Mutualistic and antagonistic effects of plant-animal and plant-fungal interactions on plant recruitment at the tree line

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Summary

Antagonistic and mutualistic species interactions provide important ecosystem functions affecting plant population dynamics and distribution. Many of these functions are important for the regeneration of plants, either by limiting or facilitating successful transition between life stages. Interactions can occur across the whole geographical range of a species and thereby encompass different environmental gradients, such as changes in temperature or water availability. Understanding the joint effects of species interactions and environmental factors on the regeneration of plants is key for understanding plant population dynamics under global change and could provide important recommendations for managing and conservation efforts.

My thesis aimed at advancing the knowledge of how species interactions depend on environmental conditions and jointly affect plant recruitment along the elevational distribution of plants. This thesis includes three chapters in which I studied the effects of animal seed deposition, seed predation, mycorrhizal and pathogenic fungi occurrences as well as abiotic and biotic environmental factors on the recruitment of Swiss stone pine (*Pinus cembra*). I conducted fieldwork in the Swiss Alps across the entire elevational distribution of the pine (1850 – 2250 m a.s.l). Over a period of three years, I recorded animal seed deposition by spotted nutcrackers (*Nucifraga caryocatactes*) and conducted seed translocation experiments. Further, I assessed fungal communities using DNA metabarcoding. I measured abiotic environmental factors such as temperature, water and light availability, pH, as well as biotic environmental factors such as distance to conspecific adults and ground vegetation cover. In my thesis, I used a broad range of community ecology approaches, from seed dispersal ecology to experimental plant ecology and microbial ecology.

First, I investigated the effects of environmental factors on four recruitment processes (i.e. seed deposition, seed predation, seed germination, seedling survival) of Swiss stone pine. Further, I aimed at identifying the most important recruitment processes potentially limiting pine regeneration across its elevational range. To investigate pine recruitment, I firstly tested how seed deposition, seed predation, seed germination and seedling survival were affected by the microhabitat characteristics ultimately determining where a seed arrives in the environment (i.e. canopy cover & ground vegetation cover). Secondly, I applied a sensitivity analysis to investigate which of the four recruitment processes poses limitation to the pines' regeneration across its range. My results reveal that the importance of particular recruitment processes varies along the pines' elevational range. I found that at the lower range margin and the distribution centre seed germination and seedling survival were the main limiting factors, whereas animal-mediated seed dispersal became especially important at the upper range

margin. My study contributes to the field with a new approach for disentangling the relative importance of recruitment processes across environmental gradients and thereby could help to project how plant recruitment might respond to future changes in environmental conditions.

The second aim of my study was to investigate how abiotic and biotic environmental factors affect the occurrence of Swiss stone pine-associated pathogenic and mutualistic fungi by combining field measurements of environmental factors with a DNA metabarcoding approach. I identified potentially important fungal interaction partners of the pine and determined drivers shaping their occurrences. My results reveal that generalist fungi were not affected by abiotic and biotic environmental factors. However, specialist pathogens showed patterns according to the Janzen-Connell framework (i.e. accumulation of pathogen close to adult plants). Interestingly, I found evidence for an "inverse" Janzen-Connell effect, i.e. high abundance of a specialist mutualist close to adult plants, potentially mitigating effects of soil pathogens close to parent trees. Further, I found that pine-associated fungi are distributed widely within and beyond the range of their host plant, adding knowledge on how mutualisms and antagonisms might be affected when plants move their distributional range upwards.

Finally, I investigated how known and unknown plant-associated fungi affect the regeneration of Swiss stone pine in an environmental context. My results suggest that seedling establishment was most strongly affected by abiotic environmental factors, such as light availability and maximum summer temperature. Further, the results indicate that seedling survival was affected by biotic environmental factors, i.e. fungal agents, with high abundances of a known fungal pathogen co-occurring with low seedling survival rates. My results also reveal that known mycorrhizal partners as well as a large number of unknown fungal operational taxonomic units (OTUs) were associated with the survival of seedlings. My findings highlight the importance of plant-fungal interactions for plant recruitment and offer a feasible approach for the identification of hidden plant-fungal associations in highly complex DNA metabarcoding datasets. This approach offers a valuable tool for investigating plant-microbe interactions, ultimately helping to understand plant population dynamics.

My dissertation adds to a deeper understanding on the linkage between plant regeneration and species interactions, especially on how plant-animal and plant-fungal interactions in concert with environmental factors shape plant recruitment. My study reveals the importance of animal-mediated seed dispersal and fungal pathogens in plant recruitment with consequences for potential range shifts of plant species. My thesis has important implications for conservation and management efforts by informing on key species interactions under environmental change.

1. Introduction

Ecosystems are important hierarchical levels of organization of life on earth (Tansley 1935; Pomeroy & Alberts 1988). They can be defined as a complex of biological communities (i.e. of plants, animals, fungi and prokaryotes) connected among each other and with their physical environment by a multitude of species interactions (Pomeroy & Alberts 1988; Leuscher 2013). These species interactions are known to be involved in a multitude of ecosystem processes, such as primary production (i.e. conversion of inorganic carbon to organic matter) or decomposition (i.e. recycling of dead organic matter; Vitousek *et al.* 1997; Hooper *et al.* 2005). Despite this knowledge, we still lack a mechanistic understanding on how species interactions act as drivers of ecosystem processes (Vitousek *et al.* 1997; Hooper *et al.* 2005; Leuscher 2013)**.**

1.1 Functional role of species interactions in ecosystem processes

Ecosystem processes, such as primary productivity, involve a multitude of species interactions across all kingdoms of life, both between organisms and with their environment (Bengtsson 1998; Tylianakis *et al.* 2010). Ecosystem processes are essential for life (Smith & Smith 2012). For example, primary productivity is the production of organic compounds by photosynthetic organisms (Smith & Smith 2012). The produced organic compounds are the base of most food chains (Smith & Smith 2012). Plants are the main primary producers in terrestrial ecosystems (Kitajima & Fenner 2000; Smith & Smith 2012). Therefore, ecosystems rely on the continuous biomass production by plants, which is facilitated by successful plant regeneration (Wang & Smith 2002; Smith & Smith 2012).

The life cycle of most plant species includes a regenerative phase (seed production, seed survival and seedling recruitment), where the successful phase transition ultimately determines the abundance and spatial distribution of plant communities (Hulme 1996). Successful plant regeneration of many plant species depends on multiple species interactions with environmental conditions. The outcome of these species interactions (i.e. survival rates) contributes to the extent of the ecosystem process primary production (Hector & Wilby 2009; Traill *et al.* 2010; van Andel 2013). In particular, mutualistic (e.g. animal-mediated plant pollination and seed dispersal, mycorrhizal symbiosis) and antagonistic species interactions (e.g. seed predation, plant pathogens) have a crucial role in plant regeneration (Callaway 1995; Hulme 1996; Bascompte & Jordano 2007; Smith & Read 2008; Bever, Mangan &

Alexander 2015). As species interactions have important functions in ecosystems, quantifying the effects of species interactions on plant regeneration is a crucial goal of ecology (Agrawal *et al.* 2007; Schupp, Jordano & Gómez 2010).

1.2 Mutualism

Mutualism describes biotic interactions in which both interacting species benefit (van Andel 2013). Mutualistic relationships can be facultative (e.g. leguminous plants can live without N-fixing *Rhizobium*-bacteria, but perform better with *Rhizobium*), or obligate (e.g. many lichens, which are a symbiosis between a fungal and an algal component, which are unable to thrive independently under natural conditions; van Andel 2013). The regeneration of most plant species depend on a multitude of mutualistic interactions, for example with animals (Bronstein 1994; Schupp, Jordano & Gómez 2017). Some of the most important plant-animal mutualisms are animal-mediated plant pollination and the dispersal of seeds to favourable sites for plant recruitment (Fontaine *et al.* 2006; van Andel 2013). Much of the focus of mutualism research has been on plant-animal mutualisms, however mutualistic interactions with microorganisms are known to play a major role in plant regeneration as well (Pomeroy & Alberts 1988). For example, the *Rhizobium*-legume symbiosis (Beringer *et al.* 1979), where nitrogen-fixing *Rhizobium* bacteria provide leguminous plants with nitrogen in exchange for favourable conditions for bacterial growth (Beringer *et al.* 1979) and the interaction of plants with mycorrhizal fungi, where plants receive minerals and water in exchange for carbon from fungal interaction partners (Smith & Read 2008).

1.2.1 Seed dispersal

Seed dispersal promotes the movement of genotypes and individuals and thereby affects the structure of plant populations as well as the distribution of individuals (Westcott & Graham 2000). Dispersal of seeds away from parental and conspecific plants promotes seedling survival (Janzen 1970; Connell 1971), the colonization of new areas (Westcott & Graham 2000) and allows range expansion under changing climate (Ettinger & HilleRisLambers 2017). Many land plants are dependent on mutualistic interactions with animals for the dispersal of their seeds (Schupp 1993; Nathan & Muller-Landau 2000; Wenny 2001; Wang & Smith 2002; Jordano 2014). The plant benefits from these interactions by the dispersal of seeds to favourable recruitment sites, whereas the animal benefits through gaining nutritional resources (Traveset, Heleno & Nogales 2000). Animal seed dispersers thereby indirectly perform an important ecosystem function by contributing to multiple ecosystem services

offered by forests, such as fruit, wood and non-timber products and carbon sequestration (Traveset *et al.* 2000).

Plant seed can be dispersed by animals in two ways, 1) epizoochorously, where seeds are transported outside an animal and 2) endozoochorously, where seeds are transported inside an animal (Gómez, Schupp & Jordano 2019). Dispersal on the outside is often incidental, where seeds are attached to the skin, fur or feather of animals (Gómez *et al.* 2019). Endozoochory can take place in two prominent ways, either by frugivorous animals, which are attracted to fleshy fruits or by granivorous animals, which harvest the seeds of the plant itself (Gómez *et al.* 2019). In the first case, animal seed dispersers feed on secondary structures instead of feeding directly on the seeds (Gómez *et al.* 2019). In the second case, the dispersers are directly attracted to the seed (Gómez *et al.* 2019). This particular form of seed dispersal is known as synzoochory, here seed predation is the prize for reliable dispersal (Janzen 1971; Hulme 1998). Synzoochorous behaviour includes the strategy of hoarding seeds as food storage for later consumption (i.e. caching; Hulme 1998). Seeds, which are not consumed directly, may escape being eaten and thereby have the chance to germinate in sites suitable for seedling establishment (Gómez *et al.* 2019).

The most effective synzoochorous dispersers are scatter-hoarding corvids and rodents, which bury seeds in small, scattered caches (Pesendorfer *et al.* 2016; Gómez *et al.* 2019). The seed dispersal by scatter-hoarding animals has been recorded in 35% of plant families and is most common in tree genera, such as *Quercus* and *Pinus*, which dominate temperate and boreal forests (Gómez *et al.* 2019). Thereby, the regeneration of keystone tree species is thought to be affected by scatter-hoarding behavior. Knowledge of the outcome of this mutualistic plant-animal interaction is important for understanding the population structure and distribution of keystone tree genera, such *Quercus* and *Pinus*.

1.2.2 Mycorrhiza

For most land plants, mutualistic interactions with mycorrhizal fungi are obligatory (Smith & Read 2008; van Andel 2013). In this interaction, both plant and fungi benefit from an exchange of minerals and organic matter (Smith & Read 2008; van Andel 2013). Fungi assist plants with the uptake of minerals and water from the substrate, and plants provide the fungi with carbohydrates (Smith & Read 2008; van Andel 2013). Four main types of mycorrhizal fungi are recognized: 1) Arbuscular mycorrhizal fungi (AM), which are associated with approximately 80% of plant species in temperate, subtropical and tropical plant communities

(Smith & Read 2008; van Andel 2013). Arbuscular mycorrhizal fungi are assumed to be highly efficient in uptake and supplying of inorganic phosphorus (Cui & Caldwell 1996), show generally very low host specificity and thereby are associated with a wide range of plant hosts (Smith & Read 2008). 2) Ectomycorrhizal (ECM) fungi, which occur mainly as symbionts of woody plants, for example members of the families *Abies*, *Picea*, *Pinus* and *Larix*. Given that only a small number of plants (approx. 3% of seed plants) are associated with ECM fungi, their global relevance results from their plant hosts' vast distribution across the terrestrial land surface and their economic value as main producers of timber (Smith & Read 2008). Along with the third type of mycorrhizal fungi, ericoid mycorrhizas, which occur mainly on plants in the order Ericales, ECM fungi are highly efficient in nitrogen uptake, and thereby beneficial in nitrogen-limited ecosystems (van Andel 2013). A fourth type, orchid mycorrhiza is recognized, where the early development of the orchid plant is completely dependent on the mycorrhizal partner (van Andel 2013).

1.3 Antagonism

Antagonistic relationships, such as herbivory, predation and parasitism, are species interactions where one species benefits (consumer), while the other species suffers (resource; van Andel 2013). Important antagonistic plant-animal interactions, involved in the regeneration of plants, are the exploitation of plant resources by animals, such as nectar robbing and the predation of plant seeds (Darwin 1841; Maloof & Inouye 2000). Plants provide resources as reward for the transfer of pollen and seeds, however nectar robbers remove nectar from flowers without having contact to pollen (Darwin 1841; Maloof & Inouye 2000) and seed predators consume seeds instead of dispersing them (Janzen 1986).

One of the most relevant plant-fungal antagonistic interactions for plant regeneration is the infection of plants by fungal pathogens (Alexander 2010; Bever *et al.* 2015). Fungal pathogens act as pests of important agricultural crops and of keystone tree species (Tomback *et al.* 2005; Dean *et al.* 2012). For example, rice blast, a disease caused by a fungal agent, can have devastating consequences on crop yield, thereby putting around one-half of the world's human population at risk, since they depend on caloric intake from rice products (Dean *et al.* 2012). Further, pathogens interacting with dominant trees species in temperate or boreal ecosystems can have severe consequences for the regeneration of keystone forest species (Tomback *et al.* 2016).

1.3.1 Seed predation

Most plant species are prone to seed predation by animals, such as insects, mammals and birds (Janzen 1971; Wenny 2000). Plant seeds are a rich source of energy, which makes them an important food source (Clark *et al.* 2007). Seed predators can have devastating effects on plant regeneration because predation usually leads to the death of seeds (Elwood *et al.* 2018). Seed predation can take place on the parent plant (i.e. pre-dispersal) or away from the parent plant (i.e. post-dispersal). Pre-dispersal predation usually is done by insects (Janzen 1971). Wind- and animal-dispersed seeds create a seed shadow around the parent plant (Janzen 1971). The heterogeneity of the created seed shadow determines the probability of post-dispersal seed predation, since many seed predators accumulate close to plants producing seeds (Janzen 1971). The accumulation of seed predators close to the seed bearing trees is part of the Janzen-Connell framework, which hypothesizes that seed germination and seedling survival increase with increasing distance from the seed source (distance-dependence), based on decreasing abundances of specialist herbivores, seed predators and pathogens (Connell 1971; Janzen 1971).

Especially mammals have been shown to strongly affect plant regeneration by predation of seeds in boreal, temperate and tropical habitats (Sork 1987). Mammalian predators in boreal and temperate forests are mostly one or two species of rodents (e.g. mice and dormice) whereas in the tropics important seed predators include multiple species of small and large mammals (e.g. agoutis, peccaries and tapirs; Hulme 1998). For many plant species, successful regeneration is only possible if the plant population produces enough seeds to satiate the predators allowing some seeds to escape (Janzen 1971; Sork 1987). The satiation of seed predators, in its most extreme form, is known as “masting” (Silvertown 1980). Masting is the synchronous production of large seed crops at extended intervals, to satiate seed predators and thereby enhance the probability of seedling survival (Silvertown 1980; Hulme 1998).

