arbuscules were highly correlated (R^2=0.93; $p < 0.001$), further statistical analysis considers only %RLC by arbuscules.
Statistical language R 3.0.2 and its corresponding packages (lme4) were used for statistical analysis.

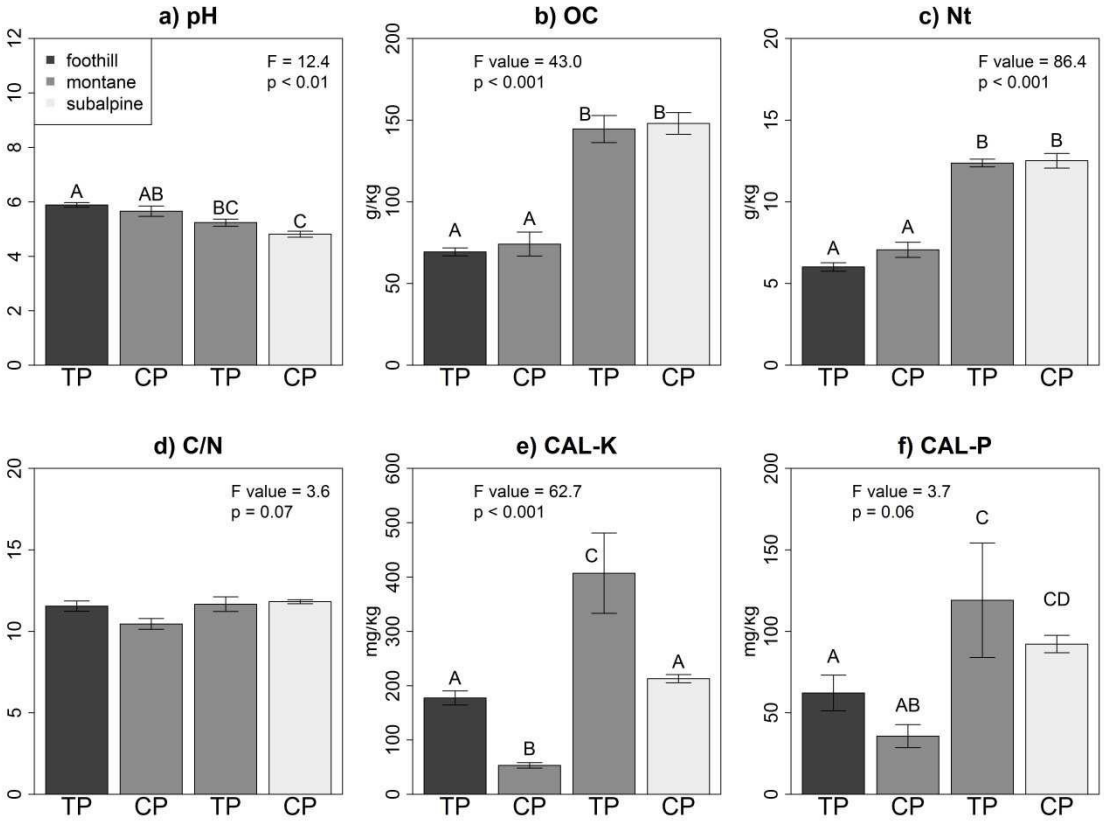

Fig. 4.2: Abiotic soil conditions for different plots and altitudes after three years of transplantation: **a)** pH, **b)** organic carbon OC (g / kg), **c)** total nitrogen Nt (g / kg), **d)** carbon to nitrogen ratio (C/N), **e)** potassium CAL-K (mg / kg) and **f)** phosphorus CAL-P (mg / kg). Letters indicate significant differences between plots and/or altitudes resulting, like indicated F- and p values, from analysis of variance (ANOVA). Error bars show standard errors ±1.

4.4 RESULTS

4.4.1 SOIL PROPERTIES

Soil pH showed a significant decrease with elevation and differed between 5.8 and 4.8 among the studied topsoils in 2013 (Fig. 4.2a). Downwards transplanted soils developed towards the pH of the local plots (pH 5.9 at CP 950 m). Soil analysis further revealed a strong impact of altitude on OC and N_t storage, which represents the net balance of organic matter input and losses, with more than twice the amounts in the subalpine zone compared to the montane zone (Fig. 4.2b, c). Interestingly, after 3 years of increased temperature and evapotranspiration simulation, OC and N_t concentrations remained unaffected in the topsoils. The C/N ratio (Fig. 4.2d) circled around 11.5 in all plots, reflecting a similar possibility of microbial activity and soil organic matter decomposition in the CPs and TPs. Concentrations of plant available K differed among soils and increased significantly in the downwards transplanted soils (Fig. 4.2e). Amount of CAL-P followed a similar pattern, but this trend was

only marginally significant (Fig. 4.2f). Measured soil moisture content at 5 cm soil depth during the four vegetation periods from 2010 to 2013 was 27% for the foothill site, 32% for the montane site and 35% for the subalpine site on average. For a more detailed information on soil hydrology at the specific sites see Della Chiesa *et al.* 2014.

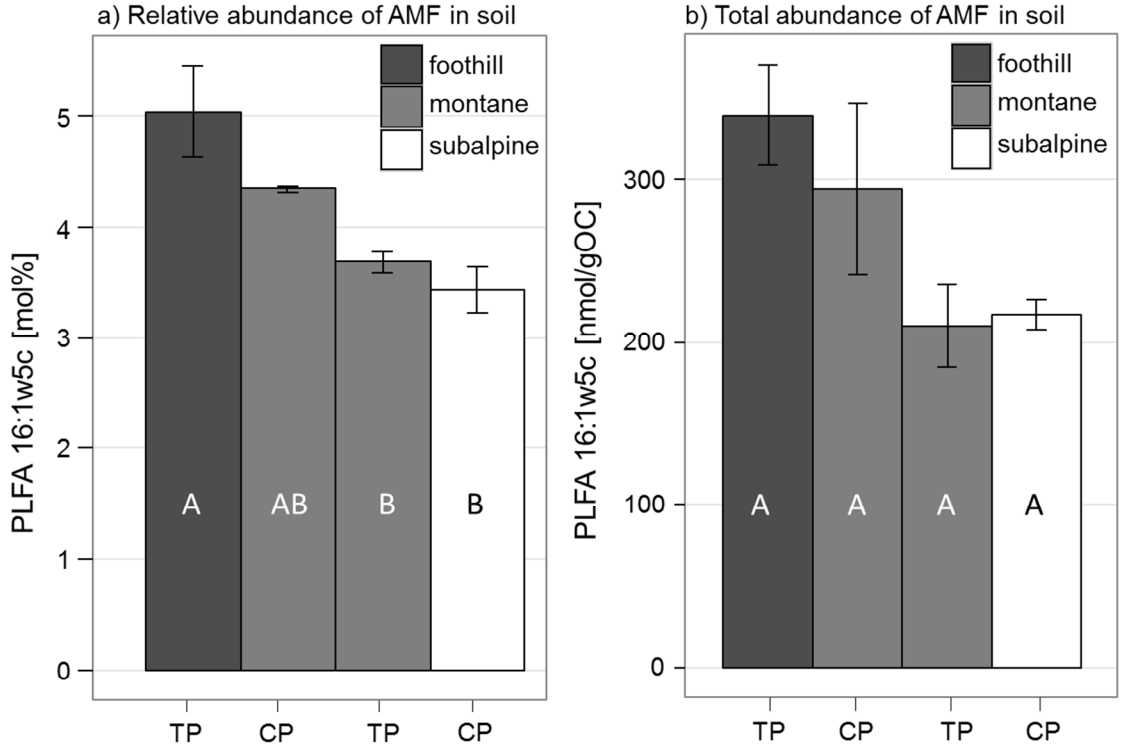

Fig. 4.3: Effects of downward transplantation (TP) on relative (a) and total (b) abundance of phospholipid fatty acid markers for AMF in the soil in comparison to control plots (CP) along the transplantation gradient form the foothill to the subalpine zone. Letters indicate significant differences between plots and/or altitudes resulting, like indicated F- and p values, from analysis of variance (ANOVA). Error bars

4.4.2 ABUNDANCE OF AMF IN THE SOIL MICROBIAL COMMUNITY

Neither the upper nor the lower transplantation affected relative AMF abundance in the soil ($p=1.000$ and $p= 0.817$ respectively; Fig. 4.3a) nor had they effect on the relative AMF abundance ($p= 0.946$ and $p=0.308$ respectively; Fig. 4.3b) in the soil microbial community.

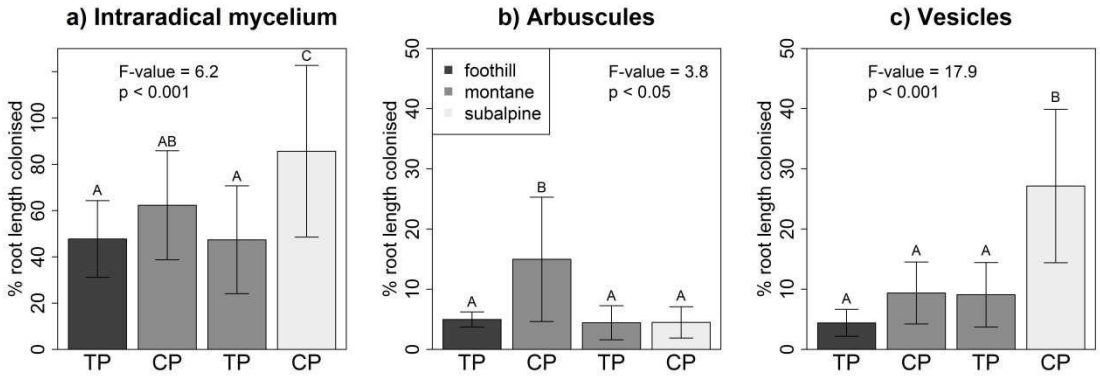

Fig. 4.4: Weighted means of % of root length colonised by **a)** intraradical mycelium, **b**) arbuscules or **c)** vesicles for downward transplanted (TP) and control plots (CP) at different altitudes. Letters indicate significant differences between altitudes resulting, like indicated F- and p-Values, from analysis of variance (ANOVA). Error bars show standard errors ±1.

4.4.3 AMF ROOT COLONISATION RATE

All nine collected plant species were colonised by AMF and all three mycorrhizal structures were present at all altitudes. We observed a difference in colonisation rate between the higher transplantation from the subalpine to the montane site and the lower transplantation from the montane to the foothill site (Fig. 4.4). Three years of exposition to a new climate induced a significant decrease of weighted means of %RLC colonised by intraradical mycelium in plants originating from the subalpine belt (Fig. 4.4a; App. 2): the subalpine control plot (CP) proved to be significantly higher colonised by intraradical mycelium than the montane transplanted plot (TP). Seven species were concerned by downward transplantation from subalpine to montane zone: *A. millefolium, A. capillaris, F. rubra, C. carvi, P. pratensis, R. acetosa, T. officinale*. On species level, a significant decrease of %RLC by intraradical mycelium was found at the montane TP for *A. millefolium, A. capillaris* and *C. carvi* (Fig. 4.5a; App. 2) while remaining species followed a similar trend, but were not significantly different. For arbuscules, downward transplantations from the subalpine to the montane belt resulted in similar weighted means of %RLC, but the montane TP's weighted means of %RLC remained about 1/3 lower compared to montane CP (Fig. 4.4b; App. 2). Individual species %RLC by arbuscules showed only a significant pattern for *F. rubra* and *P. pratensis*, and a trend for *A. millefolium*, always with a decrease of %RLC from higher to lower site, except for *T. officinale* which increased by more than five times its %RLC after transplantation (Fig. 4.5b; App. 2). Weighted means of %RLC by vesicles decreased significantly after three years of transplantation from subalpine to montane zone. The

montane TP attained similar levels of %RLC by vesicles compared to the montane CP (Fig. 4.5c; App. 2). Reflecting the pattern of the weighted means, significant decrease in %RLC by vesicles was also observed for *A. millefolium*, *C. carvi*, *F. rubra*, and *T. officinale* (Fig. 4.5c; App. 2).

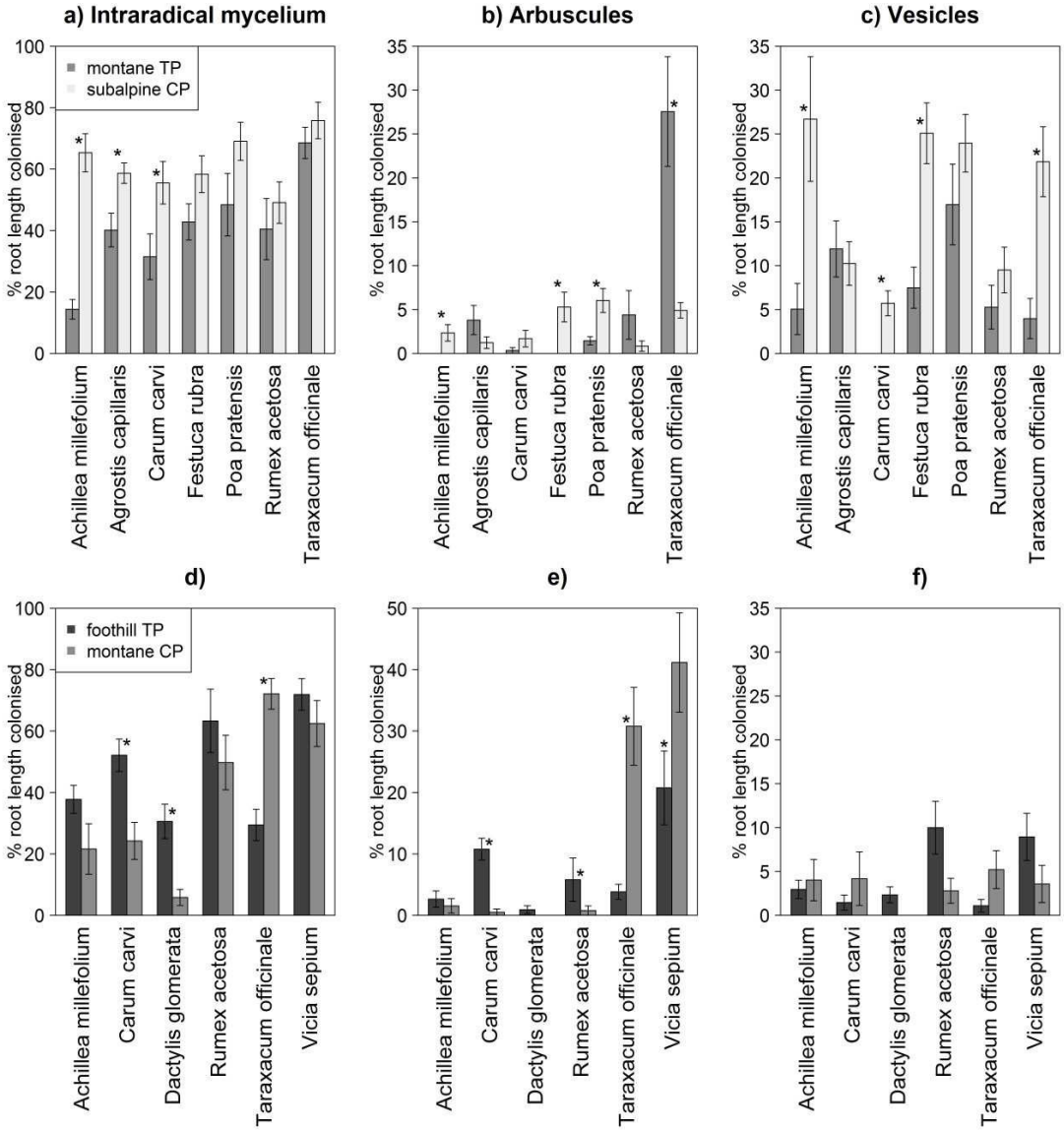

Fig. 4.5: % of root length colonised by **a)** intraradical mycelium, **b)** arbuscules or **c)** vesicles per species for transplanted (TP) and control plots (CP) after three years of transplantation form the subalpine (1950 m) to the montane (1450 m) zone and % of root length colonised by **d)** intraradical mycelium, **e**) arbuscules or **f)** vesicles per species for TPs and CPs after three years of transplantation from the montane to the foothill (950 m) zone at different altitudes above sea level. Letters indicate significant differences between altitudes resulting from analysis of variance (ANOVA). Error bars show standard errors ±1.