1.3.2 Fungal pathogens

In most terrestrial ecosystems, plant regeneration is severely affected by pathogenic fungi (van Andel 2013; Bever *et al.* 2015). Plants are prone to antagonistic interactions with fungal pathogens, where fungal agents can have diverse effects, such as killing of seeds and seedlings, or reducing plant growth by infection of leafs and roots of seedlings, saplings and adults (Alexander 2010; Bever *et al.* 2015). Plants are often associated with pathogens of

different degrees of host specialisation, ranging from generalists (many host species) to specialist species (one or few host species; Bever *et al.* 2015). Many forest trees are affected by host-specific fungal pathogens, causing blights of leaves and needles (Bever *et al.* 2015). For example, in the Rocky mountains, Whitebark pine (*Pinus albicaulis* Engelm.) is a keystone tree species, regulating snowmelt, preventing soil erosion, and providing an important food source for several seed-eating birds and mammals (Tomback *et al.* 2005). The distributional range of Whitebark pine is rapidly declining, mainly due to a host-specific fungal pathogen, the white pine blister rust (*Cronartium ribicola* J.C. Fischer; Tomback *et al.* 2005). Similar to seed predation, the accumulation of host-specific pathogens close to parent trees decreases successful seed germination and seedling growth within several meters around the seed source (Liang *et al.* 2016).

1.4 Species interactions across environmental gradients

Plant regeneration depends on multiple species interactions, such as briefly described above, but also on abiotic environmental conditions (Grebner, Bettinger & Siry 2014). Plants encounter these abiotic environmental conditions as variations in habitat characteristics along gradients across their distribution (Grebner *et al.* 2014), for example gradual changes in elevation, temperature, soil pH or soil moisture (Grebner *et al.* 2014). Plant species differ in their tolerances towards abiotic environmental conditions and are thereby found in greatest abundance on sites with a particular aspect that matches their tolerances, resulting in the typically unimodal abundance patterns (Grebner *et al.* 2014). The analysis of species distribution along environmental gradients has been conceptualized by Whittaker (1967) as "gradient analyses", which can be used to analyse responses of plant recruitment and ecosystem processes along gradients of an underlying controlling factor (Fukami & Wardle 2005).

One of the most prominent examples are elevational gradients, where the most important underlying controlling factor is temperature (Körner 2007; Jump, Mátyás & Peñuelas 2009). Along elevational gradients, an increase in elevation is correlated with changes in temperature regimes, where the increase of 167 m of elevation corresponds to a fall of approx. 1°C mean temperature (Körner 2007; Jump *et al.* 2009). One of the most distinct phenomenon observed in plant communities across elevational gradients is the tree line (Whittaker 1967; Körner 1998, 2007). This global phenomenon is characterised as a transition from forests to treeless vegetation that occurs at a particular elevational threshold

(Körner 1998). At the tree line, temperature poses a constraint on plant ranges by reducing their performance by reducing seed germination and seedling survival rates as well as growth rates (Woodward & Williams 1987; Körner & Paulsen 2004; Vitasse *et al.* 2012). In addition to temperature, plant regeneration can also be affected by other environmental gradients that co-vary with elevation, such as soil moisture or nutrient availability (Sundqvist, Sanders & Wardle 2013). Thereby, the response of a plant species to elevation is affected by the relative importance of underlying controlling abiotic factors (Sundqvist *et al.* 2013).

Environmental gradients can lead to changes in the co-occurrence of interacting species (Tylianakis & Morris 2017). For example, a plant species that occupies a large elevational range could co-occur with different assemblages of species at different positions across that range (Sundqvist *et al.* 2013). It has been shown, that the abundance of animals interacting with plants commonly changes with elevation (Sundqvist *et al.* 2013), with shifts in the dominant groups of pollinators or in the abundances of keystone seed dispersers (Sundqvist *et al.* 2013). These changes in animal abundance are related to temperature change, ultimately determining the animals' elevational range as well (Sundqvist *et al.* 2013). Further, soil environmental gradients (e.g., pH, nutrients) that co-vary with changes in elevation can alter fungal community composition (Sundqvist *et al.* 2013). Thereby, interaction partners of plants can change along their elevational distribution (Sundqvist *et al.* 2013).

The strength of species interactions can vary along environmental gradients (Sundqvist *et al.* 2013). Hence, species interaction outcomes can differ across the elevational range of a plant depending on the environmental conditions (Angert 2006; Mitchell *et al.* 2006; Sexton *et al.* 2009; Lee-Yaw *et al.* 2016). For instance, negative effects of soil pathogens on survival of tree species can decline with increasing elevation (Defossez *et al.* 2011; Sundqvist *et al.* 2013) or the benefits from mutualistic plant-fungal interactions have been shown to be higher in stressful environments with particular importance at higher elevations (Nara & Hogetsu 2004; Wagg *et al.* 2011; Sundqvist *et al.* 2013).

1.4.1 Species interactions under climate change

Species interactions may be highly sensitive to climatic changes, since they depend on the spatial overlap of favorable environmental conditions for both interacting species (Suttle, Thomsen & Power 2007; Tylianakis *et al.* 2008). Further, the strength of the interaction between plants and their animal and fungal associates can depend on environmental

conditions (Agrawal *et al.* 2007; McConkey *et al.* 2012; Morán-López, Alonso & Díaz 2015). For example, if environmental conditions are altered, this may affect animal seed dispersal through the alteration of seed quantity and quality of plant species (McConkey *et al.* 2012; Zhang & Granger 2014). Further, plant-fungal mutualism might be altered, since increased temperatures can increase root colonization as well as the production of mycorrhizal hyphae in some mutualism (Staddon, Gregersen & Jakobsen 2004; Tylianakis *et al.* 2008). Additionally, changes in climate will affect both the growth of plants as well as the diseases that attack them (Madgwick *et al.* 2011). For example, it is assumed that pathogen infections rates will increase with increasing temperature or with altered soil moisture regimes (Malmstrom *et al.* 2006; Tylianakis *et al.* 2008; Madgwick *et al.* 2011). Species interactions occurring across elevational gradients may be especially affected, since climatic changes taking place at comparatively small spatial scales and might be especially pronounced (Körner *et al.* 2016; Kueppers *et al.* 2016). Thereby the study of species interactions between plants, animals and fungal species across their elevational distribution is important to project potential effects of altered environmental conditions on plant regeneration.

2. Thesis structure and research questions

My thesis is structured into three chapters (Figure 1). The general aim was to gain detailed knowledge on the relative effects of species interactions on plant regeneration in a natural system across the variation of environmental conditions within it. In the first chapter I investigated how demographic processes involved in plant recruitment, such as seed deposition, seed predation, seed germination and seedling survival are affected by environmental factors. Further, I asked which processes limit recruitment across the elevational range of the plant depending on the environmental context (Q1). In the second chapter I investigated how abiotic and biotic environmental factors shape the occurrence of plant-associated antagonistic and mutualistic fungi (Q2). Finally, in my third chapter I investigated how seedling establishment and seedling survival were associated with the occurrence of plant-associated mutualistic and antagonistic fungi and with abiotic and biotic environmental factors (Q3).

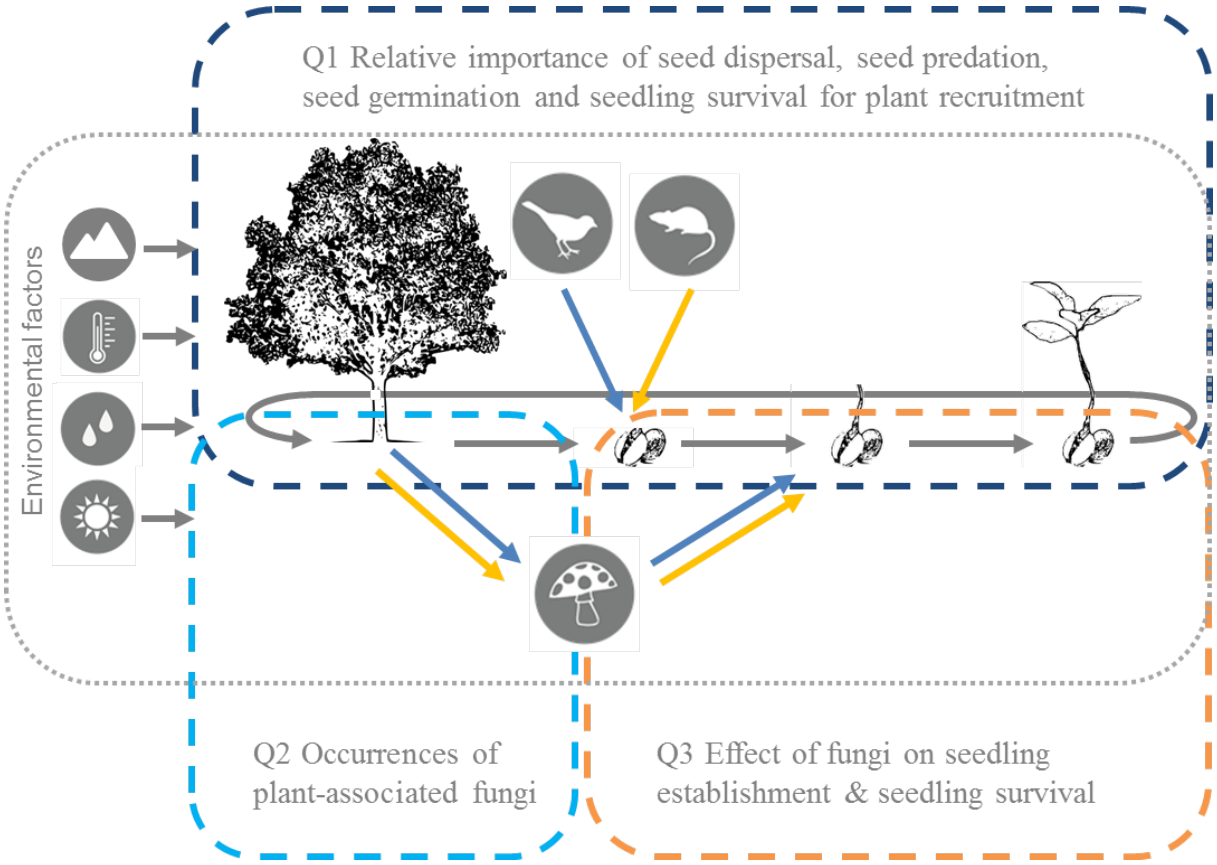

Figure 1. Conceptual framework of my main research questions. The intent of my thesis was to deepen the knowledge of the effect of plant-animal and plant-fungal interactions and environmental factors, such as elevation, temperature, water or light availability, on different stages of plant regeneration. In the first chapter of my thesis, I aimed at investigating the relative importance of four demographic processes in relation to environmental factors for the recruitment of Swiss stone pine across its distributional range (Q1). In the second chapter, I investigated the effects of abiotic and biotic environmental factors on the occurrences of pine-associated fungi (Q2). Finally, in my last chapter, I aimed to investigate the effects of Swiss stone pine-associated fungal communities as well as of abiotic and biotic environmental factors on seedling establishment and seedling survival (Q3). Mutualistic interactions are indicated with blue arrows, antagonistic interactions with yellow arrows, the inclusion of environmental factors in all chapters is indicated by the grey box.

2.1 How do environmental factors determine the Swiss stone pine recruitment and which demographic processes are the limiting factors across its elevational range? (Q1)

In the first chapter of my thesis (Appendix A1), I studied how environmental conditions shape four demographic processes (i.e. seed deposition, seed predation, seed germination, seedling survival) of Swiss stone pine recruitment. Further, I identify the most limiting demographic processes across the pines' elevational range. I monitored seed deposition by spotted nutcrackers (*Nucifraga caryocatactes*), and transplanted seeds of Swiss stone pine across and beyond its elevational distribution (1850 – 2250 m a.s.l.) to monitor predation and germination of seeds as well as the survival of emerging seedlings. First, I used generalized linear mixed models to assess how environmental factors (i.e. canopy cover and ground vegetation cover) shape the demographic processes involved in Swiss stone pine recruitment (i.e. seed deposition, seed predation, seed germination and seedling survival). Second, I performed a sensitivity analysis to assess which of the four demographic processes is most likely to limit the pines' recruitment at the lower and upper range margin as well as in the centre of its elevational range. I expected that seed deposition might be the limiting process where canopy cover is absent and ground vegetation cover is high (i.e. at the upper elevational range margin; Neuschulz *et al.* 2018). Further, I expected higher seed predator abundance and thereby higher seed predation rates under benign climatic conditions at the lower range margin and in the centre may limit recruitment (Wenny 2000). Additionally, I expected that recruitment would be limited by dry and shaded conditions negatively affecting seed germination and seedling survival (Nathan & Muller-Landau 2000).

2.2 How do abiotic and biotic environmental factors affect the occurrences of Swiss stone pine-associated antagonistic and mutualistic fungi? (Q2)

In the second chapter of my thesis (Appendix A2), I examined the effects of environmental factors and distances to their host plant (Swiss stone pine) on generalist and specialist antagonistic (i.e. pathogenic) and mutualistic (i.e. ectomycorrhizal) fungi. I collected soil samples and environmental factors across and beyond the whole elevational distribution of Swiss stone pine (i.e. 1850 – 2250 m a.s.l.) at two elevational gradients in the European Alps close to Davos, Switzerland. I assessed the soil fungal communities using a DNA metabarcoding approach and tested the effects of abiotic and biotic environmental factors on fungal communities using multivariate statistics. I expected that abundances of specialist fungal operational taxonomic units (OTUs) match the range of their host plant (Hallenberg & Kúffer 2001) in accordance with the Janzen-Connell hypothesis (i.e. high abundances close to

the host; Jumpponen & Egerton-Warburton 2005; Liang *et al.* 2016). Further, I expected abundances of generalist fungal OTUs to vary independently from the pines distribution since alternative hosts could be colonized (Jumpponen & Egerton-Warburton 2005). Additionally, I expected abiotic environmental factors to affect the abundances of all fungal OTUs, since abiotic factors have been shown to affect fungal occurrences on regional and global scales (Classen *et al.* 2003; Tedersoo *et al.* 2014; Carrino-Kyker *et al.* 2016).

2.3 How does fungal occurrence and abiotic and biotic environmental factors shape Swiss stone pine seedling establishment and survival? (Q3)

In the third chapter of my thesis (Appendix A3), I investigated if known plant-associated fungi (i.e. a subset of fungal OTUs from the DNA metabarcoding dataset identified as pine associated for the second chapter) affect seedling establishment and survival of Swiss stone pine in an environmental context. Further, I applied a null model approach on the full DNA metabarcoding dataset, in which 42% of the OTUs could not be assigned to reference sequence databases (i.e. UNITE and GenBank; Koljalg et al., 2005) or the fungal community dataset FUNGuild (Nguyen *et al.* 2016). The null model approach overcame the need for genus level identification of OTUs for inclusion in analyses. Hence, I could explore whether previously unknown fungi (i.e. taxonomically and functionally unknown fungal OTUs) could have an effect on the early stages of plant recruitment. I transplanted seeds across and beyond the elevational range of Swiss stone pine (i.e. 1850 – 2250 m a.s.l.) in the European Alps in two valleys close to Davos, Switzerland. I measured environmental factors at all transplant sites and linked the experimental and environmental data to the DNA metabarcoding dataset. I expected that seedlings might encounter different feedbacks from fungi during seedling establishment (0 – 4 months) than later stage seedling survival (5 – 15 months). I expected that seedling establishment would be most vulnerable to abiotic environmental stresses (Walck *et al.* 2011; Kueppers *et al.* 2016), whereas subsequent seedling survival might be more affected by the presence of mycorrhiza and fungal pathogens (Bardgett *et al.* 2005). I further expected that a null model approach might identify fungi affecting seedling establishment and seedling survival that are taxonomically and functionally unassigned.

3. Study area and system

3.1 Study area

I conducted this study in the Central Eastern Alps (Fig. 2). I chose two elevational gradients close to Davos, Switzerland, one in the Sertig valley (46°44′0.76″N 9°51′3.5″E) and one in the Flüela valley (46°48′0.25″N 09°54′15.38″E; Neuschulz *et al.* 2015, 2018; Merges *et al.* 2018). The vegetation structure in the valleys at the lowest elevations (about 1850 m a.s.l.) is a mixed coniferous forest, composed of European larch (*Larix decidua* Mill.), Norway spruce (*Picea abies* (L.) H. Karst) and Swiss stone pine (Fig. 2; Neuschulz *et al.* 2015, 2018; Merges *et al.* 2018). Abundance of the tree line forming Swiss stone pine is distributed in a hump-shaped pattern from 1850 m a.s.l. up to 2150 m a.s.l., where Swiss stone pine trees (> 3m tall, Harsch *et al.*, 2009) form the upper tree line. Swiss stone pine saplings can be found up to 2200 m a.s.l., but there are none growing at and over 2250 m a.s.l. pine (Neuschulz *et al.* 2015, 2018; Merges *et al.* 2018). I implemented in each valley nine elevational belts spaced by 50 m of altitude ranging from 1850 to 2250 m a.s.l. (Fig. 2, Table 1; Neuschulz *et al.* 2015, 2018; Merges *et al.* 2018).

Table 1. GPS coordinates of each established elevational belt in the Sertig and the Flüela valley, close to the city Davos, Switzerland.

Valley	Elevational belt	Latitude	Longitude
Sertig	1850	46.7331086	9.84665725
	1900	46.7326672	9.84923486
	1950	46.7329577	9.85051948
	2000	46.7337581	9.85181769
	2050	46.7344841	9.85311997
	2100	46.7345673	9.85371269
	2150	46.7351274	9.85455217
	2200	46.7354215	9.85584957
	2250	46.7363753	9.85728015
Flüela	1850	46.8002499	9.90096589
	1900	46.8003380	9.90214721
	1950	46.8008359	9.90355859
	2000	46.8014655	9.90372201
	2050	46.8020200	9.90476704
	2100	46.8023678	9.90555588
	2150	46.8023974	9.90760406
	2200	46.8021773	9.90908226
	2250	46.8028860	9.91171004

3.2 Study system: Swiss stone pine and its interaction partners

Swiss stone pine is a keystone forest species, forming the tree line in the European Alps (Fig. 2; Mattes 1982). The pine is dependent on the interaction with the spotted nutcracker (*Nucifraga caryocatactes* [L., 1758]), which serves as the sole seed dispersal agent (Mattes 1982), for its seed dispersal. The dispersed seeds are subjected to heavy post-dispersal seed predation by multiple seed predators, such as the Eurasian red squirrel (*Sciurus vulgaris* L. 1758) and bank vole (*Myodes glareolus* Schreber 1780; Mattes 1982). Further, the pine depends on an obligate mutualism with ectomycorrhizal fungi for survival (e.g. *Suillus* Gray, *Rhizopogon* Fr) under natural conditions (Moser 1967; Nierhaus-Wunderwald 1996) and can be negatively affected by antagonistic interactions with the pathogenic snow blight fungus *Gremmenia infestans* (P. Karsten) P.W. Crous. The Swiss stone pine and its interaction partners therefore provide a highly suitable study system, to address ecological question related to study plant-animal and plant-fungal interactions across environmental gradients to understand what determines plant regeneration and distributional range limits.

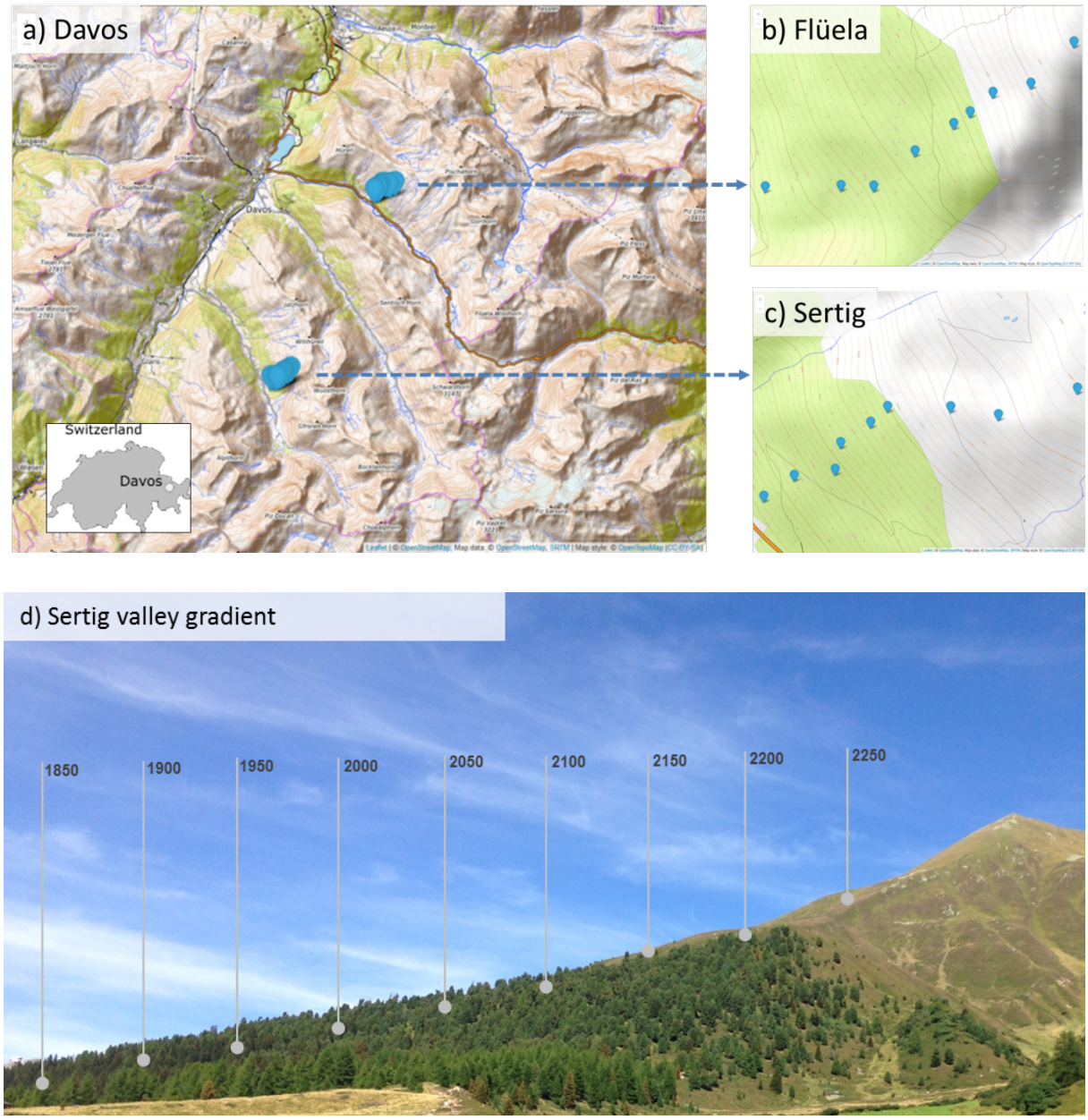

Figure 2. Map of study area and elevational belt distribution. (a) Studied elevational gradients located in the Flüela valley and the Sertig valley close to Davos, Switzerland. Elevational distribution of *Picea*/*Larix*/*Pinus* forest is shown in green colour. (b & c) Elevational belts (blue pins), ranging from 1850 to 2250 m a.s.l., spaced by 50 m of altitude. (d) Photograph of the elevational gradient in the Sertig valley. Estimated elevational belt locations with corresponding elevation in m a.s.l. are added in grey. Map images: Open street map.

3.3 Study design

3.3.1 Seed translocation experiment

To assess seed predation, seed germination and seedling establishment of Swiss stone pine, I conducted seed translocation experiments at all elevational belts in the years from 2015 – 2017. Additionally, I made use of data from 2012 – 2015 previously sampled by the working group. To space the seed translocation experiments evenly in the heterogeneous environment within each elevational belt, I applied a stratified random sampling design. For this, I chose five microhabitat types which could be found at all elevational belts: 1) close to tree trunk (or in absence of trees at elevations higher than 2200 m a.s.l.: ground covered by matgrass (*Nardus stricta* L.), 2) open soil, 3) microsite covered by snow, 4) ground vegetation cover (i.e., *Loiseleuria procumbens* (L.) Desv., *Vaccinium* spec. L., & *Rhododendron* spec. L.), 5) rocky habitat. Microhabitats were replicated between two and five times per elevational belt, depending on the sampling year (see Appendix 1, Table S1). At each seed translocation replicate, I buried five seeds of Swiss stone pine (mean nutcracker cache size; Mattes 1982). I placed the seeds on a 15 x 15 cm piece of mesh, then buried it approximately 4 cm deep in the ground and covered it with soil. I fixed these mesh bags to the ground with three metal nails and labeled them with tape, which allowed retrieval and observing the seed fate at the end of the growing season (Neuschulz *et al.* 2015). I recorded seed fate at the end of the growing season by excavating the mesh bags and visually expecting the seeds. I recorded a seed as "germinated/established" when a seed showed a radical to fully established seedling (Fig. 3). Absent seeds and seeds that showed signs of seed predation (e.g. bite marks) were recorded as "predated". Further, I observed the survival of seedlings until the end of the next growing season in the following year (Fig. 3).

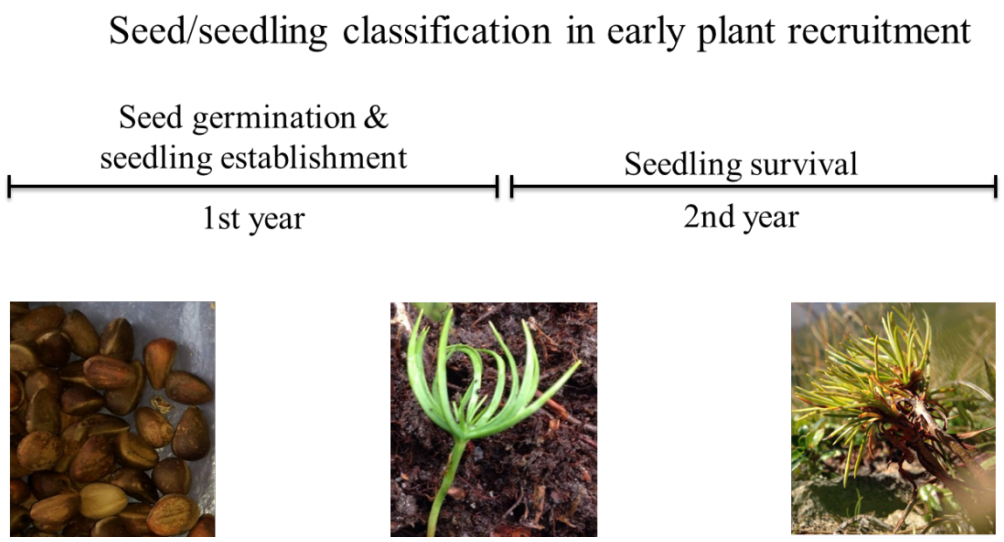

Figure 3. Successful seed germination and seedling establishment in the first year was recorded when a seed showed a radical to fully established seedling. Successful seedling survival was recorded when a seedling was present at the end of the growing season of the second year.

3.4 Environmental conditions

I recorded a range of potential biotic and abiotic drivers of Swiss stone pine recruitment across its elevational distribution. In particular I focused on drivers of early plant recruitment (described in detail below), since this is the most vulnerable period in the recruitment process, and thereby a bottleneck of plant regeneration (Vitasse *et al.* 2012).

3.4.1 Biotic environmental factors

1. Seed deposition

Swiss stone pine depends on nutcrackers for its dispersal. The seed deposition site, chosen by the pines' animal disperser, ultimately determines the microhabitat in which a seed may or may not thrive (Wenny 2000). Hence, I searched for caches (seed deposition sites) made by nutcrackers in a strategic manner at each elevation belt, by establishing two 10-metre-transects, which I divided into 20 m^2 subplots (Fig. 4). I then took a soil sample and thoroughly searched for deposited seeds in the centre of each 1 m^2 subplot (Briggs, Vander Wall & Jenkins 2009; Neuschulz *et al.* 2015, 2018). I recorded a cache presence if intact seeds or seed shells handled by nutcrackers were found. I searched for seed deposition in this manner each August and September for a total of three years (2015 – 2017) and could use

data collected in three previous years (2012 – 2014) during the main caching period mid-August until beginning of September, when Swiss stone pine cones are ripe.

Figure 4. Example of one 10-metre-transects established in 2015 in the Sertig valley at 2150 m a.s.l.. Subplots were marked with blue ribbons. At each ribbon (i.e. the centre of each subplot) I took a soil sample und searched for seeds deposited by nutcrackers.

2. Fungal communities

Swiss stone pine is an obligate mycorrhizal plant, as such it requires ECM mutualists for seedling survival under natural conditions (e.g. to acquire needed soil nutrients and resist drought stress; Smith & Read 2008). A wide range of fungi can form ectomycorrhiza with pines, such as groups of ECM forming fungi in the Basidiomycota (e.g. Russulaceae) or in the Ascomycota (e.g. Elaphomycetaceae; Bacher, Zöll & Peintner 2010; Rainer *et al.* 2015; Paz *et al.* 2017). The connection between pines and fungi is achieved through ectomycorrhizal roots, which are characterized by three main morphological structures: 1) a mantle of fungal tissue surrounding the plant root, 2) the Hartig net (i.e. inward growth of fungal hyphae between epidermal and cortical cells of the plant root and 3) an outwardly growing system of hyphae forming a mycelium which connects the ectomycorrhizal root with the soil and the fruiting bodies (i.e. sporocarps) of the fungus (Smith & Read 2008).

Further, the snow blight fungus (*G. infestans*), severely limits Swiss stone pine survival. The fungal pathogen belongs to the Phacidiaceae (Ascomycota), which contains multiple saprobic and plant pathogenic species (Crous 2014). The infection of pine needles through fungal spores takes place in late autumn (Roll-Hansen 1989; Burdon *et al.* 1992). After the primary infection of a needle by spores, the developing fungal mycelium can spread from needle to needle under snow cover, which provides suitable microclimatic conditions for fungal growth (i.e. moist and cold; Roll-Hansen 1989). In spring, after snow melt, apothecia (i.e. fruiting bodies of *G. infestans*) start to form on the infected needles (Roll-Hansen 1989). The apothecia can be recognized as small, roundish, dark flecks distributed along the needles (Roll-Hansen 1989). In late autumn, ripe apothecia release spores under moist conditions to be distributed by wind (Roll-Hansen 1989).

Following a random stratified sampling design, I collected soil samples at each elevational belt in two microhabitat types (ground vegetation cover and close to Swiss stone pine tree trunks; Merges *et al.* 2018). Within each elevational belt, I replicated each microhabitat four times and thereby collected in total 8 soil samples per elevational belt in each valley. I sampled in two seasons, one in spring 2015 (i.e. May) and one in autumn 2015 (i.e. September), thereby I collected a total of 288 soil samples over the course of the study. Soil samples were air-dried until zero humidity. Additionally, I sampled ECM roots of Swiss stone pine to create a reference database for pine-associated ECM fungi. I collected ECM roots in both valleys at 1850, 2050 and 2200 m a.s.l. in May 2015.

I used 300 mg of each root and soil sample for DNA extraction according the protocol by Cubero and Crespo (2002). I conducted the PCR according to the protocol described in Schmidt *et al.* (2013), except running two parallel PCRs (1 x 52 °C, 1 x 55 °C) before pooling of PCR products. Paired-end sequencing (2 x 350 bp) was conducted on an Illumina MiSeq sequencer by Fasteris SA, Plan-les-Ouates, Switzerland. For cleaning of the received data, I used the Illumina metabarcoding pipeline for fungi described in Bálint et al. (2014).