Six species were concerned by the lower transplantation from montane to foothill zone: *A. millefolium, C. carvi, D. glomerata, R. acetosa, T. officinale, V. sepium*. In contrast to the higher transplantation from the subalpine to the montane site, no significant decrease was observed for weighted means for all mycorrhizal structures, neither of %RLC by intraradical mycelium, nor by arbuscules or vesicles, when comparing the montane CP to the foothill TP three years after the lower transplantation (Fig. 4.4; App. 2). At species level however, we observed a significant increase of %RLC by intraradical mycelium for *C. carvi*, *D. glomerata* and *T. officinale* (Fig. 4.5d; App. 2). *C. carvi* also showed significantly higher %RLC by arbuscules (Fig. 4.5e; App. 2), as did *R. acetosa*. However, *T. officinale* and *V. sepium* revealed a significant decrease of %RLC by arbuscules (Fig. 4.5e App. 2) after three years of transfer from the montane to the foothill zone while the other species remained unaffected.

4.5 DISCUSSION

We note a shortcoming of field studies assessing the effects of warming and increased evapotranspiration on AMF dominated ecosystems in literature. As far as we know, this is the first transplantation project where a warming effect on AMF was studied under realistic conditions in the field (Wahl and Spiegelberger 2016). Moreover, our study provides new insights into the development of arbuscular mycorrhizal root colonisation of several plant species, after being subject for three years to future climatic conditions, simulated by double downward transplantation, from the subalpine to the montane vegetation belt and from the montane to the foothill vegetation belt.

Even though both transplantations induce a similar increase in temperature (+ 2.8K) and consistent precipitation quantities (about 830 mm/year), we observed different reaction to each transplantation for the intraradical AMF structures and no change for the abundance of AMF PLFA marker in the soil. When subalpine vegetation was subjected to montane climatic conditions, we observed a lower AMF colonisation rate as a rather uniform response of all plant species when subalpine vegetation was subjected to montane climatic conditions. However, contrasting individual species responses impeded a common reaction for montane vegetation when subjected to foothill conditions.

In the majority of studies addressing warming and/or drought under controlled simplified conditions, AMF reacted with increased root colonisation rates and increased spore and extraradical mycelium densities (Compant et al. 2010). In the field, short–term studies up to three years do not react to warming treatment (Yang et al. 2013; Sun et al. 2013) and a shift from extraradical mycelium to intraradical hyphae is observed from long-term studies (Rudgers et al. 2014). Yet, we observed a significant decrease of %RLC by AMF mycelium after three years of transplantation from subalpine to montane grassland and no effect on the abundance of AMF in the soil. In our study, this change goes along with the observation of a significant increase in phytomass at the montane TPs after three years of transplantation (mean increase of above-ground phytomass 2010-2012 ca. +66% or +320gm^{-2}; respectively, Niedrist, personal communication, 2015) which in turn is in accordance with some studies (Dunne et al. 2003; Kullman 2010; Frei et al. 2014), but contrasts with others (Gavazov et al. 2013; Gavazov et al. 2014). Seemingly, in our study, plant aboveground productivity, accustomed to highly variable microclimatic conditions under subalpine climate, doesn't suffer but rather profits from the warmer montane temperatures. While the application of cattle manure remained the same at all plots during 3 years of climate simulation, an increase in the soil nutrient availability of some nutrients, namely CAL-extractable K and a trend for CAL-extractable P, was noticeable for montane TPs. This seems to result from the temperature increase after transplantation to the montane vegetation belt, inducing faster mineralisation of fresh organic plant litter and the applied farmyard manure.

Plants that profit from warming and augmented nutrient availability might invest less into AMF, which can, matching our observation, result in a lower colonisation rate (Johnson et al. 2003). However, two recent field experiments on AMF and warming in dry (sub)alpine grasslands showed no significant influence on % RLC: neither one year of warming treatment of 1.3K in a Mongolian grassland with a slightly lower mean annual temperature compared to our subalpine site (2.1°C vs. 3.4°C)(Sun et al. 2013), nor three years of warming treatment of about 1.5K in an Tibetan alpine meadow (mean annual temperature -2°C) (Yang et al. 2013). This raises the question, whether some thresholds in exposition time to the warming treatment and/or the height of the induced temperature rise exists, beyond which a change in AMF colonisation rate may be observed.

Contrasting our results from transplantations from the subalpine to the montane belt, individual species showed no uniform changes for AMF colonisation rate after three years of

new climatic conditions. We observed that the increase of CAL-K and trend for increase of CAL-P was less pronounced in the soil monoliths transplanted from the montane to the foothill zone. Levels of CAL-K were either considered "sufficient" or "high" in all studied plots, only the montane CP showed a "low" nutrient supply level for grassland according to a national fertilization guide (BMLFUW 2006). Furthermore, the increase of soil pH at lower altitudes supports the retention of positively charged nutrients at sorption surfaces with variable charges in the soil matrix and increase microbial activity. Plant-available P concentrations show a general "sufficient" supply and only a "low" to "sufficient" supply in the montane CP similar as found for CAL-K (BMLFUW 2006).

Besides the difference in nutritional conditions after three years of transplantation from the montane to the foothill site, the maintenance of %RLC might additionally be attributed to the increasing evapotranspiration with declining altitude (Körner 2003). Short drought periods were recorded at the lowest site in the foothill belt and attributed to evapotranspiration (Della Chiesa et al. 2014). Those drought periods at the foothill site seemed to counteract benefits from temperature rise which could be expected to be similar to those observed for the montane TPs. The previously observed increase in plant productivity and decrease of AMF colonisation rate from the higher transplantation fail to appear at this lower transplantation. We assume that in contrast to the higher transplantation, a drought threshold seems to be passed when transplanting from the montane to the foothill zone.

Different plant species may react even to short-term droughts with species specific adaptations. These adaptions contribute to the stability of the plant community, but leaf dry matter, leaf carbon content or specific leaf area might change (Jung et al. 2014). Thus changed aboveground plant functional traits in turn can affect %RLC (Heinemeyer & Fitter 2004) and lead in consequence to species specific reactions of %RLC in different plant species, ranging from an increase to no effect or to a decrease in %RLC. Architectural and morphological root traits can respond rapidly to drought and are known to influence soil microbes such as mycorrhiza (Bardgett et al. 2014). In our study we observed for example a significant decrease in %RLC for *Taraxacum officinale* in the foothill TP. This could be linked to the specie's deep root structure providing a better access to remaining water resources and therefore less need for investment into AMF.

Some other aspect such as host selectivity (Eom et al. 2000) could also contribute to the significant interspecific differences in AMF colonisation rate for different plant species. Furthermore, warming might affect the colonisation of specialist species exclusively found in only one altitudinal zone in a different way than more generalist plant species (used for this study), which cover and are adapted to a broader range of ecological and climatic conditions.

Besides species or plant functional traits, plants can also be grouped concerning their abundance (Whittaker 1965). Subordinate plant species - present in plant communities with high frequency, but with a cumulative relative abundance of about 10% - may have more influence on below-ground processes as dominate species and may react in an opposite way to climate change (Mariotte 2014). Under unfavourable mycorrhizal conditions, dominant species can be more harmed than subordinate species (Mariotte et al. 2013). As a consequence, subordinate species seems to be more resistant to drought (De Vries et al. 2012) and also warming (Richardson et al. 2002) than dominant. At the subalpine meadow *A. capillaris* and *F. rubra* were dominant plant species, while at the montane zone all sampled species were subordinates. However, we could not find any relations between the predominance and the colonisation rate.

Usually, plants at high altitudes allocate more biomass below-ground and invest it mainly into fine roots, maybe to compensate lower AMF colonisation (Brown et al. 2013), while below-ground storage organs are similar to lower altitudes (Körner and Renhardt 1987). Plant with deeper root-systems seem to profit more from AMF colonisation than plants with shallower roots (Wang et al. 2011). We observed interspecific differences in colonisation rate that might be related to root architecture. *T. officinale* displayed a significant converse reaction for colonisation rate by arbuscules after both transplantations compared to all other species. Instead of a decrease after transplantation from the subalpine to the montane site as observed for *P. pratensis*, *F. rubra* and *A. millefolium*, root colonisation by arbusucles increased significantly for *T. officinale*. This indicates that the relationship between *T. officinale* and AMF became more mutualistic, while the opposite is true for the three other plants (Fig. 4.5b). At the lower transplantation the colonisation rate by arbuscules increased for *C. carvi* and *R. acetosa* after transplantation, indicating that they benefited more from AMF colonisation. Both observations indicate that plants with a deep-root systems profit from AMF colonisation underlining the important of the interaction between root systems and AMF colonisation.

In general, the cost-benefit ratio didn't change significantly after three years of new climatic conditions. The observed significant difference of colonisation rate by arbuscules between the montane control plot and the downwards transplanted plot originating from the subalpine zone might indicate, that a change in AMF-plant relationship can take time (Dekkers and van der Werff 2001) and that three years of new climatic conditions are not sufficient to induce a significant change in the AMF-plant community relation.

4.6 CONCLUSION

Our first hypothesis based on assumptions drawn from literature states that a rise in temperature and an increased evapotranspiration should lead to an increase in AMF colonisation rate. Most of the available literature is based on greenhouse studies. With our study in the field we could not confirm this assumption and have to reject our hypothesis.

The three-year simulation of a 2.8 K climate warming by double downward transplantation along an altitudinal gradient revealed a strong impact on plant-AMF interaction at the subalpine zone for all plant species. However, in contrast to our second hypothesis' expectation the colonisation rate decreased. While improved environmental conditions for plants due to warming led to a lower root colonisation rate, the colonisation rate in the plant species was maintained under declining environmental conditions due to drought events. No overall impact was detected for the plant-AMF interaction after three years of transplantation from the montane to the foothill belt, due to variable species-specific reaction. Even though we did not see a change in the cost and benefit for plants this might suggests that AMF mitigated plant productivity under drought conditions, conform to the third hypothesis.

Future field studies are necessary to draw a clearer picture of the effects of climate change in mountain grassland ecosystems. They should also consider a genetic approach to determine shifts in community composition of AMF as well as studying specifically groups of species such as subordinates, rare or endemic mountain species.

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5 EFFECTS OF AMF, CLIMATE CHANGE AND PLANT DIVERSITY ON PLANT PRODUCTIVITY

A field experiment's closeness to reality is advantageous to obtain realistic results. They are however more impracticable when wanting to investigate belowground events without disturbance or when trying to identify single factor effects. In contrast to field experiments, climate chamber experiments allow us to control all physical variables below and above ground. However, those studies are mainly short-term, at representativity's expense and focus on rather early successional stages (van der Heijden et al. 2003).

The here realized climate chamber experiment complements the field experiments and gives the opportunity to investigate plant competition and individual effects of different climatic factors. It was conducted by using local seeds and soil inoculum and by simulating the local climatic conditions from the field site in the French Alps. This approach stands out from other studies published up to now, where primarily commercial fungi of temperate origin were used as inoculum on mainly commercial plants or seedlings (Compant et al. 2010; Mariotte et al. 2013). In the following I present the first results obtained from this experiment.

5.1 INTRODUCTION

Global climate change is manifested in mountain ecosystems by an increase of about 2 K in the minimum temperatures and altered precipitation patterns (Körner 2003, IPCC 2013) which leads to the mass loss of glaciers, shorter periods of snow cover and subsequently to an alteration of the hydrological conditions (IPCC 2013). The climatic changes lead to a decrease of plant biodiversity (Niedrist et al. 2009) and decline in the capacity to store carbon (Sjögersten et al. 2011, Leifeld et al. 2011, Puissant et al. 2015) in (sub-) alpine grasslands.

However, overall grassland productivity increases under climate change conditions (Kullman 2010, Grabherr et al. 2010, Pauli et al. 2012). The mentioned changes also affect pastures and meadows from the montane to the alpine zone which are used for agriculture. In mountain regions such as the European Alps grasslands are important landscape elements. They are central to agricultural productivity, act as protection against soil erosion (Tasser et al. 2003), are home to a diversity of medicinal plants (Grabherr 2009), serve as carbon sink

(Follett and Reed 2010) and, with their high biodiversity (Väre et al. 2003), contribute to the landscape's attractiveness, thus adding to the touristic value (Körner 2003). The maintenance of these ecosystem services provided by alpine grasslands depends on the diversity of plant community composition and other environmental factors such as climate.

Understanding the mechanisms that drive the development of plant community composition is one of the major goals of ecological research. Most of the time, abiotic conditions alone do not provide satisfactory explanations (e.g. Ellenberg 1952). Why some plants dominate under particular conditions and others do not is further determined by the competitiveness of plants (Grime 1977). Grassland ecosystems are generally dominated by few dominant species which account for a large proportion of the total plant biomass and accompanied by numerous less abundant plant species. Even though they are numerous, those subdominant species add mainly to the diversity of a grassland ecosystem rather than to its biomass, because they are mostly of small stature (Grime 1998).

Besides competitive and thus negative plant-plant interactions, facilitation and positive plant-plant interactions (e.g. Bertness and Callaway 1994; Brooker and Callaghan 1998; Bruno et al. 2003; Michalet et al. 2006) as well as positive plant-soil interactions (van der Heijden and Horton 2009; Montesinos-Navarro et al. 2012; Ossler et al. 2015) have become central and greatly advanced plant community research during the last 20 years. Despite recent advances, research has not gone far enough to understand the role of facilitation processes in mediating climate change impact yet (Brooker et al. 2008; Powell et al. 2013; van der Heijden et al. 2015). Especially research on the role of plant-soil interactions considering ubiquitous symbiotic microbes is still incomplete (Wardle et al. 2004).