I identified pathogenic and ECM fungi by blasting Altschul *et al.* (1997) the received OTU sequences against GenBank nucleotide database (ftp://ftp.ncbi.nlm.nih.gov/blast/db/nt, downloaded on 3 March 2016) and UNITE database (Koljalg et al., 2005). Generalist (i.e. *Lophodermium* spp. Chevall.) and specialist (i.e. *G. infestans*) pathogenic OTUs were selected from this dataset. I determined the degree of host specificity by literature research. For pathogens, I searched peer-reviewed journal articles including all records until December 2016 in Web of Science using the keywords "((*Pinus cembra* OR Swiss stone pine) AND (pathogen OR pathogenic OR disease))". I found 13 articles, from which I extracted Swiss stone pine-associated pathogens. For ECM, I first used my own ECM database (derived from sequenced Swiss stone pine ECM root samples) to obtain a list of candidate ECM OTUs. Second, I taxonomically assigned these OTUs and confirmed the taxon list by searching the assigned names in Web of Science. I found 34 species reported as ECM mutualists (Appendix 2, Table S1). Following Molina, Massicotte and Trappe (1992) I then classified ECM fungi into 1) specialists (i.e. narrow host specificity with all plant hosts within the same genus) and 2) generalists (i.e. intermediate to broad host specificity, all plant hosts within the same family or plant hosts extending across multiple plant families or even orders).

3. Distance to conspecific adult

The distance to conspecific adults is regarded as an important factor in plant recruitment, since species-specific pathogens and herbivores can accumulate close to conspecific adult plants and thereby have a detrimental effect on seedling survival (Janzen-Connell hypothesis; Connell 1971; Janzen 1971). I measured the distance to conspecific adults of each replicate, subplot and soil sample by estimation and using a laser range finder (Nikon 800S) for distances over 10 m.

4. Ground vegetation cover

High ground vegetation cover might lead to nurse shrub effects, i.e. increase seed germination and seedling survival rates by ameliorating harsh environmental conditions or might compete for resource with Swiss stone pine seedlings (Castro *et al.* 2002; Maher & Germino 2006). I estimated the percentage of ground covered with vegetation at each replicate, subplot and soil sample according to Braun-Blanquet (1964). The dominant species forming ground vegetation cover were alpine azalea (*Loiseleuria procumbens*), *Vaccinium* spp. and *Rhododendron ferrugineum* L..

3.4.2 Abiotic environmental factors

1. Light availability

A lack of light might have a negative effect on seed germination and seedling survival, since triggering of germination and subsequent plant growth can be negatively affected by low light availability (Smith & Smith 2012). Therefore, I recorded light availability, as the percentage of canopy cover above each replicate, subplot and soil sample with a spherical crown densitometer (Forestry Suppliers, Jackson, USA). Canopy cover can be seen as a proxy for light availability, since high canopy cover leads to shaded conditions.

2. Temperature

Seed germination and seedling survival rates might increase with higher soil temperatures, since these are reported to favor germination and growth of plants (Smith & Smith 2012). Temperature was recorded at each replicate, subplot and soil sample in a 4 hour interval for the duration of the study with temperature loggers (iButtons, Maxim Integrated Products, Sunnyvale, USA). I calculated mean summer temperature as mean of the June-August period and mean winter temperature as mean of the December-February period for each temperature logger at each elevational belt. I also calculated maximum and minimum temperatures (Oberhuber 2004), such as mean daily maximum temperature for June-August, mean daily minimum temperature for December-February, mean temperature and mean daily maximum temperature of the hottest month (July), and mean temperature and mean daily minimum temperature of the coldest month (January). Extreme maximum temperatures can lead to desiccation of seedlings (Tingstad *et al.* 2015; Kueppers *et al.* 2016; Andrus *et al.* 2018) and

very low temperatures can lead to high seedling mortality in high-elevation systems (Lenoir *et al.* 2008; Kueppers *et al.* 2016).

3. Soil moisture

Water availability is highly important during early establishment, where seeds depend on water for germination and seedling root systems are barely developed and drought stress could lead to fast desiccation (Kueppers *et al.* 2016). Hence, I measured soil moisture, as a proxy for water availability, under dry condition at five unique locations at each replicate, subplot and soil sample with a tensiometer (Theta-Kit version 3, Delta-T Devices Ltd, Cambridge, United Kingdom).

4. pH

pH has been shown to be an important determinant for fungal community composition (Bahram *et al.* 2012; Tedersoo *et al.* 2014; Soudzilovskaia *et al.* 2015). Hence, I measured soil pH with a pH/conductivity meter CPC-401 (Elmetron) in all sequenced soil samples.

4. Main results and discussion

4.1 Environmental context determines the limiting demographic processes for plant recruitment across a species' elevational range

The first aim of my thesis was to investigate the response of four demographic processes (i.e. seed deposition, seed predation, seed germination and seedling survival) to environmental factors (i.e. dominating habitat characteristics). Moreover, I explored which of these processes might limit the recruitment of Swiss stone pine across three distributional range scenarios (i.e. lower and upper range margin, and centre of the distribution) in relation to the relative frequency distribution of the dominant habitat characteristics (i.e. canopy cover and ground vegetation cover). To do so, I first modelled the four demographic processes across gradients of canopy cover and ground vegetation cover. I used canopy cover and ground vegetation cover as explanatory variables, since these ultimately determine the seed caching behaviour of spotted nutcrackers, which sets the environment in which a dispersed seed may or may not thrive (Neuschulz *et al.* 2015, 2018). I found different optima of environmental conditions for the four demographic processes. Second, I conducted a sensitivity analysis to investigate which of these processes was the most limiting factor across Swiss stone pines' elevational

distribution. I found that the importance of the different demographic processes changed along the environmental gradient, with seed deposition being the most limiting process at the upper range margin.

My results indicate conflicts between the optima of the different recruitment processes. I found that seed deposition is highest at sites where seed germination is poor, and seed germination is highest where seedling survival is poor. These patterns have been termed "seed-seedling conflicts", stating that the most favourable sites for seed survival may not be suitable for seedling survival and vice versa (Grubb 1977; Sork 1985, 1987; Schupp 1995; Schupp & Fuentes 1995). Such within species conflicts take place when environmental conditions that are advantageous for one stage are detrimental for another stage (Schupp 1995; Schupp & Fuentes 1995). The positive effect of high canopy cover and low vegetation cover on seed depositions is potentially associated with several co-varying factors in these habitats: 1) the presence of a tree offers an important landmark for cache recovery (Mattes 1982; Tomback & Linhart 1990) and 2) dry and cold conditions preserve seeds and thereby offer long-term food storage (Neuschulz *et al.* 2015). However, for seed germination, dry and cold conditions are detrimental, since seeds need to be imbibed with water to trigger germination, and for seedling survival, high pathogen abundances close to conspecific trees could be associated with low seedling survival rates (Merges *et al.* 2018).

Which demographic processes of plant recruitment are most important for species at different range positions are largely unknown (Sagarin & Gaines 2002; Parmesan *et al.* 2005; Sagarin, Gaines & Gaylord 2006; Ettinger & HilleRisLambers 2013). I found that at lower range margins and in the centre of Swiss stone pine distribution, seed germination and seedling establishment have the potential to limit recruitment. This lack of recruitment might lead to a competitive disadvantage of Swiss stone pine against upward migrating heterospecific plants. Potential competitive interactions with heterospecific plants might hinder the persistence of pine populations at the lower range margin and at the core of the distribution (Antonovics 1976). At the upper range margin seed deposition was by far the most limiting process. This suggests that minute changes in seed deposition rates at the upper range margin could have important consequences for range expansions at the leading edge of Swiss stone pines' distribution. Thereby, my results provide important information for management and conservation efforts on potential targets to protect Swiss stone pine as a keystone forest species. My study especially highlights that the major demographic limitations of pine populations and thereby the targets for population management

(Silvertown, Franco & Menges 1996), would be different depending on the species distributional range position. Further, I deliver an approach that allows disentangling the complex plant recruitment process, and thereby offers a novel way going forward into projecting plant recruitment under changing environmental conditions.

4.2. Spatial patterns of pathogenic and mutualistic fungi across the elevational range of a host plant

In the second chapter of my thesis, I aimed at examining the effects of abiotic and biotic environmental factors on the occurrence and abundance of fungi associated with Swiss stone pine. To do so, I used multivariate statistics and generalized linear models to test the effects of elevation, temperature, soil moisture, pH, vegetation cover and distance to the closest Swiss stone pine on DNA read abundance of pathogenic and mutualistic fungal OTUs associated with Swiss stone pine. Additionally, I tested if these abiotic and biotic environmental factors had different effects on fungi of varying host specificity (i.e. host generalists, host specialists). The results revealed that all fungi included in the analysis exceeded the current distributional range of Swiss stone pine. Environmental factors (i.e. temperature, soil moisture, pH, vegetation cover, distance to host) had no effects on the abundances of generalist fungal OTUs. However, we found positive effects of Swiss stone pine on the abundance of host specialist fungi.

As expected, I found abundances of generalist fungal OTUs to vary independently from the pines distribution. Surprisingly, the occurrences of specialist fungi exceeded their host plants current range. One possible explanation for this pattern might be the efficient dispersal of fungal spores by wind or mammal dispersers across long distances (Read & Haselwanter 1981; Allen *et al.* 1992; Nuñez, Horton & Simberloff 2009; Urcelay *et al.* 2017). For example, the spores of the specialist fungal pathogen *G. infestans* can be dispersed by wind, with dispersal distances over several hundred meters (Roll-Hansen 1989). Further, *G. infestans* spore dispersal could also occur indirectly by infected needles as diaspores (Roll-Hansen 1989). After snowmelt in spring, infected needles become bleached and brittle, then needles (complete or in parts) can be spread by wind (Roll-Hansen 1989). In contrast to the wind-dispersed specialist pathogen, the specialist mutualist *Rhizopogon* belongs to the group of hypogeous fungi, which depend on animals for long distance dispersal (Massicotte *et al.* 1994). Hypogeous fungi produce their sporocarps belowground or close to the soil surface, which limits their ability to release spores into the air (Hawker 1955; Ashkannejhad & Horton

2006). Most sporocarps remain underground and decay, thereby releasing spores into the ground (Hawker 1955; Ashkannejhad & Horton 2006). However, when the fleshy sporocarps are consumed by mycophagous mammals (i.e. fungus consuming animals), such as mice and deer, the ingested spores are dispersed when the animals defecate (Cazares & Trappe 1994; Miller, Torres & McClean 1994; Colgan & Claridge 2002; Ashkannejhad & Horton 2006). Spores from hypogeous fungi found in mammal feces have been shown to be viable and to successfully form mycorrhiza on seedlings (Colgan & Claridge 2002; Ashkannejhad & Horton 2006). In the Alps, multiple ungulate species, such as red deer or roe deer, move within and beyond the current tree line (Büntgen *et al.* 2017), and could thereby potentially disperse *Rhizopogon* spores outside the current range of their host plant. Thereby, the presence of host specialist fungi outside of their hosts' current range could indicate that fungal specialists were not dispersal limited. Further, another potential explanation cloud be persistent soil spore banks, given that the tree line has been higher in the past (Gehrig-Fasel *et al.* 2007; Pini *et al.* 2017). A third explanation for the recorded pattern could be that host constraints for specialists were lifted under harsh alpine conditions (Ryberg, Larsson & Molau 2009; Botnen *et al.* 2014). For example, there is evidence that *G. infestans* was found on juniper (*Juniperus* spp.), however no *G. infestans* fruiting bodies were found in any case (Roll-Hansen 1989), and some ECM mutualists interact with ericoid bearberry (*Arctostaphylos* spp. Adans.), under harsh environmental conditions (Tedersoo *et al.* 2006; Krpata *et al.* 2007). These findings indicate that occurrences of needed mutualistic ECM fungi beyond their hosts' current distribution could provide suitable conditions for seedling establishment, allowing Swiss stone pine to track its needed climatic conditions to higher altitudes with ongoing climate warming. However, I found the same pattern for pathogenic fungi, implying that there will be no escape from enemies for Swiss stone pine in newly suitable habitats above the current tree line. My results indicate that an upward shift of Swiss stone pines' distribution under warming climates might be limited by the occurrence of antagonistic fungi, but not by the absence of mutualistic fungal partners. However, if the strength of the interaction between plants and fungi as well as the nature of the interaction (i.e. mutualistic or antagonistic) stays the same under changing environmental conditions is mostly unknown and should be the focus of future studies. Thereby understanding plant-fungal interactions is important for projecting how plants will respond to global change.

Abiotic environmental factors did not significantly affect the generalist fungal associates of Swiss stone pine. In my study, the abiotic environmental gradients might be within the climatic and environmental tolerance range of local fungal communities, given that

generalist fungi can occupy large geographic ranges encompassing pronounced changes in environmental conditions (Tedersoo *et al.* 2014). Further, I found that abundances of host specialist fungi were mostly determined by the distance to their host, where abundances decreased with increasing distances to Swiss stone pine. The negative distance dependency of pathogens is integrated in the Janzen-Connell framework (Connell 1971; Janzen 1971). The Janzen-Connell framework states that species richness of plant communities is favoured by host-specific pathogen accumulation close to conspecific adult host plants, thereby creating unfavourable conditions for conspecific offspring survival, preventing monodominance of the species and creating space for heterospecific plant species to establish (Packer & Clay 2000; Liang *et al.* 2016; Liu *et al.* 2016). Interestingly, I also found high abundance of mutualistic fungi close to the host, suggesting that negative effects of fungal pathogens on seedling survival could be mitigated by the presence of specialised mutualistic fungi enhancing seedling resistance. Enhancement of seedling resistance could be mitigated through the fast colonization of a seedling by a highly abundant specialised mutualistic fungi which might lower the stress caused by pathogens by mechanical protection against root parasites and enhancement of nutrient uptake (Marx 1972; Mukerji, Chamola & Singh 2000). In accordance with this hypothesis, Bennett *et al.* (2017) and Teste *et al.* (2017) found positive effects of ECM mutualists on plant seedlings close to conspecific adults, suggesting an "inverse" Janzen-Connell effect. However, if a general enhancement of seedling resistance could protect seedlings from infections by the specialist pathogen *G. infestans* should be the focus of future studies; especially since *G. infestans* is reported to be highly pathogenic on its primary host. My study thereby adds to the field by showing that negative distance dependence could be more important for determining specialist fungal distribution than previously assumed.

4.3 High throughput sequencing combined with null model tests reveals specific plant-fungi associations linked to seedling establishment and survival

In the final chapter of my thesis, I used the DNA metabarcoding dataset generated for the second chapter and linked it to the seed translocation dataset. Firstly, I investigated if known plant-associated fungi have an effect on seedling establishment and survival of Swiss stone pine in an environmental context (i.e. considering abiotic [light availability, temperature, soil moisture] and biotic [vegetation cover, and distance to conspecific adults] environmental factors) using generalized linear mixed models. Further, I tested whether previously unknown fungi (i.e. taxonomically and functionally unassigned fungal OTUs) have an effect on the early stages of plant recruitment by applying for the first time a null model approach to a

DNA metabarcoding dataset. As expected, early seedling establishment was most vulnerable to abiotic environmental stresses, whereas the survival of seedlings was mostly determined by the abundance of a fungal pathogen. I further identified previously unknown fungal associates of Swiss stone pine affecting the pines' seedling establishment and survival using the null model approach.

I found that seedling establishment of Swiss stone pine was mostly determined by abiotic environmental factors. High light availability (i.e. low canopy cover) and elevated soil moisture content were positively associated with seedling establishment, whereas high maximum temperatures were negatively associated with the establishment of seedlings. In line with my results, previous studies have shown the importance of cool and moist conditions for seedling establishment in alpine and arctic habitats (Tingstad *et al.* 2015; Kueppers *et al.* 2016; Andrus *et al.* 2018). Extreme temperature events, such as high maximum summer temperatures can cause severe water deficiency in early establishing seedlings and lead to desiccation (Brodersen, Germinot & Smith 2006; Andrus *et al.* 2018). Further, my results confirm the hypothesis that young seedlings are highly sensitive towards abiotic environmental factors, whereas biotic environmental factors may only play a minor role (Packer & Clay 2000; van der Heijden & Horton 2009; Hersh, Vilgalys & Clark 2012).

In contrast to seedling establishment, the survival of seedlings was severely affected by biotic environmental factors. I found lower survival rates of seedlings at sites with high abundances of the pathogenic snow blight fungus *G. infestans*. Drawing on knowledge of the life cycle of *G. infestans*, the snow blight fungus potentially infected the needles of the successfully established seedlings through spores released from ripe apothecia (i.e. fruiting bodies) of *G. infestans*-infected needles in late autumn and possibly killing the seedlings in the following winter under snow cover (Roll-Hansen 1989; Burdon *et al.* 1992). Further, the survival of the pine seedlings increased with increasing distance from parent trees and high light availability. Negative and positive distance dependency can be summarized under the Janzen-Connell framework, where an increase in survival rates could be explained due to a lack of specialised herbivores or pathogens at larger distances to parent trees, whereas a decrease with larger distances could be explained by missing mutualistic interaction with specialised plant-associated fungi (i.e. "inverse" Janzen-Connell effect; Connell 1971; Janzen 1971; Bennett *et al.* 2017; Teste *et al.* 2017; Merges *et al.* 2018).

Using the null model approach, I identified previously unknown fungi which might be associated with seedling establishment and survival of Swiss stone pine. For seedling

establishment, it became apparent that, in accordance with the generalized linear mixed model approach, seedling establishment was less affected by fungi. In comparison with the results of the null model test on seedling survival, the number of fungal OTUs potentially associated with seedling establishment was eight to 43 times lower. It is hypothesized that the sensitivity of early life stages towards biotic interactions varies, since potential positive effects of mutualists, such as mycorrhizal fungi, increase rapidly with seedlings growths, when a seedling becomes independent from its maternal resources provided by a seeds endosperm (Bardgett *et al.* 2005; Smith & Read 2008). Further, using the null model approach, I could identify ECM OTUs present in my database, as well as additional ECM fungi not been reported to be associated with Swiss stone pine before. This successful identification of ECM fungi suggests that the null model approach offers a valid tool for the identification of plant-fungal associations. Additionally, most fungi associated with seedling establishment and seedling survival could not be found in the UNITE database (Koljalg *et al.* 2005) or the fungal community dataset FUNGuild (Nguyen *et al.* 2016). However, my approach makes it possible to link these taxonomically and functionally unassigned fungi to seedling establishment and seedling survival. Further, I could reduce the complexity commonly present in DNA metabarcoding datasets, by decreasing the number of potential plant-associated fungi and creating a shortlist of candidate OTUs which could be important drivers of plant recruitment. These OTUs could be potentially located on my field sites and used for more controlled studies on plant performance in the lab.

5. Conclusion and synthesis

In my dissertation, I studied how plant-animal and plant-fungal interactions depend on environmental conditions and jointly determine the early stages of plant regeneration. I explored these cross-kingdom interactions within a relevant range of environmental conditions at the tree line in the Central Eastern Alps, where potential effects of climate change on tree lines are projected to be severe. I combined multiple approaches from different disciplines, for instance by integrating microbial ecological methods (DNA metabarcoding) with experimental plant ecology (seed translocation experiments), and used generalized linear mixed models, multivariate statistics as well as sensitivity analyses, to disentangle the different drivers ultimately explaining the regeneration and distributional ranges of a plant species. I made three main discoveries: Firstly, the relative importance of demographic processes linking recruitment changes along the elevational range of a plant species.

Secondly, the occurrence patterns of plant-associated fungi depend on the degree of host specialisation, with fungal occurrences potentially exceeding the host plants current elevational range. Finally, seedling establishment is mostly determined by abiotic environmental factors, whereas seedling survival is limited by a fungal pathogen. Overall, my dissertation contributes to a deeper knowledge of key species interactions, such as mutualistic plant-animal and plant-fungal interactions (i.e. seed dispersal and mycorrhiza) as well as antagonistic plant-animal and plant-fungal interactions (i.e. seed predation and fungal pathogens) across environmental gradients.

Understanding range dynamics of plants is a great challenge, since recruitment of plants depends on the successful passage of multiple demographic processes that are highly sensitive towards environmental factors (Parmesan & Hanley 2015). Many demographic processes of plant recruitment depend on species interactions (e.g., animal-mediated seed dispersal, seed predation), where interaction outcomes are highly context-dependent (Agrawal *et al.* 2007; Schupp *et al.* 2010). I here offer an approach to disentangle the multi-stage nature of plant recruitment and predict recruitment limitations across changing environments, potentially applicable to a wide range of habitats. My results reveal that it is particularly important that animal-mediated seed dispersal is protected and maintained to allow plants to respond to environmental change. The dispersal of seeds by animals is regarded as a critical ecosystem function that determines plant range dynamics (Traveset *et al.* 2000). However, ecological knowledge for developing conservation and management guidelines for this ecosystem function is still lacking (Traveset *et al.* 2000; García, Obeso & Martínez 2005; Gosper, Stansbury & Vivian-Smith 2005). With this approach I contribute valuable information on how species specific plant-animal interactions may limit plant recruitment under differing environmental conditions.

Plant-fungal associations are important for plant regeneration through species-specific mutualistic or antagonistic interactions (Barrett *et al.* 2009; Bever *et al.* 2015), but the factors controlling plant-associated fungal occurrence and abundance remain largely unknown (Smith & Read 2008; Tedersoo *et al.* 2014). Plant species have been reported to shift their altitudinal and latitudinal distributions upwards and northwards (Parmesan & Hanley 2015), however there is little research if microbial mutualist and antagonists act accordingly. My results show that a potential shift to higher elevations of a plant species under a warming climate might be inhibited by the presence of antagonistic fungi, but will not be limited by the absence of mutualistic fungal partners. My study is a step forward in demonstrating that, across

elevational gradients, known plant-fungal interactions might be possible in newly suitable habitats when plant species will move their distributional ranges upwards, since the occurrences of fungal interaction partners already exceeds the current range of their host plant. However, further research is needed to prove if the strength and even the nature (i.e. mutualistic or antagonistic) of interactions will stay the same. Thereby, I contribute to the understanding of plant-fungal interactions, which is key to project potential consequences for response of plant populations to global climatic change.

Understanding plant-fungal interactions is key to understanding plant community dynamics and responses to environmental change (Klironomos 2002; Wardle *et al.* 2004; Bever *et al.* 2010; Mangan *et al.* 2010). I confirm previous studies that showed the importance of fungal pathogens for plant recruitment (Packer & Clay 2000; Bell, Freckleton & Lewis 2006), in addition I propose a new method how to integrate and identify previously unknown fungal key players in plant regeneration. My results highlight the high amount of unknown fungi potentially involved in plant recruitment and outlines future research directions of the field of above- and belowground interactions research. I linked a DNA metabarcoding dataset to plant recruitment, providing a method to disentangle plant-fungal associations at previously unattainable resolution in the field. I suggest this approach could be applied to a wide range of other systems (e.g. agroecosystems to identify diseases) and other little understood taxa affecting plant health (e.g. oomycetes or bacteria).

In the last decades there has been an increasing interest in addressing the relevance of abiotic and biotic environmental factors shaping species interactions and how these feed back into ecosystem processes. So far the majority of studies have focused on understanding the mechanisms driving species interactions on either above- (e.g. pollination, seed dispersal) or belowground interactions (soil pathogens, AM/ECM research, rhizobia). To conclude, my results reveal that above- and belowground interactions together have the potential to affect plant regeneration and thereby plants responses to environmental change. Given the rapidly changing environment driven by multiple global change drivers (e.g. land use intensity or climate warming), the implementation of studies like this one that consider multiple ecosystem functions mediated by species interactions, may serve to inform global models for projections of ecosystem dynamics. Further, monitoring programs could be informed and specifically the development measure for conservation and environmental management of threatened ecosystems, such as tree line habitats in alpine and boreal landscapes.

6. Zusammenfassung

Antagonistische und mutualistische Wechselbeziehungen zwischen Arten, wie z.B. die Samenprädation oder -ausbreitung durch Tiere, wirken sich auf die Populationsdynamik und die Verbreitung von Pflanzen aus. Viele dieser Wechselbeziehungen beeinflussen den pflanzlichen Lebenszyklus, da diese den erfolgreichen Übergang zwischen unterschiedlichen Stadien, wie zum Beispiel den Übergang vom Same zum Sämling, einschränken oder ermöglichen können. Damit sind antagonistische und mutualistische Wechselbeziehungen essentiell für die Rekrutierung von Pflanzen. Da diese Wechselbeziehungen über das gesamte geografische Verbreitungsgebiet einer Pflanzenart auftreten, unterliegen diese Wechselbeziehungen unterschiedlichen Umweltfaktoren, z.B. entlang von Temperaturgradienten oder variierender Wasser- und Lichtverfügbarkeit. Allerdings ist über den Einfluss dieser Wechselbeziehungen auf die Rekrutierung von Pflanzen in Abhängigkeit von Umweltfaktoren wenig bekannt. Besonders im Hinblick auf den globalen Klimawandel ist ein Verständnis der Wechselbeziehungen zwischen Arten und deren Anhängigkeit von Umweltfaktoren wichtig, um die Dynamik von Pflanzenpopulationen sowie deren zukünftige Verbreitung vorherzusagen.

Meine Dissertation hatte das Ziel zu erforschen wie Wechselbeziehungen zwischen Arten von Umweltfaktoren abhängen und gemeinsam die Rekrutierung von Pflanzen entlang deren Höhenverbreitung beeinflussen. Die Arbeit umfasst drei Kapitel, in denen ich die Auswirkungen von Samenausbreitung durch Tiere, von Samenprädation und dem Vorkommen von Mykorrhiza- und pathogenen Pilzen, sowie die Auswirkungen von abiotischen und biotischen Umweltfaktoren auf die Rekrutierung der Zirbelkiefer (*Pinus cembra*) untersuchte. Die Zirbelkiefer ist eine über den gesamten Alpenraum verbreitete Schlüsselart, welche dort die Waldgrenze bildet und damit wichtige Funktionen übernimmt, wie z. B. den Schutz der Berghänge vor Bodenerosion oder vor Lawinen. Die Samenausbreitung der Baumart ist abhängig von dem Tannenhäher (*Nucifraga caryocatactes*), einer Vogelart aus der Familie der Corvidae. Dieser Vogel erntet die reifen Samen der Zirbelkiefer aus deren geschlossenen Zapfen, welche an die Samenausbreitung durch den Tannenhäher angepasst ist. Der Tannenhäher legt Samenverstecke an, die ihm über das gesamte Jahr als Hauptnahrungsquelle dienen. Mit der Keimung von Samen aus den Verstecken, die nicht vom Tannenhäher wiedergefunden werden, beginnt die Rekrutierung der Zirbelkiefer. Die Rekrutierung hängt von unterschiedlichen Umweltfaktoren, wie zum Beispiel der Temperatur und Bodenfeuchte sowie von Mykorrhiza ab, sie kann jedoch auch

durch pathogene Pilze beeinträchtigt werden. Die Wechselbeziehungen der Zirbelkiefer mit dem Tannenhäher sowie mit einer Vielzahl von mutualistischen und pathogenen Pilzen, sind entlang der Höhenverbreitung der Kiefer unterschiedlichen Umweltfaktoren (z. B. Temperatur- und Lichtverfügbarkeitsgradienten) ausgesetzt. Dieses System eignete sich daher aufgrund der engen Verbindungen zwischen Pflanzen, Tieren und Pilzen sowie der starken Veränderung von Umweltbedingungen auf kleinem Raum insbesondere dazu, Wechselbeziehungen zwischen Arten in Abhängigkeit von Umweltfakoren zu untersuchen. Daher führte ich meine Feldforschungen an diesem System in zwei Tälern in den Schweizer Alpen durch, in denen ich die gesamte Höhenverbreitung der Zirbelkiefer (1850 – 2200 m ü.M.) und darüber hinaus (>2200 – 2250 m ü.M.) erfassen konnte. Über einen Zeitraum von drei Jahren habe ich dort die Samenausbreitung der Zirbelkiefer durch den Tannenhäher beobachtet, indem ich zu den Hauptversteckzeiten des Tannenhähers bei Zapfenreife der Zirbelkiefer (zwischen August und September), systematisch nach Samenverstecken gegraben habe. Weiterhin habe ich Keimungsexperimente innerhalb des gleichen Zeitraumes durchgeführt, um die Prädation und Keimung von Zirbelkiefersamen sowie das spätere Überleben von erfolgreich etablierten Sämlingen zu erfassen. Dazu habe ich nach einem zufällig-stratifizierten Design Zirbelkiefersamen in beiden Tälern entlang der gesamten Höhenverbreitung und darüber hinaus gepflanzt. Außerdem untersuchte ich das Vorkommen von Pilzen über die gesamte Höhenverbreitung der Zirbelkiefer, indem ich an den Keimungsexperimenten Bodenproben sammelte und diese mithilfe eines DNA-Metabarcoding-Ansatzes analysierte. Weiterhin habe ich abiotische Umweltfaktoren, wie Temperatur, Wasser- und Lichtverfügbarkeit, pH-Wert sowie biotische Umweltfaktoren, wie z.B. den räumlichen Abstand zu adulten Zirbelkiefern zu den jeweiligen Samenverstecken und Keimungsexperimenten, sowie die Bedeckung durch Bodenvegetation gemessen. In meiner Dissertation habe ich damit ein breites Spektrum von Ansätzen verwendet, das sich über die Disziplinen der Gemeinschaftsökologie, über die experimentelle Pflanzenökologie bis hin zur mikrobiellen Ökologie erstreckt, um den Effekt von Wechselbeziehung zwischen Pflanzen und Tieren sowie zwischen Pflanzen und Pilzen in Abhängigkeit von Umweltfaktoren zu untersuchen.