Arbsucular mycorrhizal fungi (AMF) from the phylum Glomeromycota for example, are in symbiotic relationship with about 70% of the world's vascular plant species (Smith and Read 2008). AMF provide plants with nutrients, such as nitrogen and phosphorus, and water on the one hand, while, on the other hand, they receive photosynthetically derived carbon compounds from the host plant in exchange and thus affect the plant carbon budget. In 75% of all cases plant performance is improved and the plant-AMF relationship is considered to be mutualistic, but it is also described as parasitic in certain cases (Johnson et al. 1997; Klironomos 2003; van der Heijden and Horton 2009; Johnson and Graham 2013). The effect of AMF on different plant species varies because plants are not always dependent on AMF, but can also be facultative associated with AMF or completely non-mycorrhizal (Klironomos

2003; Bidartondo 2005; Smith and Read 2008). On the contrary to plants, AMF are obligate symbionts depending on the plant derived carbon compounds which are invested into intra and extra radical hyphae, spores, arbuscules and vesicles (Smith and Read 2008).

The plant-AMF relationship has been conceptualised in several frameworks and the question whether the plant community affects the AMF community or vice versa is a topical research question. The Driver/Passenger hypothesis supposes that if AMF were Drivers, they would orchestrate the changes in the plant community, whereas as Passengers, changes in AMF community composition would be a by-product of changes within the plant community (Hart et al. 2001). The Habitat hypothesis as a further framework suggests to explain concurrent changes in plant and AMF communities by abiotic conditions (Zobel and Öpik 2014). Evidence to support all three hypotheses exists and the role of abiotic drivers and which community drives which remains to be determined.

If AMF are considered as Drivers of plant communities, they could affect individual host plant productivity and, together with other soil microbes, regulate plant competition for nutrients and thus vegetation dynamics in ecosystems (Simard and Durall 2004; van der Heijden et al. 2008). Plant species from grassland vegetation are generally highly colonised by AMF (Treseder and Cross, 2006). However, results of studies that assess whether the few plant species dominating the vegetation also receive the most benefits from AMF are controversal. AMF were observed to reduce the differences in competitiveness between subordinate and dominant plant species (van der Heijden et al. 1998; Wagg et al. 2011b; Mariotte et al. 2013), enhance the competitiveness of one competitor plant species (Zobel and Moora 1995) or shift the competition from one plant species to another (Hartnett et al. 1993; Daisog et al. 2012) in different model grassland communities.

In their supposed role as Drivers of ecosystems an important function with regard to climate change related changes in ecosystem is attributed to AMF. Due to their position at the plant-soil interfaces and their mainly positive effect on plant performance, they could mitigate the effects of climate change for plants. In fact, AMF are known to improve the performance of plants under drought stress (Augé 2001; Compant et al. 2010; Mohan et al. 2014). Conversely, their effects on plants following warming treatment range from negative to positive (Compant et al. 2010; Mohan et al. 2014). Most of the studies assessing climate change effects on AMF and their relationship to plants focus on one or two factors under

rather artificial circumstances using non-natural soils, cultivated inoculum and seeds in most of the cases (Compant et al. 2010; Covacevich et al. 2012; Ohsowski et al. 2014; Mohan et al. 2014). However, while simplified greenhouse studies are important to disentangle the effect of different climate change factors and assess AMF effects by manipulating their abundance, it would be preferable that an experiment is designed close to natural conditions which allow generalisations from a greenhouse experiment to the ecosystem in question. AMF species appear for example to be adapted to their soil of origin (Johnson et al. 2010; Leigh et al. 2011), which implies that results from experiments using fungi and soil of different origins might not reflect processes occurring in the field.

The aim of the present study was to investigate the role of AMF on interspecific plant competition under current and future climatic conditions. The here presented climate chamber experiment was conducted under simplified conditions by using local seeds from dominant and subordinate grass species as well as local soil inoculum from a subalpine field site in the French Alps. The samples were either subjected to local climatic conditions or to warmer and drier conditions than at their original habitat.

We tested the following hypotheses: 1) Less dominant plant species will benefit more from AMF and competitiveness among plant species will be reduced. 2) Plant biomass production is maintained under warmer and drier conditions due to the presence of AMF. 3) Due to their ability to mitigate climate change effects and influence plant productivity, AMF effect on plant competitiveness is different under future and current climatic conditions.

5.2 MATERIAL AND METHODS

5.2.1 SEED AND SOIL COLLECTION

Native seeds were collected from an extensively used pasture (45°05'15.2", N 6°03'16.9" E, 1640 m asl) on a limestone plateau at at Alpe d'Huez in the French Central Alps in August and September 2013. We had assessed the vegetation type beforehand with several relevées and found it to be in a transgression state from *Arrhenatherion* to *Cirso-Brachypodion*. We chose seeds of the two dominant grass species *Brachypodium pinnatum* and *Arrhenatherum elatius* and the two subordinate plant species *Bromus erectus* and *Dactylis glomerata* as target species and collected them randomly from plant individuals all over the field site. Seeds were air-dried, cleaned and then vernalized at 4 °C until further use. Their germination capacities

were assessed in a germination experiment in 2014 which turned out successful for all plant species. 20 soil blocks of 15 cm depths and about 0.25 m^2 were collected from in August 2014 from the same field site. Stones, aboveground plant structures as well as macro fauna were removed by hand. The remaining soil-root mixture was then well mixed with a shovel, sieved at 5 mm and stored at 4°C until further use.

5.2.2 EXPERIMENTAL DESIGN

The growth chamber experiment was set up as a randomised split-block design (Fig. 1) with four factors: (1) Presence of AMF (high/low), (2) temperature treatment (current/warmer), (3) water availability (current/lower) and (4) plant mixture type (monoculture, 4-species mix of *A. elatius, B. erectus, B. pinnatum,* and *D. glomerata*). Five replicates were produced for each treatment (Fig. 1).

5.2.3 SOIL STERILISATION AND MICROBIAL WASH

Half of the soil to be used as control was sterilised by γ-irradiation at 15,4 kGy at IONISOS (Parc Dombes Côtières Activités, 01120 Dagneux, France). Then, both soils were thoroughly mixed with autoclaved sand (1:1 / v:v). In total, 260 rectangular pots (9cm x 9cm x 9,5cm) were filled with 0,4 l of substrate each. A microbial wash was produced by passing 2 kg of unsterilized original field soil and 10 l deionised water through a 20 µm nylon mesh to remove AMF (Ames et al. 1987). This wash was distributed evenly between all pots containing the sterilised substrate one week before the start of the experiment. It was applied to reintroduce the remaining microbial community (smaller than 20 µm). Unsterilized pots received the same amount of deionised water.

5.2.4 SEED GERMINATION

Six weeks before the start of the experiment, 1200 seeds per target species were surface sterilised and dispersed on 1% autoclaved Agar solution containing 1/10 of Murashige and Skoog nutrient basal medium in germination trays with humidity lids. Germination was conducted in a greenhouse cabin. During the first 12 days conditions were at 25°C day temperature and 15°C night temperature and then changed to 20°C day time temperature and 10 at night.

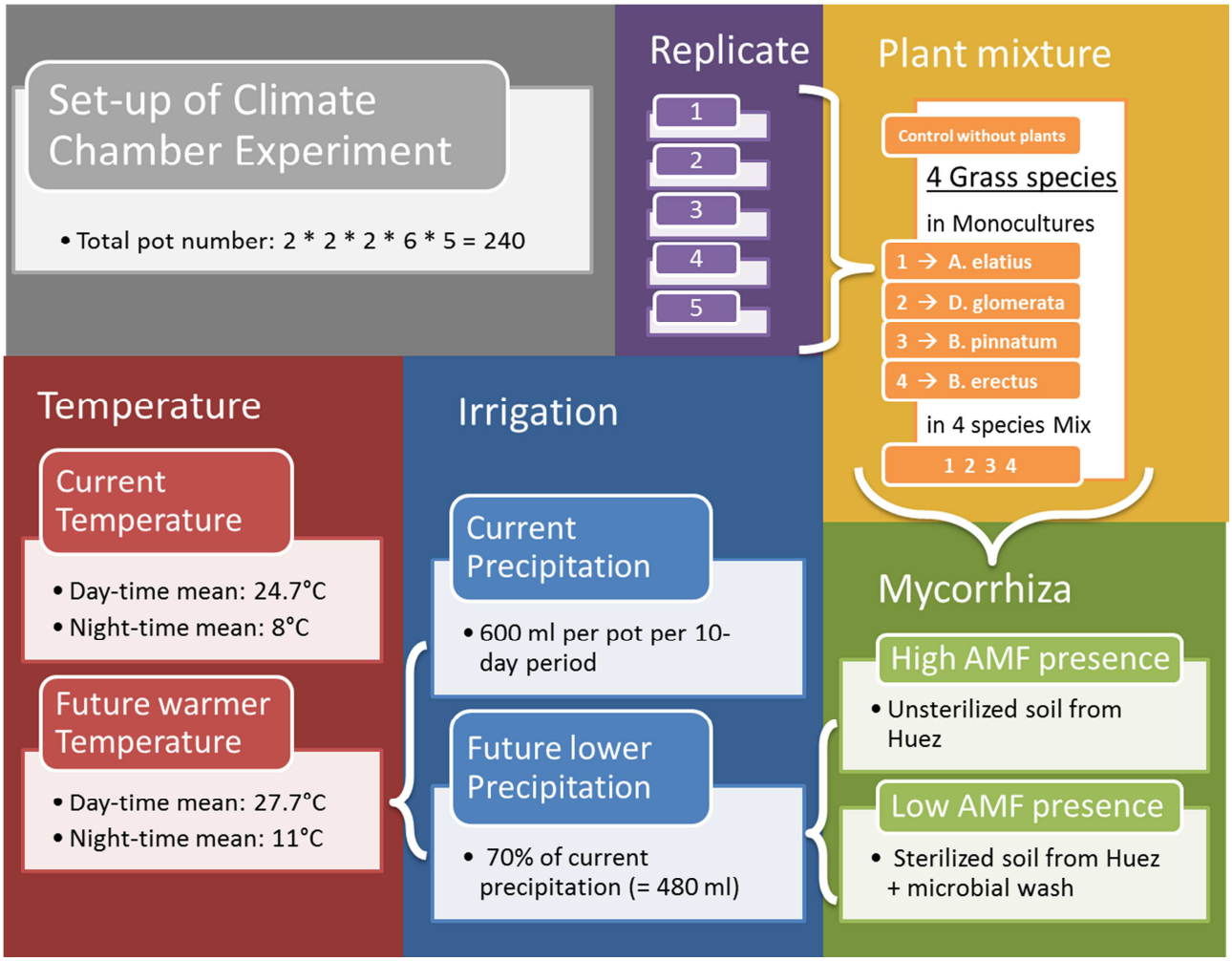

Fig. 5.1: Schematic diagram of the experimental set-up: Five replicates (1-5) were produced for each treatment. Six different plant mixtures were investigated: The monoculture pots (1x) contained four individuals (A. elatius, B. erectus, B. pinnatum, and D. glomerata) of the same grass species , 4-species mix one individual per grass species and the Control pots were plant free. They were either grown in unsterilized soil (High AMF) or in treated soil (Low AMF) and either subjected to normal irrigation (Current Precipitation) or low irrigation (Future Precipitation) as well as to two different temperature regimes (Current Temperature, Future Temperature). Different temperatures were simulated alternatingly in two separate climate chambers. All other treatments were placed mixed on trolleys and their position in the climate chamber changed randomly following each irrigation event.

All experimental pots were planted in the days preceding the first 10-day period of the experiment. The monoculture pots (mono) contained four individuals of the same grass species (*A. elatius, B. erectus, B. pinnatum,* and *D. glomerata)* and the 4-species mix (quarto) one individual of all four grass species. Plants were planted with even distance between the plants and to the pot walls.

5.2.5 TEMPERATURE AND AIR HUMIDITY

One vegetation period was simulated during ten 10-day periods in two climate chambers from March to July 2015 at the research Institute Agroscope Reckenholz in Zurich, Switzerland. Mean temperatures were increasing steadily for the first six 10-day periods and then decreasing. Simulation started with 5 °C minimum temperatures during night time and 17 °C maximum temperatures during day time for the first 10-day period. Starting with the second 10-day period, the temperature regime for the current climate (CT) was simulated for half of the pots according to the temperatures recorded at the weather station of Alpe-d'Huez-Altiport (45°05'16" N, 6°05'08" E, 1860 m a s l), which is situated 3 km from the field site. Simulation of future warmer temperatures (FT) was also started in the second 10-day period and was always 3 °C higher than the current temperature referring the prediction of IPCC's climate scenarios A1B (+2.8°C). The highest maximum day temperatures and minimum night temperatures were simulated during the 6^{th} and 7^{th} 10-day period. During these 20 days, CT peaked at 27.5 °C and FT at 30.5 °C (Fig. 5.2). To exclude chamber effects, the warming treatment alternated between the two chambers in a 10 day rhythm and positions of pots within each climate chamber were randomized during this exchange and after each irrigation event.

5.2.6 IRRIGATION

During the experiments full duration two different precipitation scenarios were simulated by different irrigation regimes. The current precipitation pots (CP) of the pots were watered with 600 ml of demineralised water per ten day periods (5 irrigation events à 120 ml in 10 days). The other half of the pots received only 425 ml of demineralised water per ten day period, which is about 30% less, to simulate future lower precipitation (FP) during the vegetation period. 20ml standard NPK-fertiliser was added per 3 l irrigation water during the fourth, sixth and eights 10-day period.

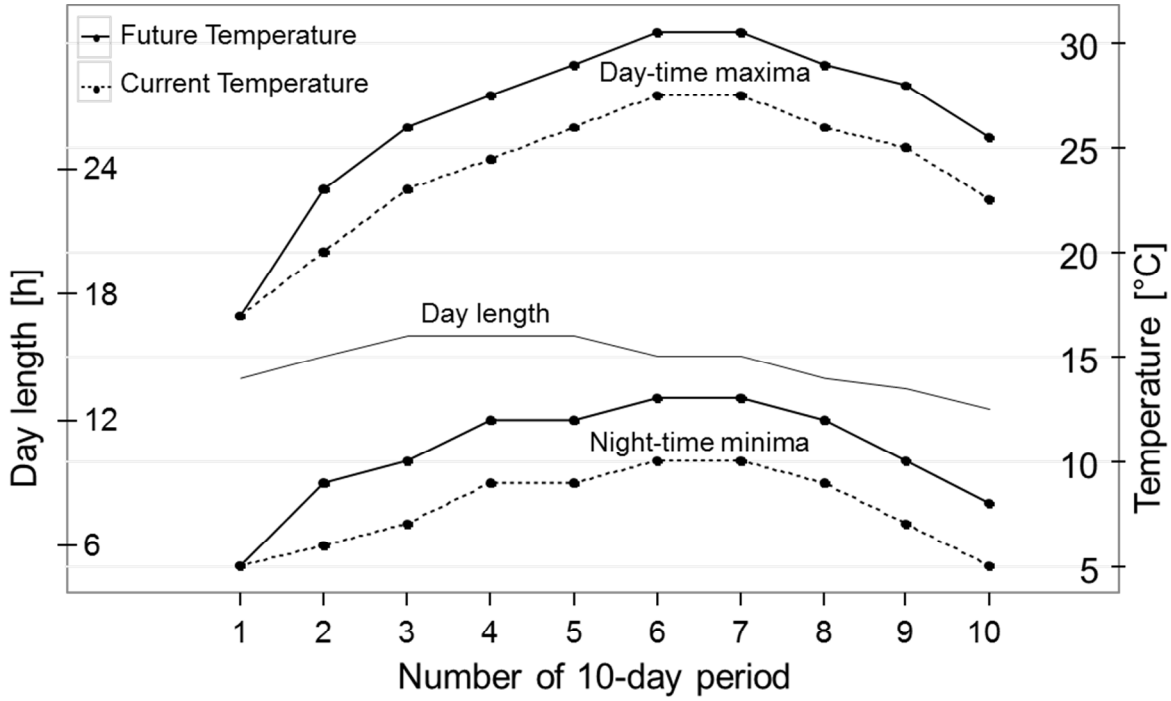

Fig. 5.2: Day length and temperature during the experiment. The mean current and future minimum and maximum temperatures as well as hours of day length are displayed for the ten 10-day periods of simulation.