Das erste Ziel meiner Thesis war es, die Auswirkung von Umweltfaktoren auf vier für die Rekrutierung der Zirbelkiefer wichtige demographischen Prozesse (Samenausbreitung, Samenprädation, Samenkeimung, das Überleben von Sämlingen) zu untersuchen. Insbesondere hatte ich zum Ziel die wichtigsten demographischen Prozesse zu identifizieren, welche die Rekrutierung der Kiefer in Abhängigkeit ihrer jeweiligen Höhenverbreitung

einschränken könnten. Dies ist besonders wichtig, da unter derzeit prognostizierten Klimaveränderungen erwartet wird, dass Pflanzen ihre Verbreitung entlang von Höhengradienten nach oben verlagern müssen, um für sie günstige klimatische Standorte zu besiedeln. Um den relativen Einfluss von demographischen Prozessen auf die Rekrutierung der Zirbelkiefer entlang ihrer Höhenverbreitung zu verstehen, untersuchte ich zunächst, wie die Samenausbreitung durch den Tannenhäher, die Samenprädation, die Samenkeimung und das Überleben der Sämlinge durch die vorherrschenden Umweltfaktoren beeinflusst wurden. Ich identifizierte dazu die zwei wichtigsten Umweltfaktoren, die Kronenbedeckung und die Bedeckung durch Bodenvegetation. Weiterhin analysierte ich die relative Zusammensetzung von Kronenbedeckung und der Bodenvegetation über die Höhenverbreitung der Zirbelkiefer. Anhand der Veränderung beider Umweltfaktoren über die Höhenverbreitung der Zirbelkiefer führte ich eine Sensitivitätsanalyse durch. Damit untersuchte ich, welcher der vier demographischen Prozesse die Rekrutierung der Kiefer am unteren und oberen Rand des Verbreitungsgebiets sowie im Zentrum des Verbreitungsgebiets in Abhängigkeit der Umweltfaktoren am stärksten einschränkte. Meine Ergebnisse zeigen, dass Kronenbedeckung und Bodenvegetation maßgeblich beeinflussen, wo ein Same vom Tannenhäher im Umweltraum abgelegt wird, und zwar in Mikrohabitaten mit hoher Kronenbedeckung und geringer Bedeckung durch Bodenvegetation. Ich fand heraus, dass die Samenkeimung und das Überleben der Sämlinge im unteren Bereich und im Zentrum des Verbreitungsgebietes die wichtigsten begrenzenden Prozesse waren, wohingegen die Samenverbreitung durch den Tannenhäher im oberen Bereich der Höhenverbreitung besonders relevant war. Die Samenausbreitung durch den Tannenhäher in den oberen Höhenlagen ist aufgrund fehlender Kronenbedeckung, sowie der hohen Bedeckung durch Bodenvegetation sehr unwahrscheinlich. Änderungen dieser vom Tannenhäher bevorzugten Umweltfaktoren können sich daher sehr stark auf die Versteckwahrscheinlichkeit und somit auf die Rekrutierung der Zirbelkiefer auswirken. Meine Studie liefert einen neuen Beitrag zum Forschungsgebiet, da ich zeigen konnte, wie die relative Bedeutung von unterschiedlichen demographischen Prozessen über die Höhenverbreitung einer Pflanze variiert. Dadurch trägt meine Arbeit dazu bei, die möglichen zukünftigen Szenarien der Verbreitung von Pflanzen unter dem Einfluss sich verändernder Umweltbedingungen projizieren zu können.

Nachdem ich mich in meinem ersten Kapitel mit den Auswirkungen der tierischen Samenausbreitung und den Kaskadeneffekten der Samenausbreitung durch den Tannenhäher auf die Rekrutierung der Zirbelkiefer beschäftigt habe, war das zweite Ziel meiner Studie die Verbreitung von mit der Zirbelkiefer assoziierten pathogenen und mutualistischen Pilzen zu

erforschen. Hierfür untersuchte ich, wie abiotische und biotische Umweltfaktoren die Verbreitung dieser pathogenen und mutualistischen Pilze beeinflussen. Entlang der Höhenverbreitung der Zirbelkiefer sammelte ich Bodenproben und nahm Messungen von abiotischen (Temperatur, Wasser- und Lichtverfügbarkeit, pH-Wert) sowie von biotischen Umweltfaktoren (Abstand zu adulten Zirbelkiefern, Bedeckung durch Bodenvegetation) vor. Aus den Bodenproben isolierte ich „environmental DNA“ (direkt aus dem Boden verfügbare, freie Desoxyribonukleinsäure [DNA]) und sequenzierte diese mit einem Hochdurchsatz-Verfahren. Mit diesem DNA-Metabarcoding-Ansatz generierte ich eine Liste von pilzlichen taxonomischen Einheiten („operational taxonomic units“, OTUs), aus der ich wichtige Pilzinteraktionspartner der Zirbelkiefer identifizierte. Anschließend analysierte ich welche Faktoren deren Vorkommen bestimmen. Dabei ging ich insbesondere auch auf unterschiedliche Wirtsspezifitäten ein, da spezialisierte Pilze, im Gegensatz zu generalistischen Pilzen, stärkere Effekte auf ihre Wirte haben können. Meine Ergebnisse zeigen, dass generalistische Pilze, d.h. Pilze die nicht auf die Zirbelkiefer als Wirt angewiesen sind, nicht von abiotischen und biotischen Umweltfaktoren beeinflusst wurden. Jedoch zeigten pathogene Pilze, die auf die Zirbelkiefer als Wirtspflanze spezialisiert sind, Muster gemäß der Janzen-Connell-Hypothese (d. h. erhöhte Abundanz von Pathogenen in der Nähe von adulten Pflanzen). Interessanterweise fand ich Hinweise auf einen „inversen“ Janzen-Connell-Effekt, d. h. ein erhöhtes Vorkommen von Mutualisten in der Nähe von adulten Zirbelkiefern. Diese erhöhte Abundanz von Mutualisten könnte möglicherweise die negativen Auswirkungen von Bodenpathogenen auf Sämlinge in der Nähe von adulten Zirbelkiefern mildern. Außerdem stellte ich fest, dass mit Zirbelkiefern assoziierte Pilze über dem derzeitigen Verbreitungsgebiet ihrer Wirtspflanze heraus vorkommen. Somit ergänzt meine Arbeit bestehendes Wissen darüber, wie mutualistische und antagonistische Wechselbeziehungen beeinflusst werden können, wenn Pflanzen ihr Verbreitungsgebiet bergaufwärts verlagern.

In meinem letzten Kapitel untersuchte ich, wie die mit der Zirbelkiefer assoziierten pathogenen und mutualistischen Pilze, die Etablierung sowie das Überleben von Zirkelkiefersämlingen in Abhängigkeit vom abiotischen und biotischen Umweltfaktoren beeinflussen. Weiterhin nutzte ich eine Nullmodel-Methode um herauszufinden ob Pilze, welche nicht mit Hilfe gängiger Referenzdatenbanken identifiziert werden konnten, ebenfalls mit der Etablierung sowie dem Überleben von Zirbelkiefernsämlingen zusammenhängen. Dafür nutzte ich die Daten aus den Keimungsexperimenten und kombinierte diese mit den Daten des DNA-Metabarcoding-Ansatzes. Meine Ergebnisse deuten darauf hin, dass die

Etablierung von Sämlingen am stärksten von abiotischen Umweltfaktoren wie Temperatur und Lichtverfügbarkeit beeinflusst wurden. Ferner zeigten die Ergebnisse, dass das Überleben von Sämlingen hauptsächlich durch biotische Umweltfaktoren bestimmt wurde. Insbesondere fand ich heraus, dass besonders niedrige Überlebensraten von Sämlingen dort zu beobachten waren, wo besonders hohe Abundanzen von pathogenen Pilzen nachgewiesen wurden. Basierend auf der Nullmodel-Methode zeigen meine Ergebnisse auch, dass bekannte Mykorrhizapartner der Zirbelkiefer, sowie eine große Anzahl unbekannter Pilz-OTUs mit dem Überleben von Sämlingen assoziiert waren. Dies deutet darauf hin, dass deutlich mehr Pilze mit der Zirbelkiefer assoziiert sind, als zuvor bekannt war. Daher unterstreichen meine Ergebnisse die Bedeutung von Pflanze-Pilz-Wechselbeziehungen für die Rekrutierung von Pflanzen und bieten einen praktikablen Ansatz für die Identifizierung zuvor unbekannter Pflanze-Pilz-Assoziationen mithilfe von Keimungsexperimenten und DNA-Metabarcoding. Dieser Ansatz bietet eine wertvolle Methode zur Untersuchung der Wechselbeziehungen zwischen Pflanzen und Pilzen und hilft letztendlich dabei, die Populationsdynamik und die Verbreitung von Pflanzen besser zu verstehen.

Meine Dissertation trägt zu einem tieferen Verständnis der Wechselbeziehungen zwischen Arten und deren Bedeutungen für den Lebenszyklus von Pflanzen bei. Insbesondere konnte ich zeigen, wie Pflanze-Tier- und Pflanze-Pilz-Wechselbeziehungen gemeinsam mit Umweltfaktoren die Rekrutierung von Pflanzen beeinflussen. Meine Studie verdeutlicht, dass die Samenausbreitung durch Tiere sowie die Verbreitung von Pilzen entlang von Gradienten bestimmter Umweltfaktoren einen bedeutenden Einfluss auf die Verbreitung von Pflanzen haben und damit diese Wechselbeziehungen zwischen Pflanze und Tier, sowie Pflanze und Pilz direkte Konsequenzen für potenzielle Verbreitungsverschiebungen von Pflanzenarten haben könnten. Meine Thesis kann daher einen wichtigen Beitrag zur Erhaltung und zur Entwicklung von Schutzbemühungen für die Zirbelkiefer leisten, indem sie über wichtige Wechselbeziehungen unter sich verändernden Umweltbedingungen informiert.

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Appendices

Appendix 1: Environmental context determines the limiting demographic processes for plant recruitment across a species' elevational range

Appendix 2: Spatial patterns of pathogenic and mutualistic fungi across the elevational range of a host plant

Appendix 3: High throughput sequencing combined with null model tests reveals specific plant-fungi associations linked to seedling establishment and survival

Appendix 4: Curriculum Vitae

Appendix 1. Environmental context determines the limiting demographic processes for plant recruitment across a species' elevational range

Authors:

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Title:

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Author Contributions:

1. Development and planning:
   DM 80%, JA, KBG, MS and ELN in total 20%
2. Field work/data collection:
   DM collected data on seed deposition (50%) with help of ELN (50%). **DM** conducted seed transplant experiment (50%) with help of ELN (50%).
3. Compilation of data sets and figures/tables:
   DM assembled the data sets and prepared the figures (100%).
4. Data analyses and interpretation:
   DM performed the statistical analyses (90%) with the supervision from JA, MS and ELN (in total 10%). **DM** interpreted results (80%), JA, MS, ELN contributed with the interpretation of the results (in total 20%).
5. Preparation of manuscript:
   DM 80%; MS and KBG in total 5%, JA and ELN in total 15%

Environmental context determines the limiting demographic processes for plant recruitment across a species' elevational range

Keywords: Elevational gradient, *Nucifraga caryocatactes*, plant regeneration, *Pinus cembra*, distributional range margin, seed dispersal, seed predation, seedling establishment, sensitivity analysis, transplant/translocation experiment

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Plant recruitment is a multi-stage process determining population dynamics and species distributions[1–3]. So far, we have only limited understanding of how the different recruitment processes linking plant life history stages depend on environmental context[4,5]. We conducted a large-scale transplant experiment to study four recruitment processes (i.e., seed deposition, predation, germination and seedling survival) of Swiss stone pine (*Pinus cembra*) over a period of six years. We recorded seed deposition by its single seed disperser (*Nucifraga caryocatactes*)[6], and transplanted seeds across and beyond the pines' elevational range to monitor seed predation, seed germination and seedling survival. We quantified the effect of environmental conditions on each demographic process and performed a sensitivity analysis to identify the most limiting demographic processes in recruitment across and beyond the species' current elevational range. We found that the importance of particular recruitment processes changed across the distributional range. Seed germination and seedling survival were the main limiting factors at the lower range margin and the distribution centre, whereas seed deposition was the most sensitive process of pine recruitment at the upper range margin. Our results imply that even slight changes in seed deposition, for instance due to a decline of animal seed dispersers, would result in lower pine recruitment at the upper range margin. Our long-term experiment shows the critical role of animal mutualisms in determining the distributional range of plant species, and demonstrates that environmental context determines the local relevance of particular plant recruitment processes. We conclude that conducting experimental studies to identify limiting demographic processes controlling plant species distributions is key for projecting future range dynamics of plants.

Plant recruitment is a complex process where several plant life stages (e.g., seeds, seedlings, saplings, adults) are connected by demographic processes, such as seed dispersal, predation, germination, or survival[1–3]. The overall recruitment probability of a plant is thus given by the product of successive process-specific transition probabilities[1]. Consequently, a failure in one of the demographic processes could limit the entire recruitment process[1,3,7]. The processes linking plant life stages are affected by the environmental conditions at the sites where recruitment takes place, and can thereby be highly dependent on the environmental context[4,5]. Further, many demographic processes depend on biotic interactions, such as the dispersal or predation of seeds by animals, or interactions with fungal pathogens or mutualists[8,9], which, in turn, are influenced by the environmental context.

Across their distributional ranges, plants are exposed to a variety of environmental conditions[10], which could differentially affect the demographic processes involved in recruitment (Figure 1)[11,12]. This context-dependency of plant recruitment is especially important at species' range margins, where the limitation of a single demographic process may limit a species' range expansion[11]. Due to the complex interactions between demographic processes, biotic interactions and abiotic conditions, we so far have a very limited understanding of how environmental context shapes plant recruitment across species distributional ranges[12]. Identifying the limitations and bottlenecks in plant recruitment, is, however, key for projecting future range dynamics of plants[13,14].

In this study, we investigate how environmental conditions shape the demographic processes that determine the recruitment of Swiss stone pine (*Pinus cembra*; Fig. 1), a keystone species forming and stabilizing the treeline in parts of the Alps[6]. To this end, we assessed the relative importance of four demographic processes (i.e., seed deposition, seed predation, seed germination and seedling survival) for pine recruitment in relation to the environmental context across its elevational range (Fig. 1). To measure the relative sensitivity

of plant recruitment toward slight changes in the transition probability of each demographic process, we conducted a context-specific sensitivity analysis at three range positions (lower and upper range margin and centre of the elevational range), accounting for the environmental variability in terms of canopy and ground vegetation cover across the species range. We expected seed deposition to depend on canopy cover and to be a limiting process where canopy cover is absent[15]. Further, we expected high predator densities and associated high seed predation rates under high canopy cover and high ground vegetation cover[4]. Finally, we expected reduced seed germination and survival in dry and shaded conditions under canopy cover[2].

Consistent with our expectations, we found that each demographic process was differently affected along the gradients of canopy and ground vegetation cover (Fig. 2 a-d; Table 1). This resulted in the highest overall recruitment at microhabitats with no canopy or ground vegetation cover (i.e., 0.02 % per sown seed over 2 growing seasons; Fig. 2e), where seed germination and seedling survival were estimated to be highest (Fig. 2c,d; Table 1). Overall recruitment was reduced by about 50 % to 0.01 % at sites with high canopy cover and low ground vegetation cover, where low rates of seed germination and seedling survival counteracted the positive effects of high seed deposition rates (Fig. 2a,c,d; Table 1). Overall recruitment was estimated to be lowest (~0.002 %) at sites without canopy and high ground vegetation cover, where the combination of low seed deposition and low survival rates, as well as high seed predation rates, diminished the positive effects of high seed germination rates (Fig. 2a-e; Table 1).

We found substantial environmental variation across the species elevational range (Fig. 3a). The majority of microhabitats at the lower range margin (1850 – 1950 m a.s.l.) and at the centre of the elevational distribution (2000 – 2100 m a.s.l.) of Swiss stone pine were characterized by high canopy cover and low ground vegetation cover (Fig. 3a). In contrast, at

the upper range margin (2150 – 2250 m a.s.l.) encompassing the treeline, microhabitats were predominantly characterized by the lack of canopy cover and high ground vegetation cover (Fig. 3a). Environmental variation across the three range positions resulted in pronounced differences in the sensitivity of the recruitment process of Swiss stone pine to changes in seed deposition, seed predation, seed germination and seedling survival (Fig. 3b). At the lower range margin and at the range centre, plant recruitment was most sensitive to changes in the probability of seed germination and first-year seedling survival (Fig. 3b). Plant recruitment was much less sensitive to changes in seed deposition and predation in this environmental context (Fig. 3b). In contrast, at the upper range margin, plant recruitment was most sensitive to changes in seed deposition, but much less sensitive to changes in seed predation, seed germination and seedling survival.