5.2.7 PLANT SAMPLING

At the end of the experiment plants were removed from the pots and most of the soil shaken off. They were then washed and their roots were carefully separated from each other, roots cut from the shoots, and the shoot section further divided into dead and living plant material. All plant material was then dried for 48 h at 60 °C and dry weights recorded individually. The root-shoot ratio was calculated by dividing root dry-weight by shoot dry-weight. The shoot vitality was calculated as the percentage of vital shoot on the total shoot biomass. To check for AMF colonization in the roots of plants from the low AMF treatment we randomly selected one root sample per species per low AMF treatment. It was rehydrated in water for one hour, bleached for 15 min in 10% potassium hydroxide at 90°C, rinsed twice with tap water and acidified for 10 min in 3.7% hydrochloric acid. Afterwards roots were stained for 5 min in a 5% ink-vinegar solution (5 ml Waterman Serenity Blue Ink in 95 ml 5% acetic acid) at 90 °C. By 30 min of decolourisation of plant cell structures in pure vinegar, mycorrhizal structures were visible in the roots (Vierheilig et al. 1998). For each sample, 100 fields of

view were checked for the presence or absence of mycorrhizal structures inside the root at 300fold magnification with a Nikon Optiphot microscope, using the line-intersect method (McGonigle and Miller 1990) in a modified way.

5.2.7 SOIL SAMPLING

The remaining soil for each pot was well mixed and soil samples of about 10 g were taken, fresh weight and dry weight determined and its carbon and nitrogen content analysed. Further fresh soil samples of 0.5 g from the monoculture pots planted with *Arrhenatherum elatius* as well as from the plant free control pots were taken for sequencing and microbiological analysis.

5.2.8 DNA EXTRACTION AND QUANTIFICATION

Soil samples (0.5 g) were subjected to three consecutive nucleic acid extractions using the bead beating procedure described by Bürgmann et al. (2001). Soil DNA was resuspended in TE (10 mM Tris/Cl, 1 mM EDTA, pH 8) at 1 ml g-1 dry weight equivalent of extracted soil. Quality of DNA was examined by electrophoresis in 1 % (w/v) agarose gels and ethidium-bromide staining. DNA was quantified fluorometrically with PicoGreen (Invitrogen, Carlsbad, CA) according to Hartmann et al. (2005). DNA concentration of the extracts was adjusted to 10 ng ml-1. Amplicon-based metagenetic analyses as well as downstream bioinformatics and statistics were performed as described by Frey et al. (2016) and are briefly described in the following sections.

5.2.9 PCR AMPLIFICATION OF TARGET REGIONS

The V3-V4 region of the prokaryotic (bacterial and archaeal) small-subunit (16S) rRNA gene was amplified with slightly modified versions of primers 341F (CCTAYGGGDBGCWSCAG;) and 806R 170 (GGACTACNVGGGTHTCTAAT; (Frey et al. 2016). The internal transcribed spacer region 2 (ITS2) of the eukaryotic (fungal and some groups of protists and green algae) ribosomal operon was amplified with degenerate versions of primers ITS3 (CAHCGATGAAGAACGYRG) and ITS4 (TCCTSCGCTTATTGATATGC) described by Tedersoo et al. (2014). The 5’ ends of the primers were tagged with the CS1 (forward) and CS2 (reverse) adapters required for multiplexing samples using the Fluidigm Access Array™ System (Fluidigm, South San Francisco, 175 CA, USA). PCR amplification was performed in a total volume of 25 µl reaction mixture containing 20 ng of template DNA, 1x PCR-buffer (Qiagen, Hilden,

Germany), 2 mM MgCl2, 0.2 mM of each primer, 0.4 mM deoxynucleoside triphosphate (dNTP; Promega, Dübendorf, Switzerland), 0.6 mg ml-1 bovine serum albumin (BSA; Fluka, Buchs, Switzerland), and 2 U HotStar Taq polymerase (Qiagen). The PCR amplification conditions for the prokaryotic 16S and eukaryotic ITS2 fragments consisted of an initial denaturation at 95°C for 10 min, 38 (16S) or 42 (ITS2) cycles of denaturation at 95°C for 40 s, annealing at 58°C for 40 s and elongation at 72°C for 1 min followed by a final elongation at 72°C for 10 min. Each sample was amplified in triplicates and pooled prior to purification with Agencourt AMPure XP beads (Beckman Coulter, Berea, CA) and quantification with the Qubit® 2.0 fluorometric system (Life Technologies, Paisley, UK). Amplicon pools were sent to the Génome Québec Innovation Center at McGill University (Montréal, Canada) for barcoding using the Fluidigm Access Array technology (Fluidigm) and paired-end sequencing on the Illumina MiSeq v3 platform (Illumina Inc., San Diego, CA, USA).

5.2.10 CONTROL OF SEQUENCE QUALITY, OTU CLUSTERING, AND TAXONOMIC ASSIGNMENT

Quality filtering and clustering into operational taxonomic units (OTUs) was performed with a customized pipeline that was largely based on UPARSE (Edgar 2013; Edgar and Flyvbjerg 2015) implemented in USEARCH v.8 (Edgar 2010), but with some additional modifications. Paired-end reads were merged using the USEARCH 190 fastq_mergepairs algorithm (Edgar and Flyvbjerg 2015), allowing staggered alignment constructs in order to accommodate potentially short ITS2 amplicons. BayesHammer (Nikolenko et al. 2013) was used to correct for substitution errors originating from phasing events during Illumina sequencing. PCR primers were detected and trimmed using Cutadapt (Martin 2011) allowing for one mismatch. Reads not matching the primers or with read lengths below 300 (16S, V3-V4) or 200 bp (ITS2) were discarded. Trimmed reads were quality-filtered using the 195 USEARCH fastq_filter function with a maximum expected error threshold of one. Sequences were de-replicated to retrieve information on abundance distribution and singleton reads were removed prior to clustering in order to avoid artificial OTU inflation (Edgar 2013). Sequences were clustered into OTUs at 97% sequence identity using the USEARCH cluster_otu function that includes an “on-the-fly” chimera detection algorithm (Edgar 2013). The remaining centroid sequences were tested for the presence of ribosomal signatures using Metaxa2 (Bengtsson-Palme et al. 2015) or ITSx (Bengtsson-Palme et al. 2013), respectively. Centroid sequences with insufficiently supported ribosomal origin were discarded. Finally, all quality-filtered reads were mapped against the final set of centroid sequences using the USEARCH

usearch_global 205 algorithm with the most comprehensive search criteria (maxrejects 0, maxaccepts 0, top_hit_only). For taxonomic classification of the OTUs, corresponding centroid sequences were queried against selected reference databases using the naïve Bayesian classifier (Wang et al. 2007) implemented in MOTHUR (Schloss et al. 2009) and a minimum bootstrap support of 80%. Prokaryotic (16S, V3-V4) sequences were queried against 210 GREENGENES (DeSantis et al. 2006; McDonald et al. 2012), whereas eukaryotic ITS2 (Abarenkov et al. 2010) sequences were first queried against a custom-made ITS2 reference database retrieved from NCBI GenBank (Benson et al. 2015) and sequences assigned to fungi were subsequently queried against the fungal ITS database UNITE (Abarenkov et al. 2010). Additionally, centroid sequences of OTU assigned to Glomeromycota were blasted against MaarjAM (Öpik et al. 2010), a specialized Glomeromycota database, to verify and refine the assignments. Only sequences assigned to Bacteria, excluding chloroplast and mitochondrial sequences, and Fungi were retained for downstream analysis.

5.2.11 COMPETITIVE EFFECT

Competitiveness of different plant species in the 4-species mix was assessed based on relative total biomass production: the mean individual biomass per plant species in the mixture pots was divided by the mean individual biomass per species in the monocultures (Parrish and Bazzaz 1982). When monoculture performance equals performance in the mixture the competitive effect is 1. When plant species perform better in the monocultures than in the mixture the competitive effect is lower than 1 and vice versa. We compared the competitive effect of different plants species for all treatments to assess the effect of AMF presence in the soil and detect possible climate effects.

5.2.12 STATISTICAL ANALYSIS

Statistical analysis was performed using the R statistical computing version 3.2.3 (Wooden Christmas-Tree, R Core Team 2015). The biomass parameters, Root-shoot ratio and shoot vitality were analysed using linear models (error-type III) with irrigation, temperature, plant mixture and AMF presence as explaining variables. Before the analysis, root biomass, shoot biomass and total biomass data were square root transformed, root-shoot ratio was log and shoot vitality ratio was arc-sine transformed to improve the fit of the model. Normality and homoscedasticity were ascertained using Shapiro and Bartlett's Test. Tukey HSD post-hoc tests were used to discriminate interactive effects.

Average Bray-Curtis distances were calculated with MOTHUR using the function dist.shared and 10'000 iterative subsampling steps based on the lowest read counts in a sample (7'368 sequences for fungi and 16'393 sequences for Bacteria). PCoA were calculated using the function cmdscale in R. PCoA were plotted in R using the function plot_ordination from the phyloseq package. Microbial α-diversity was assessed using the function rarefy of the vegan package, which estimates the OTU richness of samples randomly subsampled to the lowest read counts in a sample. Differences in β-diversity were assessed by PCoA, followed by permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) using vegan's adonis function with 99'999 permutations.

From the initial 1'937'324 fungal and 1'676'679 bacterial sequences, 1'236'101 and 997'239 sequences were retained after filtering. At least 7'368 fungal and 16'393 bacterial reads were obtained in all samples. Thus, we discarded 36% and 41% of the fungal and bacterial sequences respectively. The remaining sequences were clustered into 1'352 fungal and 4'432 bacterial OTU.

5.3 RESULTS

5.3.1 GLOMEROMYCOTA AND MICROBIAL COMMUNITY

In total, 50 OTU were assigned to Glomeromycota when using the UNITE database. The assignment to Glomeromycota could be further refined for the OTU with a closely related reference sequence in the specialized MaarjAM database. This allowed assigning 39 OTU at the genus level, where Glomus, with 23 different OTU, represented the most diverse genus. The other detected genera were Archaeospora (6 OTU), Claroideoglomus (5 OTU), Entrophospora (3 OTU), and Dominikia (2 OTU). 9 OTU were assigned to family level, 8 of which to Glomeraceae, and 1 to Claroideoglomeraceae. The remaining two Glomeromycota OTU could not be assigned to lower taxonomic levels.

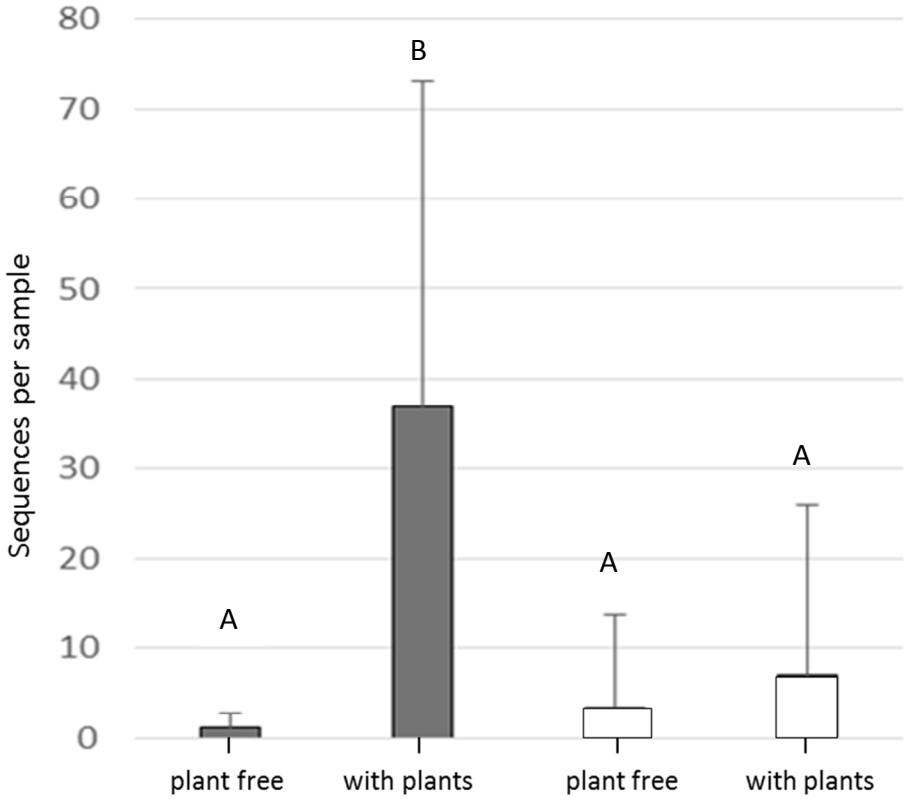

Figure 5.3: Average Glomeromycota sequences detected in each sample by soil treatment and presence and absence of *Arrhenatherum elatius* plants in monocultures. Error bars display standard deviations. Grey fill colour: without microbial wash → high AMF treatment; White fill colour: sterilisation and microbial wash → low AMF treatment. The sequence number detected in the samples with high AMF treatment and plants differs significantly from sequence numbers detected in all other treatments ($p < 0.05$), all other differences in sequence number are not significant.

Irradiation and addition of microbial wash successfully reduced the presence of Glomeromycota (Figure 5.3). Spot tests confirmed that colonisation was low but not completely prevented in the irradiated soil. Furthermore, the diversity of both, bacteria and fungi, is highly reduced by irradiation and addition of microbial wash (Fig. 5.4). The average OTU richness of fungal and bacterial communities is reduced by 60.8% and 42.9%, respectively in the irradiated samples in comparison to the non-irradiated samples. We also observed an interactive effect of the temperature treatment and soil treatment on microbial diversity: Fungi showed a lower diversity when they were subjected to increased temperature in the untreated soil samples (Fig. 5.4a). However, diversity was still significantly higher than in the irradiated samples. Bacterial communities show also a reduced diversity in samples incubated at higher temperatures, but mainly in sterilised samples (Fig. 5.4b). Irrigation did

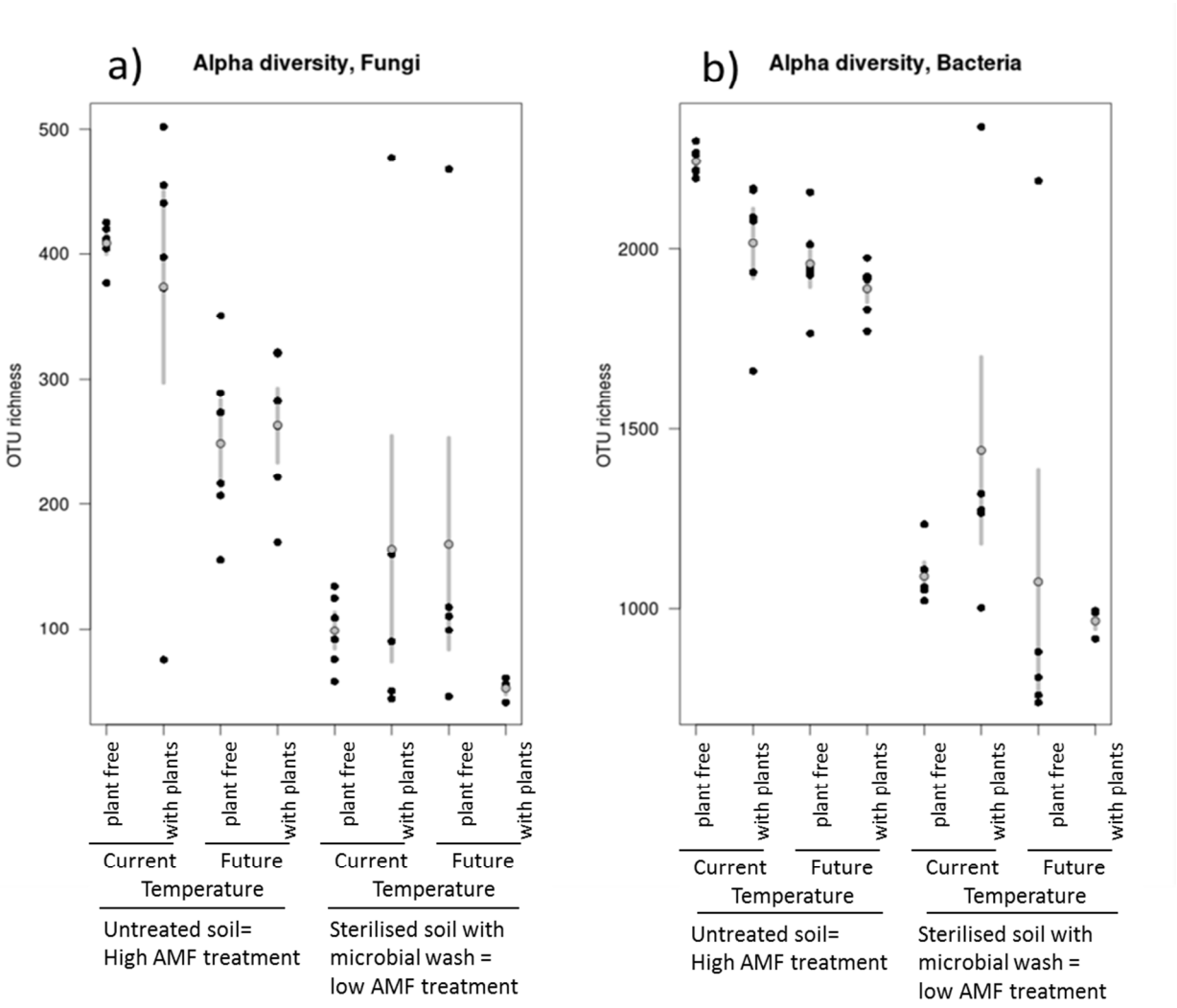

Figure 5.4: Alpha-diversity of all samples grouped by soil and temperature treatment as well as plant presence (*Arrehanatherum elatius* Monocultures). Grey dots: Mean values per treatment; grey lines: standard deviation per treatment.

not significantly change fungal or bacterial community structures.

Principal coordinates analyses (PCoA) show very well the beta-diversity of microbial communities in the samples, with 59.6% and 45.4% of explained variances for bacterial and fungal communities respectively. Both, bacterial and fungal communities were clearly different between the non-treated and irradiation soil treatment. Furthermore, these communities were influenced by the presence of plants, and the incubation temperature (Tables 5.1 and 5.2). In this experiment, the influence of plants is more pronounced on bacterial community structures, while the temperature plays a more important role for the fungal communities.

Table 5.1: PERMANOVA based on Bray-Curtis distances of bacterial communities between samples. The soil treatment (M_N), temperature, presence and absence of plants, and the interactions of the different factors (except Plants:Temperature) significantly influence bacterial community structure: significant with $p < 0.05$.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	P-value	
Temperature	1	0.3194	0.31937	3.1427	0.03848	0.02347	*
Precipitation	1	0.0967	0.09666	0.9512	0.01165	0.37371	
M_N	1	2.8784	2.87841	28.3248	0.34677	1.00E-05	***
Plants	1	0.6314	0.63138	6.213	0.07606	0.00109	**
Temperature:Precipitation	1	0.0796	0.07959	0.7832	0.00959	0.50101	
Temperature:M_N	1	0.2598	0.25981	2.5566	0.0313	0.04386	*
Precipitation:M_N	1	0.0833	0.08331	0.8199	0.01004	0.46941	
Temperature:Plants	1	0.1192	0.11923	1.1732	0.01436	0.26473	
Precipitation:Plants	1	0.0615	0.06151	0.6053	0.00741	0.6846	
M_N:Plants	1	0.3649	0.36492	3.591	0.04396	0.01353	*
Temperature:Precipitation:M_N	1	0.1388	0.13878	1.3657	0.01672	0.19905	
Temperature:Precipitation:Plants	1	0.1288	0.1288	1.2674	0.01552	0.23124	
Temperature:M_N:Plants	1	0.2386	0.23858	2.3477	0.02874	0.0573	.
Precipitation:M_N:Plants	1	0.0838	0.0838	0.8246	0.0101	0.46994	
Temperature:Precipitation:M_N:Plants	1	0.0726	0.07262	0.7146	0.00875	0.56492	
Residuals	27	2.7438	0.10162		0.33055		
Total	42	8.3006			1		

Table 2: PERMANOVA based on Bray-Curtis distances of fungal communities between samples. The soil treatment (M_N), temperature, and the presence and absence of plants, and the interactions of the different factors, all significantly influence fungal community structure: significant with $p < 0.05$

	Df	SumsOfSqs	MeanSqs	F.Model	R2	P-value	
Temperature	1	1.0785	1.07853	4.9446	0.07373	0.0001	***
Precipitation	1	0.2034	0.2034	0.9325	0.01391	0.4606	
M_N	1	2.9239	2.92394	13.4049	0.19989	1.00E-05	***
Plants	1	0.818	0.81798	3.7501	0.05592	0.00129	**
Temperature:Precipitation	1	0.152	0.15204	0.697	0.01039	0.72244	
Temperature:M_N	1	0.7778	0.77776	3.5657	0.05317	0.00187	**
Precipitation:M_N	1	0.2246	0.22461	1.0297	0.01536	0.37289	
Temperature:Plants	1	0.4866	0.48657	2.2307	0.03326	0.02855	*
Precipitation:Plants	1	0.1898	0.18984	0.8703	0.01298	0.52189	
M_N:Plants	1	0.5068	0.50683	2.3236	0.03465	0.0225	*
Temperature:Precipitation:M_N	1	0.2617	0.26169	1.1997	0.01789	0.25826	
Temperature:Precipitation:Plants	1	0.3191	0.31914	1.4631	0.02182	0.1447	
Temperature:M_N:Plants	1	0.417	0.41695	1.9115	0.0285	0.05418	.
Precipitation:M_N:Plants	1	0.2482	0.24818	1.1378	0.01697	0.2975	
Temperature:Precipitation:M_N:Plants	1	0.1309	0.13093	0.6002	0.00895	0.82808	
Residuals	27	5.8894	0.21812		0.40262		
Total	42	14.6277			1		

5.3.2 EFFECTS OF AMF PRESENCE ON PLANT BIOMASS PRODUCTION

We observed a significant negative effect of AMF presence on biomass in monoculture pots over all treatments: Root ($F_{1,114}=22.443$; $p<0.001$) and shoot biomass ($F_{1,114}=24.017$; $p<0.001$), and therefore total biomass ($F_{1,114}=28.233$; $p<0.001$) of all species was reduced when mycorrhiza were present (Fig. 5.5a). The significant negative effect of AMF presence on root ($F_{1,101}=7.332$; $p<0.001$), shoot ($F_{1,98}=4.550$; $p<0.01$) and total plant biomass ($F_{1,99}=7.440$; $p<0.001$) on the plants in the 4-species mix was however plant species dependent (Fig. 7b). The root-shoot ratio was also significantly reduced by the presence of AMF regardless if plants were grown in monocultures ($F_{1,114}=5.595$; $p<0.05$) or in the 4-species mix ($F_{1,109}=5.572$; $p<0.05$). High AMF presence significantly improved shoot vitality ($F_{1,114}=51.545$; $p<0.001$) in monoculture pots but had no effect on plants in 4-species mix.

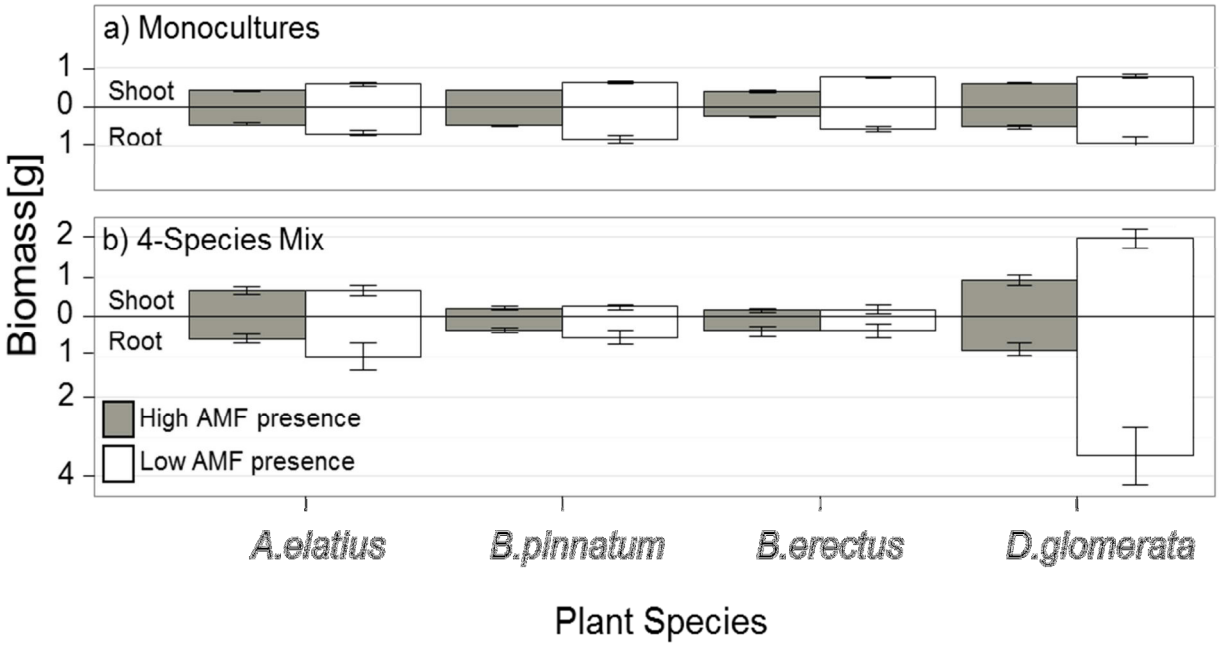

Fig. 5.5: Effect of high mycorrhizal presence on root and shoot biomass of the different plant species in a) monocultures and b) 4-species mix.

5.3.3 EFFECT OF FUTURE CLIMATE FACTORS ON PLANT BIOMASS PRODUCTION

Warmer temperatures (FT) reduced root ($F_{1,120}=8.737$; $p<0.01$), shoot ($F_{1,120}=6.058$; $p<0.05$) and total biomass ($F_{1,120}=8.832$; $p<0.01$) in monocultures (Fig. 8a). The shoot biomass was also reduced in the dry (FP) monoculture pots ($F_{1,120}=7.676$; $p<0.01$), and a trend towards less total biomass following lower irrigation for total biomass ($F_{1,120}=3.591$; $p<0.07$) was observed. In monocultures, FT had a significant negative effect on shoot vitality ($F_{1,120}=4.372$; $p<0.05$) but not on the root-shoot ratio of all species.

In the 4-species mix FP had no significant effect on plant performance effect and the effect of FT differed among plant species ($F_{1,118}=3.551$; $p<0.05$): FT led to lower shoot biomass for *A. elatius* and *D. glomerata* in the 4-species mix and to increased shoot biomass for *B. erectus* and *B. pinnatum* (Fig. 8b). In the 4-species mix, temperature also affected root-shoot ratio ($F_{1,116}=6.251$; $p<0.05$) as well as shoot-vitality ($F_{1,119}=4.562$; $p<0.05$) of all plant species, which were both lower for plants in warmer FT pots.

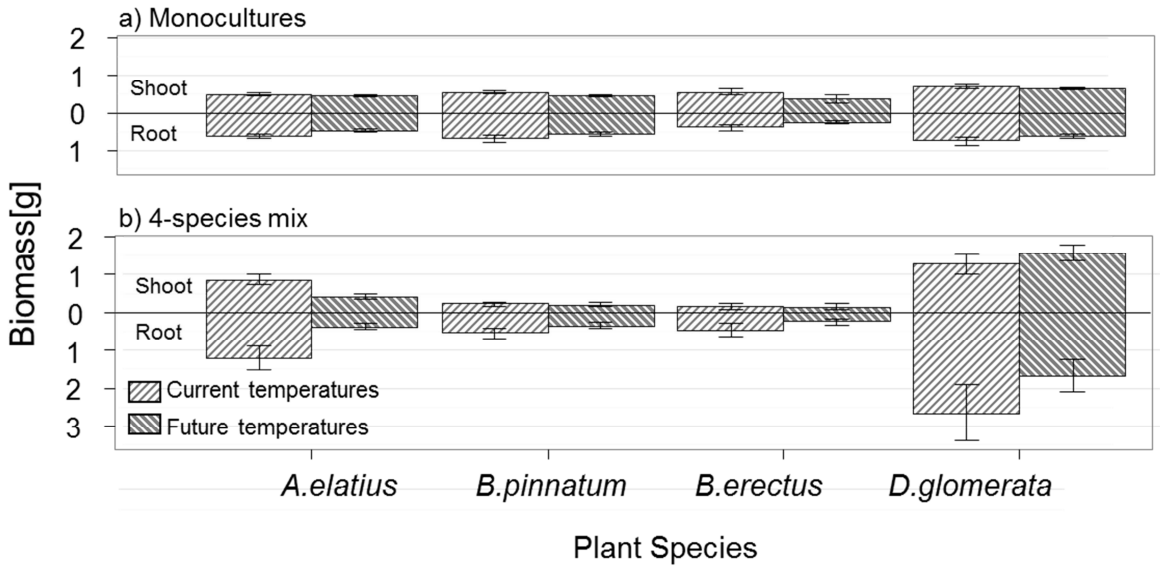

Fig. 5.6: Effect of current and warmer future temperature on root and shoot biomass of the different plant species in a) monocultures and b) 4-species mix.