We found that the relative importance of particular demographic processes changed across the species' elevational range, due to variability in the environmental context. As a result, different demographic processes have the potential to limit pine recruitment depending on the range position[11]. Our result suggests that demographic processes related to seed germination and seedling survival were crucial for recruitment in the centre of a plant's distribution, whereas seed deposition was the main limiting process at the upper range boundary. Swiss stone pine depends on a single seed-dispersing bird, the Spotted nutcracker, which extracts pine seeds from closed cones to deposit them in caches beneath the soil surface for later consumption[15]. The bird's selection of caching sites is driven mainly by two microhabitat characteristic (i.e., canopy and ground vegetation cover)[15,16]. Beyond the treeline, the preferred caching microhabitats of nutcrackers are absent (i.e., high canopy cover, low ground vegetation cover) and the probability of seed deposition is low[16]. Thereby, the distinct environmental conditions at the upper elevational range boundary cause a dispersal limitation for Swiss stone pine. Under these conditions, already little changes in seed deposition have a pronounced effect on overall recruitment (Fig. 3b). Dispersal limitation by

animal-mediated demographic processes in peripheral populations have been proposed to be critical bottlenecks when plants are forced to respond to rapid environmental changes[17,18]. Indeed animal-mediated seed dispersal may be highly sensitive to environmental changes, since this plant-animal mutualism depends on a spatial overlap of favorable environmental conditions for both interacting species[19,20]. Moreover, the strength and even the mutualistic nature of the interaction between plants and their animal associates can be context dependent[21–23]. For example, if environmental conditions are altered, this may affect animal seed dispersal through alteration of seed quantity and quality[23] as well as through context-specific changes in animal behaviour[24]. The context-dependency of animal-mediated demographic processes can have severe impacts on future range dynamics of plants in most terrestrial ecosystems, for instance ~ 42 % of woody species in temperate coniferous forest and up to 90 % in tropical rainforest are adapted to animal seed dispersal[25]. Our findings enforce the notion that animals play a pivotal role in driving plant range dynamics and determine a plant's capacity to effectively colonize new habitats, especially in the light of rapid climate and land-use change.

At the lower elevational range margin and the centre of Swiss stone pine distribution, recruitment was most sensitive to changes in seed germination and seedling establishment. Seed germination and seedling survival were reduced by high canopy cover. Canopy cover causes dark and dry environmental conditions and thereby hinders seed germination and seedling survival (Supplementary Table 1)[26]. Further, sites with high pine canopy cover are associated with high accumulation of specialist fungal pathogens of the pine, potentially affecting seed germination and survival[27]. In addition, ground vegetation cover reduced overall recruitment, which could be related to the occurrence of unsuitable fungal communities for pine recruitment, associated with ericoid vegetation[27]. Lack of recruitment as a result of poor seed germination and seedling survival at the lower range margin and at the range centre might put Swiss stone pine at a competitive disadvantage against upward

migrating heterospecific plants. Therefore, competitive interactions could hamper the persistence of peripheral plant populations at the lower range margin and the persistence at core populations[28]. Importantly, the major demographic bottlenecks of such populations, and the targets for population management[29], would be distinct from those at the species' upper elevational range.

We conclude that the relative importance of demographic processes of plant recruitment vary substantially across the distributional range of a plant. Identifying limiting plant recruitment processes across a species range are especially relevant when keystone species are facing severe environmental changes. Hence, for understanding plant range dynamics under climate change, it is crucial to evaluate the relative importance of different demographic processes across a species range[12,30]. Our long-term experiment shows how the relevance of demographic processes can be assessed for a range of plant life forms and taxa. In our study case, we reveal that animal-mediated demographic processes play a crucial role in shaping plant recruitment at the range margin and are likely to be pivotal for future range expansion of keystone species such as the Swiss stone pine.

Methods

Study area and design

We conducted this study within the geographical distribution centre of Swiss stone pine in the Central Alps. We chose two elevational gradients close to Davos, Switzerland, encompassing the whole elevational distribution of Swiss stone pine*;* one in the Sertig valley (46°44′0.76″N, 9°51′3.5″E) and one in the Flüela valley (46°48′0.25″N, 09°54′15.38″E). The forest structure at the lowest elevational belts (about 1850 m a.s.l.) is a mixed coniferous forest, mainly composed of European larch (*Larix decidua*) and Norway spruce (*Picea abies*). The

abundance of Swiss stone pine is distributed unimodally from 1850 m a.s.l. up to 2150 m a.s.l., where pine trees (> 3m tall, Harsch *et al.*, 2009) form the upper tree line. Young pine trees can be found up to 2200 m a.s.l., but none are growing at and beyond 2250 m a.s.l. [16]. In each valley, we established nine elevational belts spaced by 50 m of altitude ranging from 1850 to 2250 m a.s.l. reaching across and beyond the elevational distribution of the pine.

Demographic processes

Seed deposition sites (i.e., seed caches deployed by Spotted nutcrackers) were recorded by randomly selecting a 2 x 10 metre plot within each elevation belt. Each 20 m^2 plot was composed of 20 1 m^2 subplots. In the centre of each subplot, we took a soil sample and thoroughly searched for deposited seeds. Intact seeds or seed shells handled by nutcrackers were recorded as cache presence and marked as a seed deposition site. We recorded seed deposition during the main seed-caching season in mid-August until beginning of September over six years (2012-2017), resulting in a total number of 2156 soil samples. In these soil samples, we found 256 (12 %) seed caches deposited by nutcrackers across the elevational gradient in both valleys and all years.

To determine seed predation, seed germination and seedling survival rates, we conducted a seed transplant experiment across the nine elevational belts. According to a random-stratified sampling design, we selected five microhabitat types at each elevational belt for the experiment (1. soil covered by ericaceous vegetation, 2. close to adult Swiss stone pine individuals [i.e., up to a distance of 1 m], 3. open soil, 4. rocky habitat, 5. microsite covered by snow [i.e., late snow lie areas]). For elevational belts above the tree line (at 2250 m a.s.l.), the microhabitat “close to adult Swiss stone pine” was replaced by matgrass (*Nardus stricta* L.) dominated sites, to guarantee an equal sample size in each elevational belt. At the beginning of the growing season (i.e., end of May), we placed mesh bags with Swiss stone pine seeds in two to six replicates per microhabitat at each elevational belt, resulting in a total

number of 1980 seed sowing replicates, including 6858 seeds monitored during the study (for detailed information on the number of replicates deployed per year see Supplementary Table 2). Each mesh bag contained five Swiss stone pine seeds in a 1.5 mm net, simulating the average number of seeds per cache deposited by nutcrackers [6]. From the total number of 1980 mesh bags, 540 mesh bags were protected by 1.5 mm wire-mesh to prevent loss of seeds. Mesh bags were buried about 4 cm deep in the soil and fixed with metal pins. To break dormancy of the seeds, seasonal variation was simulated in a wet clay-sand mixture by exposure to temperature shifts between 5-25°C for 22 weeks. At the end of the growing season (i.e., end of September), we evaluated whether seeds had been preyed upon or germinated. Further, we monitored the survival of seedlings until the end of the subsequent growing season in the following year. Out of 6858 seeds monitored in the six study years 3023 seeds (44 %) were preyed upon or removed by rodents and other animals and 451 (7 %) germinated within the first growing season (i.e., the period between May and September). Of 319 seedlings 65 (20 %) survived to the end of the following growing season (i.e., the period from September to September of the following year) in two years (i.e., 2014-2015, 2015-2016).

Environmental variables

Canopy cover was measured at each seed deposition subplot and seed sowing replicate with a spherical densitometer. On the same sites, ground vegetation cover was assessed by estimating the percentage cover of dominant ground flora species: *Juniperus communis* L., *Loiseleuria procumbens* (L.) Desv., *Vaccinium* spp. L. and *Rhododendron ferrugineum* L. within 1 m² (Braun-Blanquet, 1964). In total, we measured canopy cover and ground vegetation cover at 4136 1 m² plots, i.e., at 2156 seed deposition subplots and 1980 seed sowing replicates.

We characterised the frequency distribution of canopy and ground vegetation cover across three range positions. To do so, we used the data collected at the 2156 1 m^2 seed deposition subplots across the 9 elevational belts and categorized these into three range positions: 1) three lowest elevational belts (i.e., lower range margin, 1850-1950 m a.s.l.), 2) three central elevational belts (i.e., centre, 2000-2100 m a.s.l.) and 3) three high elevational belts (i.e., upper range margin, 2150-2250 m a.s.l.). The relative frequency of canopy and ground vegetation cover for each range position was determined, taking into account the interaction between the two measures. To this end, each 1 m^2 subplot was assigned to a cell of a 10 x 10 grid of values for canopy cover and ground vegetation cover ranging from 0 to 100 % in steps of 10 %. Each combination of canopy cover and ground vegetation cover was counted as the sum of subplots within the respective combination of values.

In addition, we tested how the microhabitat characteristics (canopy and ground vegetation cover and their interaction term) control microclimatic environmental conditions in terms of soil surface temperature and soil moisture. Soil surface temperature was recorded every four hours over the duration of the study using iButton data loggers (Maxim) at 1343 seed deposition and seed transplant sites across the elevational gradient and years. We calculated the mean of daily temperatures of the hottest three months of each year (i.e., June, July, August). Soil moisture was recorded on 4136 seed deposition and seed transplant sites across the elevational gradient and years. Soil moisture was recorded under dry weather conditions in September by averaging five tensiometer (Theta-Kit version 3) measurements at each seed deposition and seed transplant site. Using linear mixed models, we found soil surface temperature during the hottest three months and soil moisture to be negatively associated with high canopy and ground vegetation cover (Supplementary Table 1). Thereby, our results suggest that the microhabitat characteristics are the main cause for site by site variation in microclimatic conditions, such as variations in soil surface temperature and soil moisture. Hence, we focussed on environmental variation in canopy cover and ground

vegetation cover, as the most appropriate descriptors of the variation in microhabitat conditions across the species range.

Statistical analyses

We assessed the recruitment probability across and beyond the whole elevational range of Swiss stone pine. To do so, we fitted four models describing the determinants of seed deposition (i.e., the presence or absence of cached seeds in soil samples taken at each subplot), seed predation (i.e., absent seeds and seeds that showed signs of seed predation [e.g., bite marks] at seed translocation sites), seed germination (i.e., seed germination and seedling establishment within the first growing season), and seedling survival (i.e., survival from the end of the first growing season to the end of the second growing season). We used generalized linear mixed models with a binomial error distribution in the R package 'lme4' [31]. We included canopy cover, ground vegetation cover and their interaction as continuous explanatory variables into the models. In addition, we included plot ID, site and year as random factors to account for spatial and temporal non-independence. An observation-level random factor was included in the seed predation and seed germination models to account for overdispersion [31,32]. All predictors were mean centred and scaled to unit variance to allow for comparison of effect sizes across predictor variables and models. We used the models to predict the expected probability of seed deposition, predation, germination and first-year seedling survival across the gradients of canopy cover and ground vegetation cover (Fig. 2a-d). Then, we calculated the overall recruitment probability as the product of the expected transition probabilities (P) from the four individual models of each demographic process: $P_{\mathbf{Recruitment}} = P_{\mathbf{Deposition}} \times (1 - P_{\mathbf{Predation}}) \times P_{\mathbf{Germination}} \times P_{\mathbf{Survival}}$ (Fig. 2e).

We conducted a context-specific sensitivity analysis to evaluate which of the four demographic processes limits recruitment most at the three different range positions (i.e., lower range margin, centre, upper range margin). Sensitivity analysis allows the calculation of

sensitivity of overall recruitment to changes in the transition probabilities between the successive demographic processes (i.e., seed deposition, seed predation, seed germination, and seedling survival)[33,34], i.e., it measures how strongly changes in the specific transition probabilities affect overall recruitment [33–35]. For example, if a small change in seed deposition probability significantly affects the plant recruitment probability at a given range position, then seed deposition might be a target for conservation and managing efforts [36]. For this context-specific sensitivity analysis, we first used the realized frequency distribution of canopy and ground vegetation cover at each range position to calculate the weighted mean of the expected transition probability of each demographic process at the respective range position (Fig. 1). Then, we calculated for each range position the sensitivity of the recruitment probability s_i to small changes in the transition probability of each demographic process i as $s_i = \partial P_{\text{Recruitment}} / \partial P_{\text{Demographic}_i}$, where $\partial P_{\text{Recruitment}}$ is the change in recruitment probability following a change in the transition probability (here 0.1 %) of demographic process i ($\partial P_{\text{Demographic}_i}$)[33,37]. To allow for comparisons of the relative importance of each demographic process across the three range positions, we calculated the relative importance s'_i as $s'_i = s_i / \sum_{i=1}^{I} s_i$. To gain an estimate of uncertainty (confidence intervals) for the sensitivities, we performed a bootstrap analysis with 1000 replicates.

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Authors Contribution

D.M., K.B.G. and E.L.N. conceived and designed the project. D.M. and E.L.N. collected the data. D.M. performed the analyses with input from J.A., M.S. and E.L.N. D.M. and E.L.N. led the writing of the manuscript. All authors contributed to the various drafts and gave final approval for publication.

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Data accessibility

Data from this paper are deposited in the Dryad Digital Repository

Table 2: Summary of generalized linear mixed models testing for effects of canopy cover, ground vegetation cover and their interaction on four demographic processes (i.e., seed deposition, seed predation, seed establishment and seedling survival). Plot ID, site and year were included as random effects (RE) in all models. An observational level random effect was used in the seed predation and establishment models accounting for overdispersion. Given are standardized effect sizes and 95% confidence intervals (CI) as a measure of support. For REs standard deviations (SD) are shown. Estimates with CIs not passing zero boundaries are considered as significant and highlighted in bold. Sample sizes (*n*) for each model are given below the response variables.

Response	Variable	Estimate	2.5% CI	97.5% CI
Seed deposition	Canopy cover	**0.9**	**0.57**	**1.24**
n_{obs} = 2156,	Vegetation cover	**-0.31**	**-0.55**	**-0.08**
n_{year} = 6,	Canopy cover x vegetation cover	-0.14	-0.39	0.1
n_{site} = 18	RE year	1.38	0.94	2.14
	RE site	1.19	0.7	2.47
Seed predation	Canopy cover	0.31	-0.14	0.77
n_{obs} = 1381,	Vegetation cover	0.23	-0.09	0.55
n_{year} = 6,	Canopy cover x vegetation cover	0.22	-0.06	0.51
n_{site} = 18	RE year	3.35	3.05	3.69
	RE site	1.94	1.3	3
	RE observation	2.99	1.83	6.05
Seed germination	Canopy cover	**-0.21**	**-0.4**	**-0.02**
n_{obs} = 915,	Vegetation cover	0.04	-0.13	0.2
n_{year} = 6,	Canopy cover x vegetation cover	0.01	-0.14	0.16
n_{site} = 18	RE year	0.83	0.63	1.04
	RE site	0.23	0	0.48
	RE observation	1.69	1.02	3.45
Seedling survival	Canopy cover	-0.43	-0.98	0.07
n_{obs} = 319,	Vegetation cover	-0.26	-0.67	0.13
n_{year} = 2,	Canopy cover x vegetation cover	**0.4**	**0.02**	**0.79**
n_{site} = 18	RE year	0.97	0.58	1.64
	RE site	0.78	0.18	3.67