5.3.4 EFFECTS OF AMF PRESENCE ON PLANT COMPETITIVENESS

As expected, the different plant species displayed different competitiveness in the 4-species mix ($F_{1,104}=15.777$; $p<0.001$). Only *D. glomerata* was identified as a real competitor in most of the pots of our studies (median competitive effect = 2.353). *A. elatius* displayed and overall competitive effect close to 1 (1.025) and was only considered a weak competitor over all treatements whereas *B. erectus* and *B. pinnatum* showed no competitiveness in our experiment (competitive effect < 1; Fig. 5.7). The increased presence of AMF in the soil only significantly affected the competitiveness of *D. glomerata*, which was reduced in presence of AMF (Fig. 5.7).

5.3.5 EFFECTS OF FUTURE CLIMATE FACTORS ON PLANT COMPETITIVENESS

The competitiveness of *A. elatius* was favoured under cooler temperatures, where it dominated the pots together with *D. glomerata.* Warming reduced the competitiveness of *A. elatius* and only *D. glomerata* dominated in the warmer pots (Fig. 5.8).

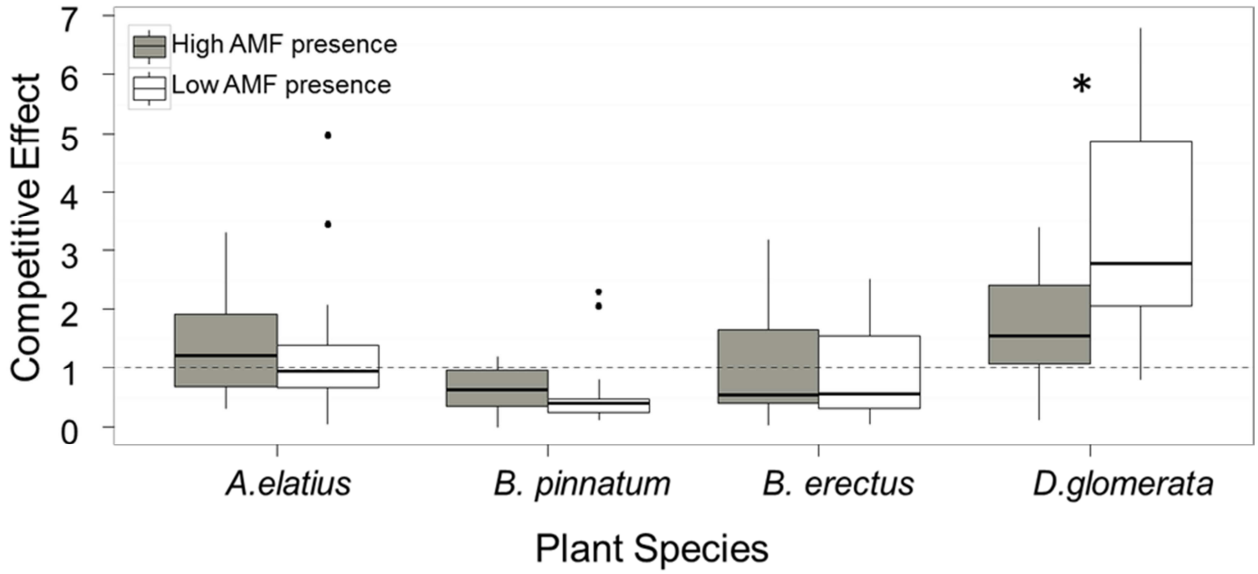

Fig. 5.7: Plant species' competitiveness with and without high AMF presence in the 4-species mix. Significant effects are marked with *.

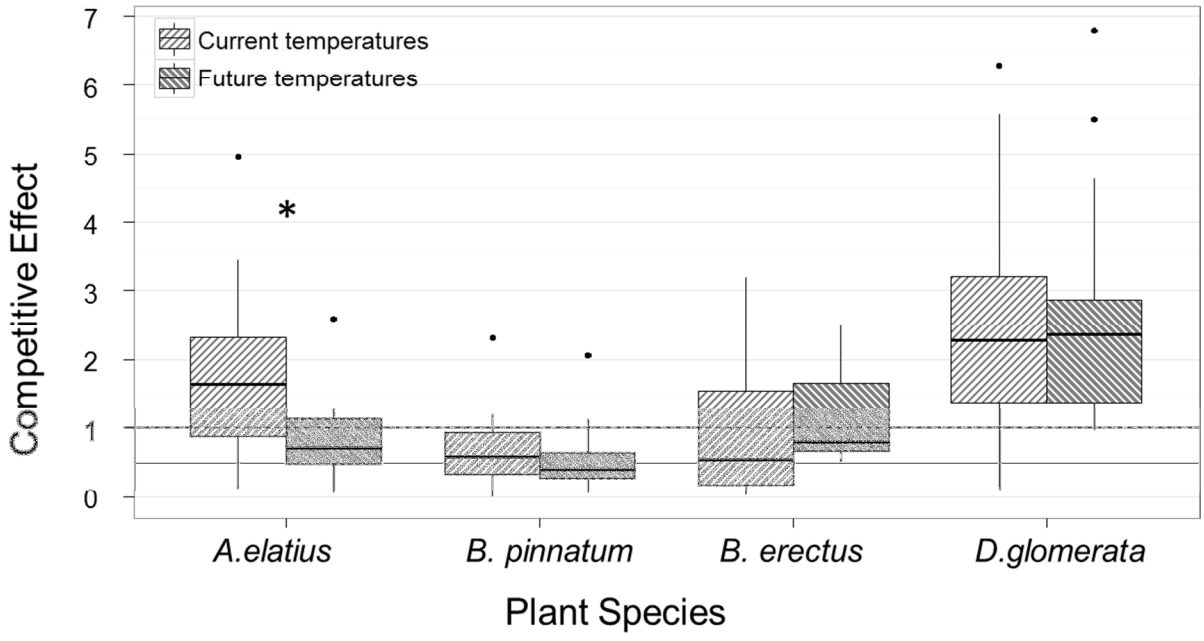

Fig. 5.8: Plant species' competitiveness following different temperature regimes in the 4-species mix. Significant effects are marked with *.

5.4 DISCUSSION

By taking local soil and seeds from the field site in the French Central Alps and by simulating the on site climatic conditions we designed our expermiment as close as possible to nature to allow generalisations of results from a greenhouse experiment to the subalpine ecosystem.

We expected that the higher presence of AMF in half of the pots of our study would have improved plant growth as an outcome (Smith and Read 2008). General nutrient availability was kept low to promote AMF-plant interaction. Still, we observed less biomass production of all plant species in all mixtures to increased presence of AMF. By mixing field soil with sand we lowered the general nutrient conditions and altered soil structure in our experiment from conditions in the field. This might have affected the experimental outcome because effects of different AMF species on plant productivity have been shown to depend on sand proportion in the soil in both, pot experiments (Wagg et al. 2011, Zaller et al. 2011) and under natural conditions in the field (Coutinho et al. 2015). In addition, the observed negative effect of higher AMF presence in half of the pots could be due to a nutrient limitation of the fungi themselves (Treseder and Allen 2002, Liu et al. 2012), as they are known to use for example N compounds to enhance their own fitness (Hodge and Fitter 2010, Hodge et al. 2010). It is known that γ- irradiation is better suited for control purposes because the physical and chemical changes to the soil are fewer compared to autoclaving (Berns et al. 2008). However it is still possible that sterilisation by γ- irradiation affected the plants in the control pots due to induced changes in soil organic matter structure and a slight increase of soil surface area (Berns et al. 2008). Therefore nutrient accessibility could be better in the control pots and improved growing conditions for plants even with lower AMF presence.

As the higher presence of AMF led to decreased biomass production we presume that the plant-AMF relationship in this experiment acted at the parasitic end of the parasitism-mutualism continuum (Johnson et al. 1997, Klironomos 2003, Smith and Smith 2013, 2015, Mariotte et al. 2013). Further information on whether the AMF effect on plant is positive or negative might be gained from the assessment of AMF root colonisation type and rate (Duckmanton and Widden 1994, Dekkers and van der Werff 2001), but not according to all current opinions (Klironomos et al. 2000, Smith and Smith 2013). However, their higher presence in the soil still affects plant physiology in a positive way, as is visible form the higher shoot vitality for plants from monoculture pots with high AMF presence. Other

positive effects such as increased nutrient content in plant matter and better seed viability could also be possible, but were not assessed in this study.

In contrast to our expectation we observed some colonisation by AMF in roots from the irradiated soil with microbial wash and detected their presence in the soil. Fungi are generally more susceptible to γ- irradiation than bacteria, and 10 kGy were observed to suffice to eliminate virtually all viable fungi (McNamara et al. 2003). The detection of AMF in the soil as well as root reveals that either the irradiation with 15.4 kGy did not inhibit all AMF propagules or that some propagules were reintroduced to the pots with the microbial wash. As we do not have samples from only irradiated soil we cannot further analyse the reason for the presence of AMF in the soil that was designated to be AMF free. Still, the presence of AMF in the treated roots and soil was strongly reduced and therefore allowed us to assess the effect of AMF on plant biomass.

Different plant species are generally known to have varying reactions to a relationship with AMF (Klironomos 2003, Mariotte et al. 2012, Bunn et al. 2015). In this experiment, an overall negative effect of higher AMF presence on biomass was observed for all species in monoculture. However, plant biomass in the monocultures was consistent with that in the 4-species mix when presence of AMF was high, which shows that the individual plant species maintained their productivity even under conditions of plant-plant competition. When AMF presence was lower a strong increase of plant biomass of one species (*D. glomerata*) was observed, which dominated over the other plant species. We deduce therefore that the higher abundance of AMF in the soil levelled out plant productivity under conditions of interspecific plant competition.

Interestingly, *D. glomerata* was also the only plant species which turned out to be a clear competitor in our experiment, although *B. pinnatum* and *A. elatius* were the dominant species at the original field site. In particular the root biomass of *D. glomerata* was increased more than three fold in the 4-species mix compared to the monoculture. The higher root biomass means that *D. glomerata* also had a higher surface for nutrient acquisition, which gives this species an advanced access to nutrients in comparison with the other species. This could probably be the reason for *D. glomerata*‘s competitive success in the low AMF pots, were root competition is more important (Höpfner et al. 2015). The importance of AMF for nutrient acquisition in our study is supported by the significant reduction of *D. glomerata*’s competitive effect with high AMF presence. It seems that the interspecific plant competition

is levelled out in the pots with the higher presence of AMF, despite their overall negative effect on plant biomass. This result corroborates the findings of other studies were AMF were found to act (even) more negatively on the dominant competitor species than on the subordinate less competitive species (Fitter 1977, Rinaudo et al. 2010, Wagg et al. 2011, Mariotte et al. 2013). There are other studies however, where performance of competitor plants is reported to be poorer without AMF and the competitive success of the dominant plant species is dependent on their presence in the soil (Hartnett et al. 1993, Zobel and Moora 1995). A synthesis of 304 studies reveals that AMF affect the competitiveness of plant species in different magnitudes and directions dependent on their different plant functional groups (Lin et al. 2015). However, the here studied grasses are all C3 grasses (Osborne et al. 2014) and do not differ in regard to their functional groups.

Despite the varying results among studies researching AMF effects on plant competition, the conclusion that AMF are important drivers of plant-plant interaction and community composition is possible. This is support for the driver hypothesis (Hart et al. 2001, Zobel and Öpik 2014) and emphasizes the importance to include plant-microbe interactions into new ecological frameworks explaining plant community composition (Schenk 2006, Dickie et al. 2015, Hacquard and Schadt 2015). However, additional environmental factors such as land use pressures and climate have influence on vegetation and soil microbes and add to complexity of such frameworks (Wahl and Spiegelberger 2016).

Warming reduced all biomass parameters regardless of mycorrhizal treatment while low irrigation had only a negative effect on shoot biomass. Warming effects on AMF and their capacity to mitigate adverse effects of warming for plants have been reported to be positive, neutral and negative, while effects of drought are almost always mitigated by AMF (Augé 2001; Compant et al. 2010; Mohan et al. 2014).

Warming significantly decreased the competitiveness of *A. elatius* but not that of *D. glomerata*, who were both dominating the pots under current temperature. The different reaction to warming could be explained by the species' ecological requirements. According to the Ellenberg indicator values, which assign the position of the realised ecological niche to plant species regarding a certain environmental gradient ranging from 1 to 9 (Bartelheimer and Poschlod 2016), *D. glomerata* is indifferent to temperature whereas the Ellenberg temperature number 5 (moderate temperatures) is assigned to *A. elatius*. The ecological niche range of *A. elatius* is adapted to moderate temperatures and its performance with warming is

therefore reduced. Warming might consequently affect plant community in the field in favour of *D. glomerata* and warmth adapted species. However, the warmth adapted species in our experiment B. *pinnatum* and *B. erectus* did not show a positive biomass response to warming and their competitiveness was not significantly increased in the time of the experiment.

Warming also let to a decrease of Glomeromycota richness in the soil. Different AMF species have different traits and differ in their effect on plant species and a change of community composition will therefore affect plant-plant interaction (van der Heijden and Scheublin 2007; Chagnon et al. 2013).

Effects of warming and drought on AMF can be direct as well as indirect dependent on the studied plant species, the soil type and the AMF community composition (Compant et al. 2010) and the combination of these two climatic factors was expected to have similar effects. However, in our study irrigation had no effect and no interaction effects of temperature and irrigation were found. It seems that warming was the main driver of plant AMF interactions in our study.

5.5 CONCLUSIONS

In this study we disentangled the effects of AMF, warming and drought on plant biomass and interspecific competitiveness. Based on our findings, the first hypothesis, which states that AMF presence in the soil will reduce the competitiveness of high competitor species and thus level out plant competitive interactions, was supported, although the observed patterns were not exactly as we expected. High AMF presence in the soil reduced the biomass of the competitive species in the pots and, against our expectation, also that of the subdominant species. However, the effect on the competitor species was significantly stronger and its competitiveness was significantly reduced. This still gave an advantage to the subordinate species and ultimately levelled out the interspecific plant competition.

The second and third hypothesis, which concern the capacity of AMF to mitigate climate change impacts for plants could not be confirmed because we observed mainly separated negative effects of both, warming and AMF presence in the soil and no irrigation effect.

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6 SYNTHESIS

The main objective of this dissertation was to investigate the interactions between AMF and plants along altitudinal gradients under both, natural conditions and simulated future climate change conditions. Further objectives were to disentangle the effects of AMF, water availability and warming on plant productivity and competition. Altogether it contributes to current research questions in ecology, climate change mitigation and plant –soil interactions, because it addresses the role of AMF in mountain grassland ecosystem, investigates the effects of climate change and provides a new framework concerning the functioning of the AMF-plant relationship ranging from parasitism to mutualism.

6.1 A NOVEL FRAMEWORK TO ASSESS THE AMF-PLANT RELATIONSHIP ALONG ALTITUDINAL GRADIENTS

In Chapter 2 of this dissertation two working hypotheses for the functioning of the AMF-plant relationship along altitudinal gradients are presented. Since AMF-plant interactions are highly variable their functioning can be graded along a continuum ranging from parasitism to mutualism (Johnson et al. 1997; Johnson and Graham 2013) just as plant-plant interactions can range from competition to facilitation depending on the environmental context (Menge and Sutherland 1987; Daleo and Iribarne 2009). We think that the stress gradient hypothesis, which conceptualises positive and negative interactions along environmental stress gradients (Bertness and Callaway 1994; Bruno et al. 2003; Michalet et al. 2006; Michalet et al. 2014) could also be applied for assessing the functioning of AMF-plant interactions: Just like competition shifts towards facilitation with increasing environmental stress, we hypothesised that the AMF-relationship shifts along the mutualism–parasitism continuum following changing environmental stress along the altitudinal gradient and might be most mutualistic at the subalpine zone. Furthermore it is to be expected that this predicted shift along the parasitism-mutualism continumm differs under global change conditions. Assessing AMF-plant relationships along altitudinal gradients by approaching it via the stress gradient hypothesis will provide much needed information about their role and importance in mountain ecosystems. Moreover it will contribute to identifying whether AMF-plant relationships in those ecosystems are mainly driven by abitiotic conditions as suggested by the Habitat hypothesis (Zobel and Öpik 2014) or if they are the driving forces or merely

by-products of the plant community as proposed by the Driver/Passenger hypothesis (Hart et al. 2001).

6.2 AMF IN MOUNTAIN GRASSLAND ECOSYSTEMS

The importance of the presence and functioning of AMF-plant associations in mountain ecosystems remain unclear (Gardes and Dahlberg1996) and invesitigations on dispersal, disturbance, succession, host availability and climatic suitability are currently investigated. The review presented in this dissertation (Chapter 2) confirms the omnipresence of AMF in mountain grassland ecosystems at all altitudes up to 5400 m asl around the world. It reveals that fundamental steps towards a better description of AMF community composition and towards the understanding of the role of AMF in mountain grassland ecosystems and altitudinal gradients have been taken. It also points out that the drivers of their distribution and community composition in mountain ecosystems are not yet clear and that the functioning of their symbiosis is barely studied. All in all it was not possible to frame the available literature as evidence for or against the stress gradient hypotheses we stated because few studies report the effect of AMF on plants in mountain grassland ecosystems.

Altitudinal gradients spanning over several vegetation zones are particularly well suited for the study of AMF-plant interactions because abiotic conditions differ predictably (Körner 2007). Research resulting from altitudinal gradients could also be transferred to latitudinal gradients (Körner 2000) and therefore could provide information on the variation of AMF-plant interactions on a general scale. In the study along an Italian dry altitudinal gradient in the Italian Central Alps (Chapter 3) high root AMF colonisation rate and abundance in the soil was observed at all altitudes, which is in contrast to other studies from the European Alps. However, as conditions at the altitudinal gradient are drier than at other gradients it is possible to conclude that AMF might play a more important role in drier mountain ranges and are therefore more abundant. The study is also a good example of how even under equalised nutrient and irrigation conditions along the altitudinal gradient altitudinal effects can be blurred and make it difficult to assess the actual drivers of AMF abundance. Different plant species seemed to benefit differently at different altitudes and a general shift along the parasitisum-mutualism continuum as predicted by the working hypothesis could not be confirmed. It becomes clear that a context-dependency of AMF effects on plants in mountain grassland ecosystems under climate change makes it difficult to generalize about these interactions from single plant species at individual studies sites.

To sum up my research in this thesis for AMF along altitudinal gradients I conclude: First, AMF are also ubiquitous in mountain ecosystems, but a decrease in their abundance with increasing altitude is dependent on the overall climatic context. Second, their relationship to plants is however strongly dependent on the host plant species as well as the biotic and abiotic context. Third, a shift of the AMF-relationship along with altitude is expected but will quite possibly also depend on the plant species identity. Fourth, to fully assess the suggested working hypotheses for AMF-plant interactions field studies must be conducted at different spatial scales and covering different mountain systems.

6.3 CLIMATE CHANGE EFFECTS ON AMF IN MOUNTAIN GRASSLAND ECOSYSTEMS

While many prior studies focused on evaluating the mycorrhizal status of plant species along altitudinal gradients and assessing the distribution of AMF species (Read and Haselwandter 1981; Cripps and Eddington 2005; Gai et al. 2009; Gai et al. 2012; Li et al. 2014), this dissertation provides also information on plant-AMF interactions along the altitudinal gradients and their reaction to climate change. AMF are known to mitigate effects of certain climate change factors on plant performance (Compant et al. 2010; Mohan et al. 2014). However, many studies on AMF and climate change focus on the interaction of one or several plants with one or several mycorrhizal species. These studies are important to understand physiological functioning and to disentangle different factors, but may be limited in their relevance to complex ecological communities in real nature, because of their relative simplicity. Field studies can give a more accurate idea of AMF and plant species response to climate change.

In addition to their qualities for studies along gradients of environmental stress, altitudinal gradients provide a good opportunity to simulate climate change conditions under natural conditions (Körner 2007). Downward transplantation of soil monoliths provides a space for time substitution for future climate change conditions. The results obtained after three years of climate change simulation in such an experiment (Chapter 4) showed that the effects of warming and increased temperature are not the same at all altitudes. A uniform decrease of colonisation rate is displayed by all plant species after three years of downward transplantation from the subalpine to the montane zone and plants seem to benefit from the increased nutrient availability and warming. They might therefore be less dependent on AMF what could be a reason for lower root colonisation rates. As no such overall effect was observed after transplantation from the montane to the foothill zone, where evapotranspiration

led to reoccurring drought events during the vegetation period, it seems that at the lower transplantation a certain threshold has been passed and that plants maintained investment into AMF colonisation to mitigate their in response to the warming induced drought events. This could be interpreted as a plant realted shift of AMF colonisation is support for the Passenger hypothesis (Hart et al. 2001). Unfortunately AMF presence was not assessed and a clear deduction of a shift from parasitism to mutualism along the altitudinal gradient under climate change was impeded.

As the presented field experiment (Chapter 4) did not offer the possibility to manipulate and assess the effect of AMF presence on plants in the field, I planned and carried through a climate chamber experiment under near natural conditions. It revealed independent effect of warming and AMF presence on the productivity of the different plant species and no effect of lower water availability. Mitigating effects of AMF were plant species dependent.

Even under current climatic conditions it is difficult to identify drivers of AMF-plant relationships with certainty, due to the strong complex context-dependency of this relationship under natural conditions that becomes apparent from literature research (Chapter 2) and from the field study on AMF along an altitudinal gradient (Chapter 3). Adding additional factors such as warming and increased evapotranspiration increases this complexity. Still, a major conclusions can be drawn from the here presented experiments on climate change (Chapter 4 and 5). From the field experiment it becomes apparent that warming and lower water availbility affect the plant AMF relationship only after passing a certain threshold and that its reaction to warming is therefore not equal at all altitudes. It was not possible to determine the shift with certainty and to validate the second working hypothesis stated in Chapter 2 because not all underlying factors could be assessed. The climate chamber experiment revealed species dependent effects of warming on the AMF-plant realtionship. that even when simulating near natural conditions, the prevalent effects are not the same.

6.4 OUTLOOK

The present dissertation suggests a novel framework for the functioning of the AMF-plant relationship along altitudinal gradients and stress gradients in general. Studies to validate the presented hypothesis will help to identify important mechanisms underlying plant-AMF interaction and with that the mediation of plant-plant interactions by AMF.

However, as it was not possible to manipulate the AMF presence in the field during the time of the thesis and the functioning of the AMF relationship could only be assessed roughly via the ratio of colonisation by arbuscules to total colonisation in the root. Further experiments that assess the cost and benefit of AMF by manipulating AMF presence in the field are needed for a generalisation of these results.

Furthermore, assessing the changes that climate change evokes on AMF species community composition and their specific functional traits, such as along altitudinal gradient under climate change conditions will make it possible to state more precise predictions about soil aggregation and ecosystem productivity.

6.5 CONCLUSION

In conclusion, my thesis provides insight into AMF-plant relationship in mountain ecosystems and their response to climate change. Research questions were addressed by a review of the literature followed by field and climate chamber studies. I addressed questions about the abundance along altitudinal gradient, about the functioning of the AMF-plant relationship along altitudinal gradients based on the stress gradient hypothesis and the effects of climate change.

I disentangeled biotic and abiotic factors that contribute to the variation in mycorrhizal abundance in soil and roots as well as to the varying AMF effects on plant productivity and plant-plant interactions.

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APPENDICES

APPENDIX 1: SUPPLEMENTARY MATERIAL FOR CHAPTER 2

Tab. 2.1: List of all reviewed studies concerning mountain grassland ecosystems grouped according to their primary study aim, novelty and author.

Author	Year	Mountain Range	Country	Gradient or altitude above sea level [m]		Ecosystem/ Habitat type	AMF variables
Altitudinal gradient							
Coutinho et al	2015	Serra do Cipo Mountain Range	Brazil	800	1400	rupestrian grassland	spore density, spore morphotypes
Liu et al	2015	Qinghai-Tibet Plateau	China	4149	5033	alpine grassland	DNA extraction
Li et al	2014	Qinghai-Tibet Plateau	China	3446	4556	montane to alpine grassland	colonisation rate, spore density, extra radical hyphae, DNA extraction from roots
Shi et al	2014	Qingling Mountains	China	1050	3750	montane to alpine grassland	colonisation rate, spore density, spore morphotypes

Vanesa et al	2013	Argentinian Andes	Argentina	4123	4327	hypersaline high-altitude wetland	colonisation rate
Gai et al	2012	Segrila Mountain	China	1990	4648	sub-tropical to alpine grassland	colonisation rate, spore density, spore morphotypes
Lugo et al	2012	Puna Highland	Argentina	3320	4314	steppe	colonisation rate
Gai et al	2009	Qinghai-Tibet Plateau	China	3500	5200	montane to alpine grassland	spore density, spore morphotypes
Zubek et al	2009	Carpathian Mountains	Poland	1000	2050	montane to alpine grassland	colonisation rate, spore morphotypes
Lugo et al	2008	Puna Highland	Argentina	3320	3870	steppe	spore density, spore morphotypes
Schmidt et al	2008	Rocky Mountains (Colorado) and Andes	USA, Peru	4700	5391	high alpine vegetation	colonisation rate
Kagawa et al	2006	Japanese South Alps	Japan		2800	alpine grassland	colonisation rate, spore density
Cripps and Eddington	2005	Rocky Mountains (Montana,	USA	2950	3250	alpine grassland	colonisation rate

		Wyoming)					
Ruotsalainen et al	2004	Mount Paras	Norway	0	1400	boreal to high alpine grassland	colonisation rate
Väre et al	1997	Kilpisjärvi area	Finnland	600	900	boreal to nival grassland	colonisation rate, spore morphotypes
Lesica and Antibus	1986	Rocky Mountains (Montana, Wyoming)	USA	2200	3300	alpine fell field communities	colonisation rate, spore density
Read and Haselwandter	1981	European Alps	Austria	1600	3200	subalpine to nival grassland	colonisation rate
Haselwandter and Read	1980	European Alps	Austria	1600	3200	subalpine to nival grassland	colonisation rate
Crush	1973	Mountains in Otago Province	New Zealand	472	2000	herbfield to snow tussock	colonisation rate, spore density, spore morphotypes
Climate change							
Kim et al	2014	Inner Mongolian Plateau	China		1324	steppe	spore density, extra radical hyphae, DNA extraction from

							soil
Rudgers et al	2014	Rocky Mountains (Colorado)	USA		2920	subalpine grassland	colonisation rate, extra radical hyphae
Zhang et al	2014	Qinghai-Tibet Plateau	China		4659	alpine grassland	PLFA
Sun et al	2013	Inner Mongolian Plateau	China		1324	steppe	colonisation rate, spore density, spore morphotypes
Yang et al	2013	Qinghai-Tibet Plateau	China		3200	alpine grassland	colonisation rate, spore density, extra radical hyphae, DNA extraction from roots
Budge et al	2011	European Alps	Switzerland	1665	2525	alpine grassland	PLFA
Facilitation effects							
Casanova-Katny	2011	Chilean Andes	Chile	3200	3600	alpine cushion plant vegetation	colonisation rate, spore morphotypes
Haselwandter	1987	European Alps	Austria	1600	3200	subalpine to nival	colonisation rate
Fertilisation							

Liu et al a	2015	Qinghai-Tibet Plateau	China		3500	alpine grassland	DNA extraction from soil
Liu et al b	2015	Qinghai-Tibet Plateau	China		3500	alpine grassland	colonisation rate, spore density, extra radical hyphae, DNA extraction from soil
Tischer et al	2015	Andes	Equador		1930	tropical mountain pasture	colonisation rate, extra radical hyphae, PFLA
Zheng et al	2014	Qinghai-Tibet Plateau	China		3200	alpine grassland	colonisation rate, spore density, spore morphotypes, extra radical mycelium
Blanke et al	2012	European Alps	Switzerland		2000	subalpine grassland	colonisation rate
Liu et al	2012	Qinghai-Tibet Plateau	China		3500	alpine grassland	colonisation rate, spore density, spore morphotypes, extra radical hyphae, DNA extraction from roots
Ruotsalainen et al	2011	Kilpisjärvi area	Finnland	600	700	mountain tundra	colonisation rate
Habitat patchiness							

Ranelli et al	2015	Rocky Mountains (Colorado)	USA	2634	4025	montane to alpine grassland	colonisation rate
Becklin and Galen	2009	Rocky Mountains (Colorado)	USA	3598	3698	alpine grassland	colonisation rate
Barnola and Montilla	1987	Sierra de la Culata	Venezuela		3800	tropical high mountains	colonisation rate
Host specificity							
Li et al	2015	Qinghai-Tibet Plateau	China	4149	5033	alpine grassland	colonisation rate, spore density, extra radical hyphae, DNA extraction from roots
Becklin et al	2012	Rocky Mountains (Colorado)	USA	3590	3630	alpine grassland	DNA extraction from roots
Zubek et al	2011	Pamir Alay Mountains	Tajikistan	1600	2630	steppe to wet meadow	colonisation rate, spore morphotypes
Sýkorová et al	2007	European Alps	Switzerland	1820	2010	montane meadow	DNA extraction from roots
Haselwandter and Read	1982	European Alps	Austria	Pot experiment		High alpine plant community	Colonisation rate

Land use change							
Zubek et al	2014	Pamir Alay Mountains	Tajikistan	1270	2400	mountain agroecosystem	spore density, spore morphotypes
Duchicela et al	2013	Bolivian Altiplano	Bolivia	3966	4195	alpine agroecosystem	DNA extraction from soil
Schmid et al	2008	European Alps	Switzerland		2671	alpine grassland	spore density, spore morphotypes
Schmidt et al	2008	European Alps	Switzerland	2671	2671	high alpine vegetation	spore density, spore morphotypes
Su et al	2007	Inner Mongolian Plateau	China	1180	1250	steppe	spore density, spore morphotypes
Börstler et al	2006	Thuringian Highland	Germany	640	705	mountain meadow	DNA extraction from roots and spores
Frank et al	2003	Yellowstone Plateau	USA		2000	mountain grassland	colonisation rate, spore density, spore morphotypes
Lugo et al	2003	Sierra de Cordoba	Argentina		2250	mountain grassland	colonisation rate
Seasonal variation							

Lugo et al	2002	Sierra de Cordoba	Argentina		2250	mountain grassland	spore density, spore morphotypes
Succession							
Welc et al	2014	European Alps	Switzerland	1966	2025	alpine glacier foreland	DNA extraction from roots and soil
Liu et al	2011	Qinghai-Tibet Plateau	China	4524	4773	high altitude grassland	colonisation rate, spore density, DNA extraction from roots and spores
Oehl et al	2011	European Alps	Switzerland		2000	subalpine glacier foreland	spore density, spore morphotypes
Wu et al	2007	Mount Fuji	Japan	1500	1930	Scoria (volcanic rock)	spore morphotypes, DNA extraction from roots and spores
Cazares et al	2005	North Cascade Mountains (Washington)	USA		1800	subalpine glacier foreland	colonisation, AMF propagules in soil
Fujiyoshi et al	2005	Mount Fuji	Japan	1550	1650	subalpine volcanic desert	colonisation rate, spore density
Tsuyuzaki et al	2005	Mount Koma	Japan	550	800	different volcanic	colonisation rate

						deposits	
Wu et al	2004	Mount Fuji	Japan	1500	1930	scoria (volcanic rock)	colonisation rate
Titus and Tsuyuzaki	2002	Mount Koma	Japan		1000	different volcanic deposits	colonisation rate
Titus and Del Moral	1998	Mount St. Helens	USA	1150	1300	different volcanic deposits	colonisation rate
Titus et al	1998	Mount St. Helens	USA	1150	1300	different volcanic deposits	colonisation rate, spore density
Allen et al	1992	Mount St. Helens	USA	1100<	>2540	different volcanic deposits	colonisation rate, spore density
Allen et al	1987	Rocky Mountains (Montana)	USA	2975	3050	alpine grassland	colonisation rate, spore density
Allen et al	1984	Mount St. Helens	USA	1100<	>2540	different volcanic deposits	spore density

APPENDIX 2: SUPPLEMENTARY MATERIALS FOR CHAPTER 4

Tab. A: The following table gives an overview of applied tests, transformations and p-values:

Single species or weighted means	Response variable	Explicative variable	Transformation	Test	df	F- or χ^2-value	p-value
Achillea millefolium	Arbuscules	TP1450 - CP1950	-	Kruskal-W.	1	3,6	0,056
	Mycelium	TP1450 - CP1950	-	anova	1	35,6	**<0,001**
	Vesicles	TP1450 - CP1950	sqrt(x)	anova	1	7,0	**0,017**
	Arbuscules	TP950 - CP1450	-	Kruskal-W.	1	0,3	0,568
	Mycelium	TP950 - CP1450	-	anova	1	3,4	0,084
	Vesicles	TP950 - CP1450	-	Kruskal-W.	1	0,1	0,757
Agrostis capillais	Arbuscules	TP1450 - CP1950	-	Kruskal-W.	1	0,9	0,332
	Mycelium	TP1450 - CP1950	-	anova	1	8,9	**0,009**
	Vesicles	TP1450 - CP1950	-	Kruskal-W.	1	0,2	0,665
Carum carvi	Arbuscules	TP1450 - CP1950	-	Kruskal-W.	1	1,6	0,206
	Mycelium	TP1450 - CP1950	-	anova	1	5,6	**0,030**
	Vesicles	TP1450 - CP1950	-	Kruskal-W.	1	8,9	**0,003**
	Arbuscules	TP950 - CP1450	-	Kruskal-W.	1	11,0	**<0,001**
	Mycelium	TP950 - CP1450	-	anova	1	12.0	**0,003**
	Vesicles	TP950 - CP1450	-	Kruskal-W.	1	0,2	0,656
Dactylis glomerata	Arbuscules	TP950 - CP1450	-	Kruskal-W.	1	1,0	0,323
	Mycelium	TP950 - CP1450	-	anova	1	8,1	**0,013**
	Vesicles	TP950 - CP1450	-	Kruskal-W.	1	3,0	0,085
Festuca rubra	Arbuscules	TP1450 - CP1950	-	Kruskal-W.	1	7,0	**0,008**
	Mycelium	TP1450 - CP1950	-	anova	1	2,9	0,105
	Vesicles	TP1450 - CP1950	sqrt(x)	anova	1	15,1	**0,001**
Poa pratensis	Arbuscules	TP1450 - CP1950	-	Kruskal-W.	1	5,0	**0,025**
	Mycelium	TP1450 - CP1950	-	anova	1	3,3	0,084
	Vesicles	TP1450 - CP1950	sqrt(x)	anova	1	1,3	0,267
Rumex acetosa	Arbuscules	TP1450 - CP1950	-	Kruskal-W.	1	1,4	0,229
	Mycelium	TP1450 - CP1950	-	anova	1	0,6	0,47
	Vesicles	TP1450 - CP1950	-	anova	1	1,3	0,275
	Arbuscules	TP950 - CP1450	-	Kruskal-W.	1	4,3	**0,037**
	Mycelium	TP950 - CP1450	-	Kruskal-W.	1	1,9	0,223
	Vesicles	TP950 - CP1450	-	Kruskal-W.	1	3,1	0,077
cum offi cina	Arbuscules	TP1450 - CP1950	-	Kruskal-W.	1	8,5	**0,004**

	Mycelium	TP1450 - CP1950	-	anova	1	0,9	0,365
	Vesicles	TP1450 - CP1950	sqrt(x)	anova	1	23,4	**<0,001**
	Arbuscules	TP950 - CP1450	sqrt(x)	anova	1	21,3	**<0,001**
	Mycelium	TP950 - CP1450	-	anova	1	35,7	**<0,001**
	Vesicles	TP950 - CP1450	-	Kruskal-W.	1	2,7	0,097
Vicia sepium	Arbuscules	TP950 - CP1450	-	anova	1	4,3	0,054
	Mycelium	TP950 - CP1450	-	anova	1	1,1	0,294
	Vesicles	TP950 - CP1450	-	Kruskal-W.	1	2,3	0,129
weighted means for 7 respectively 6 TP / CP samples	Arbuscules	TP950 - CP1450 - TP1450 - CP1950	cbrt(x)	anova	3	3,8	0,010
	Mycelium	TP950 - CP1450 - TP1450 - CP1950	cbrt(x)	anova	3	6,2	**<0,001**
	Vesicles	TP950 - CP1450 - TP1450 - CP1950	cbrt(x)	anova	3	17,9	**<0,001**

Tests are shown regarding their response variable, both on species and weighted means level: LA = local area, TP = transplanted plot and CP = control plot. 950, 1450 and 1950 refer to the respective plot's altitude above sea level in meters. Sqrt(x) indicates $\sqrt[2]{x}$, and cbrt(x) $\sqrt[3]{x}$ transformation. Weighted means for TP950 - CP1450 were calculated by using the following six species in relative abundance: *Achillea millefolium, Carum carvi, Dactylis glomerata, Rumex acetosa, Taraxacum officinale, Vicia sepium*; Weighted means for TP1450 - CP1950 were calculated by using the following seven species in relative abundance: *Achillea millefolium, Agrostis capillaris, Carum carvi, Festuca rubra, Poa pratensis, Rumex acetosa, Taraxacum officinale*.

CURRICULUM VITAE

Anne-Lena Wahl was born on April 12, 1985 and grew up in Achern, Germany. She graduated from Gymnasium Achern in 2004. In 2005, after one year of volunteer work in a Northern Irish wild flower nursery, she went to study landscape ecology at the University of Münster, Germany. During her studies, she spent 2 semesters (year 2007 – 2008) at the University of Lisbon, Portugal, where she studied marine ecology. She earned her Diploma in Landscape Ecology with a study on the effects of fertilisation on mycorrhiza in the Swiss Alps at Agrosopce Zürich in 2011. After her studies Anne-Lena worked as botanical field assistant for the University of Berne, Switzerland, and monitored carnivores in Borjomi-Kharagauli National Park,Georgia. In 2012 she worked at the planning office Frinat in Freiburg, Germany, which specialises on bat ecology. In 2013 Anne-Lena moved to Grenoble, France, to start her doctoral thesis at irstea Grenoble, under the guidance of Thomas Spiegelberger and Jean-Jacques Brun. Since 2014 she is part of the MICMoR Helmholtz Research School on Mechanisms and Interactions of Climate Change in Mountain Regions. She is going to continue her research on AMF in changing mountain ecosystems focusing on carbon cycle during her Post-doc position at Agroscope Zürich in Switzerland.

PUBLICATIONS AND PRESENTATIONS

Publication	ARBUSCULAR MYCORRHIZAL FUNGI IN CHANGING MOUNTAIN GRASSLAND ECOSYSTEMS – A CHALLENGE FOR RESEARCH	BOTANY, SPECIAL ISSUE ON "MYCORRHIZA AND GLOBAL CHANGE" DOI: 10.1139/CJB-2015-0255
Poster	Arbuscular Mycorrhizal Fungi in Mountain Grassland show altitude and host plant dependent responses to future climatic conditions	8th International Conference on Mycorrhiza: 3-7 August 2015, Flagstaff, Arizona, USA

RÉSUMÉ

Bien que les champignons mycorhiziens arbusculaires (AMF) soient présents des habitats collinaires aux habitats alpins, les recherches sur leur rôle dans l'écosystème montagnard sont encore incomplètes. Les objectifs de cette thèse sont d'analyser l'écologie et le fonctionnement des AMF ainsi que leur réponse au changement global dans les écosystèmes montagnards. Nous tentons de répondre aux questions de recherche suivantes : quels sont les effets de l'altitude sur les AMF indépendamment des effets des autres gradients et de la relation AMF-plante hôte ? Quelles relations de facilitation se développent dans les écosystèmes montagnards et quels bénéfices les plantes tirent-elles des AMF ?

Avec l'augmentation d'un stress environnemental la symbiose AMF-plante doit théoriquement devenir plus mutualiste. Nous émettons l'hypothèse d'une modification du fonctionnement des interactions AMF-plante selon un gradient altitudinal dans les conditions environnementales actuelles, puis dans les conditions futures. Afin de vérifier ces hypothèses, une expérimentation in situ a été mise en place dans les Alpes Centrales d'Italie pour évaluer les variations des taux de mycorhization et leur abondance dans la communauté microbienne du sol, étudier la nature des relations entre plantes et mycorhizes ainsi que la productivité végétale le long d'un gradient altitudinal. De plus, les influences d'une augmentation de la température et d'une réduction des précipitations sont analysées séparément dans une chambre de croissance sous conditions contrôlées, ce qui permet de distinguer leurs effets respectifs sur la productivité des plantes et sur les interactions plantes – mycorhizes.

Cette thèse montre que les AMF sont omniprésents dans les écosystèmes de montagne et qu'une diminution de leur abondance avec l'altitude dépend du contexte climatique global. D'autre part, la relation des AMF avec les plantes est fortement dépendante de la plante-hôte, ainsi que du contexte biotique et abiotique. Troisièmement, un changement des interactions AMF-plante avec l'altitude est suggéré par des indices indirects, mais est également très probablement dépendant de l'identité de la plante hôte. Cette thèse propose aussi une nouvelle orientation de recherche pour bien évaluer les hypothèses présentées. Il est nécessaire de réaliser des études sur le terrain où la présence des AMF est contrôlée et les interactions AMF-plante peuvent être évaluées. Afin de généraliser les résultats, ces expérimentations doivent être menées à différentes échelles spatiales et représenter différentes aires géographiques.

Il est particulièrement important de comprendre et de qualifier ces processus en zone montagnarde pour prévoir leur évolution possible dans un contexte de changement global. Nos expérimentations montrent en effet que le réchauffement est un facteur important car il aggrave les conditions de sécheresse en basse altitude et entraine une baisse de la productivité des plantes. Nous démontrons que la présence de mycorhizes atténue l'impact du changement climatique sur la productivité des plantes mais que le niveau de cette atténuation varie selon les espèces de plantes.

Les connaissances actuelles concernant les AMF en milieu montagnard sont peu développées sur les processus en jeu dans les interactions AMF-plantes. Grâce aux hypothèses présentées et à leur approche expérimentale cette thèse offre de nouvelles perspectives sur l'analyse de ces processus.

ABSTRACT

Even though arbuscular mycorrhizal fungi (AMF) are present from foothills to all alpine habitats, research on their role in mountain ecosystems remains incomplete. The main objective of this dissertation was to investigate interactions between AMF and plants along altitudinal gradients under both, natural conditions and simulated future climate change conditions.

A novel framework is suggested for the functioning of the AMF-plant relationship along altitudinal gradients based on the stress gradient hypothesis. The first hypothesis expects the AMF-relationship to shift along the mutualism–parasitism continuum following changing environmental stress along the altitudinal gradient. The relationship might be most mutualistic at the subalpine zone. In a second hypothesis, this shift along the mutualism-parasitism continuum is predicted to be different under climate change conditions, and the most mutualistic expression of the AMF-plant relationship expected in the montane and alpine zone. Studies to validate the presented hypotheses will help to identify important mechanisms underlying plant-AMF interaction and with that the mediation of plant-plant interactions by AMF. In the scope of this thesis, the framework was addressed in field experiment as well as under controlled conditions in a climate chamber experiment.

From a literature review and from a field experiment along a dry inner-alpine altitudinal gradient this thesis proposes the following conclusions: First, AMF are also ubiquitous in mountain ecosystems, but a decrease in their abundance with increasing altitude is dependent on the overall climatic context. Second, their relationship to plants is however strongly dependent on the host plant species as well as the biotic and abiotic context. Third, a shift of the AMF-relationship along with altitude is expected but will quite possibly also depend on the plant species identity. Fourth, to fully assess the suggested working hypotheses for AMF-plant interactions field studies must be conducted at different spatial scales and covering different mountain systems.

It is particularly important to understand and investigate the drivers of AMF plant relationship in mountain ecosystems to be able to make sound predictions for AMF-plant interactions under future climate change conditions. The presented field and climate chamber experiments on climate change show that temperature is an important factor because it aggravates the conditions of drought in lowland and a threshold is surpassed. It becomes also clear that whether AMF mitigate climate change effects for plants or not is dependent on the plant species. Altogether this thesis contributes to current research questions in ecology, climate change mitigation and plant–soil interactions, because it addresses the role of AMF in mountain grassland ecosystem, investigates the effects of climate change and provides a new framework concerning the functioning of the AMF-plant relationship ranging from parasitism to mutualism.