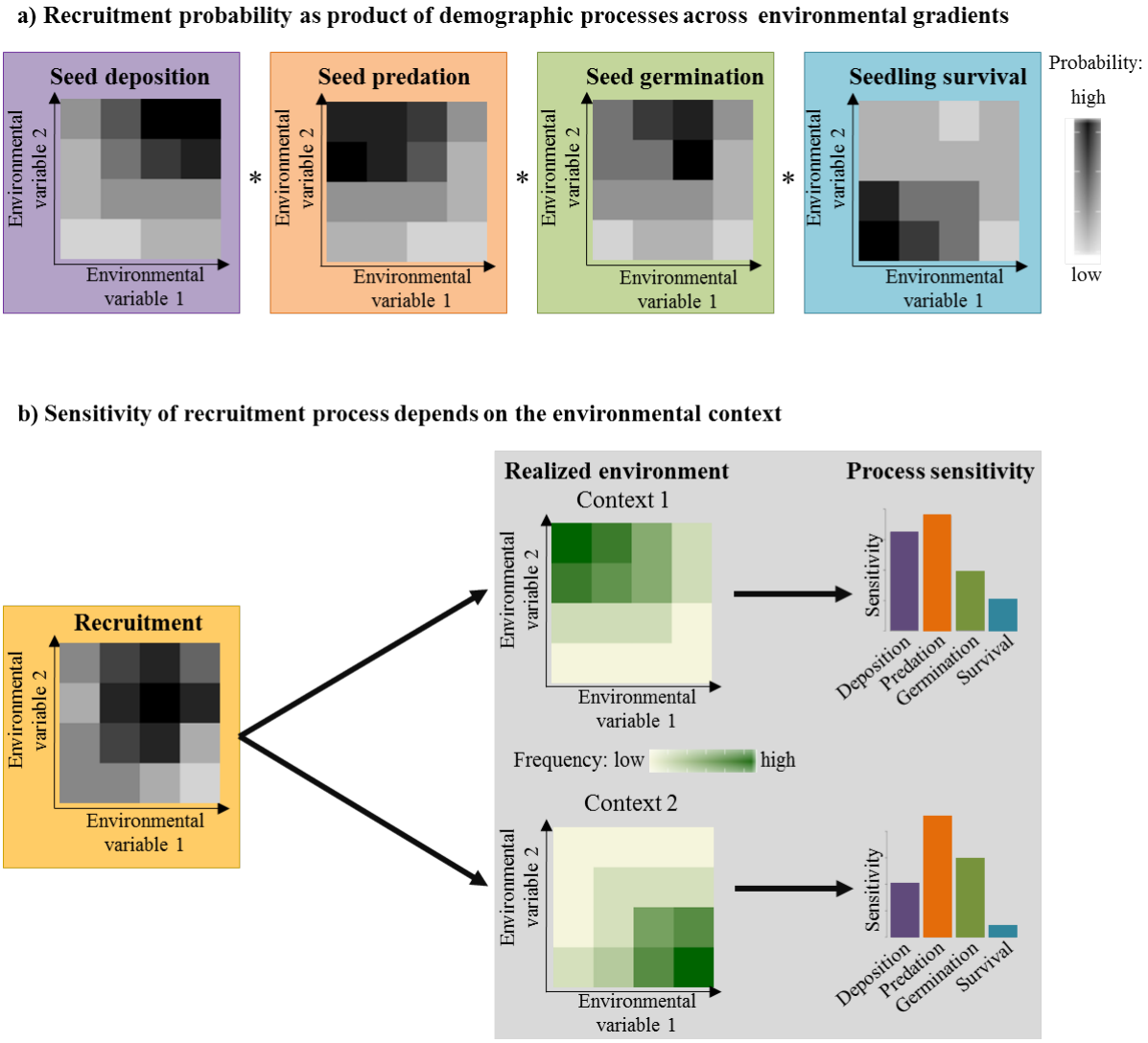

Figure 1: a) The multi-stage process of plant recruitment is the product of seed deposition, the proportion of seeds not predated (i.e., [1-seed predation]), seed germination and seedling survival. Each process varies independently as a function of the environmental context (i.e., environmental variable 1 & 2; grey colour shading). b) Recruitment of a species occurs under different environmental contexts, e.g., environmental conditions may vary between peripheral and central populations across a species range. Consequently, the sensitivity of recruitment to the individual demographic processes is likely to be context-dependent, e.g., compare the hypothetical differences in the frequency of the specific environmental condition between Context 1 & 2 (green colour shading).

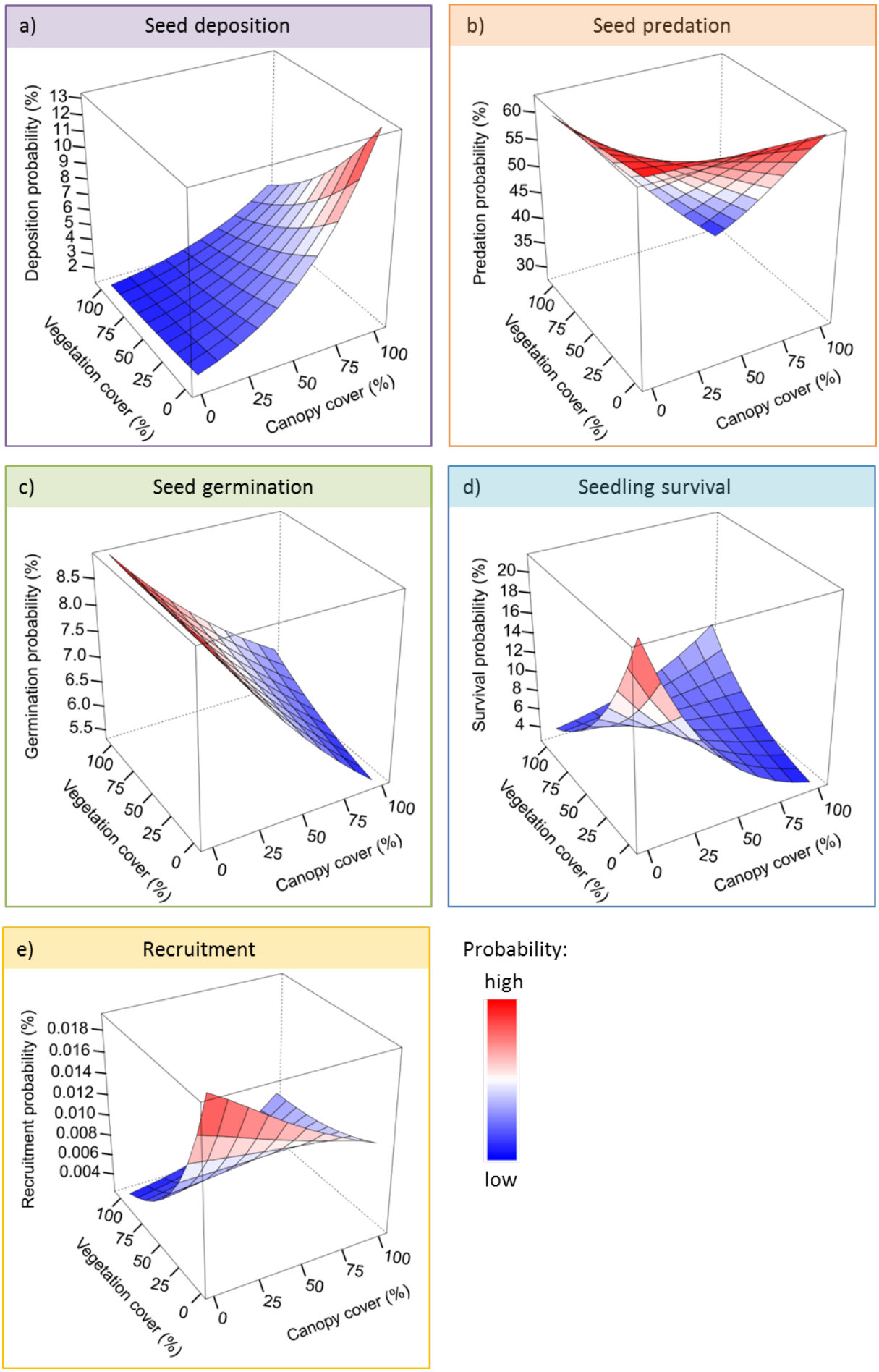
a) Seed deposition
Deposition probability (%)
13
12
11
10
9
8
7
6
5
4
3
2
Vegetation cover (%)
100
75
50
25
0
Canopy cover (%)
0
25
50
75
100
b) Seed predation
Predation probability (%)
60
55
50
45
40
35
30
Vegetation cover (%)
100
75
50
25
0
Canopy cover (%)
0
25
50
75
100
c) Seed germination
Germination probability (%)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
Vegetation cover (%)
100
75
50
25
0
Canopy cover (%)
0
25
50
75
100
d) Seedling survival
Survival probability (%)
20
18
16
14
12
10
8
6
4
Vegetation cover (%)
100
75
50
25
0
Canopy cover (%)
0
25
50
75
100
e) Recruitment
Recruitment probability (%)
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
Vegetation cover (%)
100
75
50
25
0
Canopy cover (%)
0
25
50
75
100
Probability:
high
low

Figure 2: Probability of four demographic processes across the full environmental gradient in terms of canopy cover and ground vegetation cover. a) Probability of seed deposition was highest under closed canopy with no ground vegetation cover (n_{obs} = 1381). b) Probability of seed predation was high under all environmental conditions (n_{obs} = 915). c) Seed germination probability was highest under low canopy cover (n_{obs} = 2156). d) Probability of seedling survival was highest under low canopy cover and low ground vegetation cover (n_{obs} = 319). e) Probability of Swiss stone pine recruitment as product of the predicted probabilities of four demographic processes (i.e., seed deposition, proportion of seeds not predated (i.e., [1-seed predation]), seed germination, seedling survival). Recruitment probability was highest at open microhabitats (i.e., low canopy cover and low ground vegetation cover).

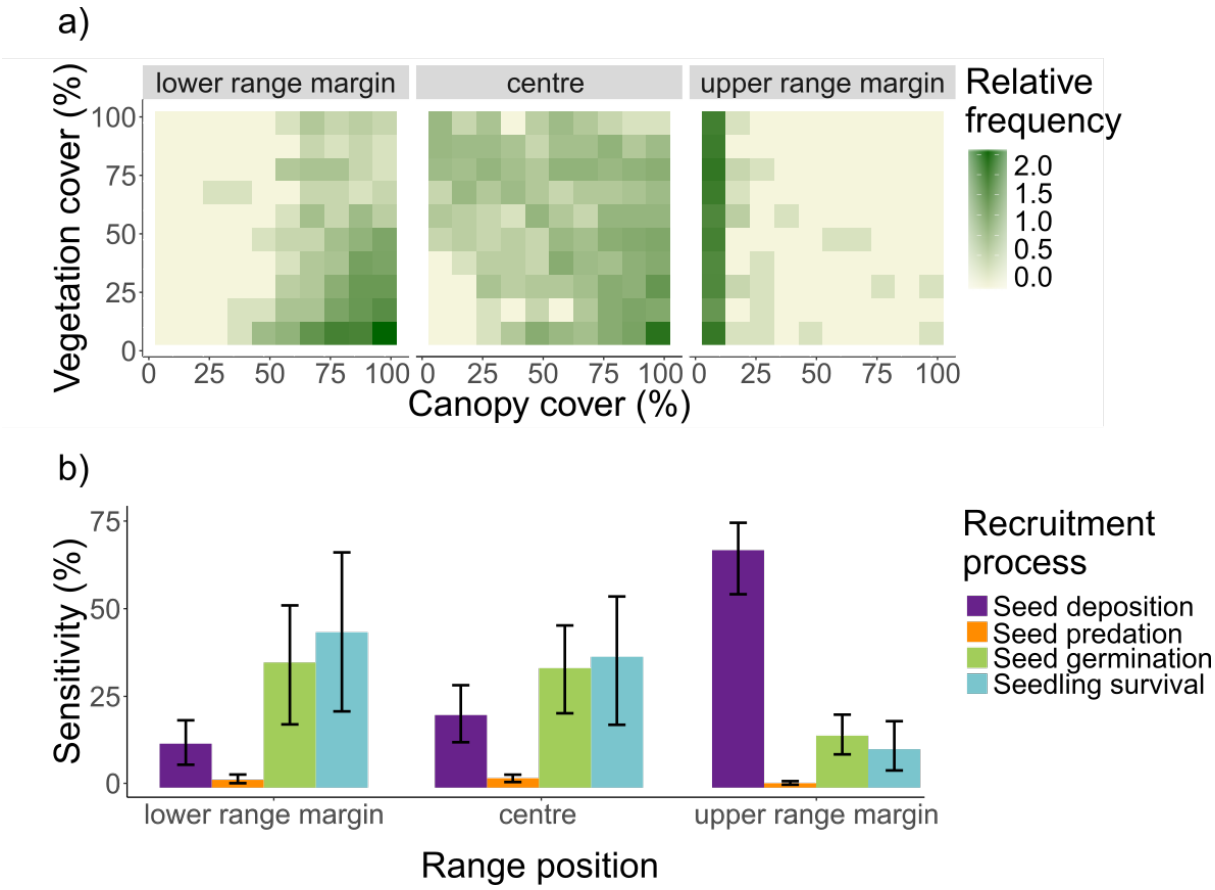

Figure 3: a) Relative frequency (log scale) of environmental conditions in terms of canopy and ground vegetation cover measured at each range position of Swiss stone pine. Frequencies are based on the sum of 1 m^2 seed deposition subplots with the respective combination of canopy and ground vegetation cover in a 10 x 10 matrix of the values ranging from 0 to 100 % in steps of 10 %. b) Sensitivity of overall recruitment toward small changes in transition probabilities of the respective demographic processes at each range position based on the locally realized environmental context. Recruitment in the lower range margin and the centre were most sensitive toward small changes in seed germination and seedling survival, whereas slight changes in seed deposition had strong effects on recruitment at the upper range margin of Swiss stone pine.

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Supplementary material

Supplementary material for Merges *et al.* "Environmental context determines the limiting demographic processes for plant recruitment across a species' elevational range".

Supplementary Table 1: Summary of linear mixed models testing the effects of canopy cover, vegetation cover and their interaction on factors potentially important for recruitment processes (i.e. mean soil temperature, mean soil moisture). The sample size (n) results from sites where respective variables were measured on a microhabitat level. Year and site were included as random factors in all models. Shown are effect estimates with 95% confidence intervals (CI) as a measure of support. For REs standard deviations are shown. Estimates with CIs not passing zero boundaries are considered as significant and highlighted in bold.

Response	Variable	Estimate	2.5% CI	97.5% CI
Mean soil temperature n_{obs} = 1343, n_{year} = 6, n_{site} = 18	Canopy cover	**-0.83**	**-0.95**	**-0.72**
	Vegetation cover	**-0.09**	**-0.17**	**0**
	Canopy cover* vegetation cover	**0.1**	**0.02**	**0.17**
	RE year	0.48	0.34	0.72
	RE site	1.09	0.62	2.01
Mean soil moisture n_{obs} = 4136, n_{year} = 6, n_{site} = 18	Canopy cover	**-11.31**	**-12.25**	**-10.36**
	Vegetation cover	**-1.17**	**-1.81**	**-0.54**
	Canopy cover* vegetation cover	**3.19**	**2.59**	**3.8**
	RE year	7.93	5.71	11.43
	RE site	5.58	3.21	10.71

Supplementary Table 2: Summary of seed translocation experiment conducted over a 6 year period. “Elevation” refers to elevation in m a.s.l.. “Treatment” refers to an open treatment (meshbag) without protection from predation or to an exclosure treatment where seeds were protected from predation by wire-meshbags. “Overall seeds” as product of “Seeds per bag”, “Replicates”, “Micro-habitats”, “Elevational belts” and “Valleys”.

Year	**Elevation**	**Treatment**	**Seeds per bag**	**Replicates**	**Micro-habitats**	**Elevational belts**	**Valleys**	**Overall replicates**
2012	1850-2250	open	5	2	5	9	2	180
2013	1850-2250	open	5	4	5	9	2	360
2014	1850-2250	open	5	2	5	9	2	180
	1850-2250	exclosure	5	2	5	9	2	180
2015	2100-2250	open	5	6	5	4	2	240
	1850-2250	exclosure	5	4	5	9	2	360
2016	2100-2250	open	5	6	5	4	2	240
2017	2100-2250	open	5	6	5	4	2	240
							Total =	1980

Appendix 2: Spatial patterns of pathogenic and mutualistic fungi across the elevational range of a host plant

Authors:

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Author Contributions:

1. Development and planning:
   DM 80%, MB, IM, KBG and ELN in total 20%
2. Field work/data collection:
   DM collected soil samples (95%) with help of MB (5%).
3. Compilation of data sets and figures/tables:
   DM assembled the data sets and prepared the figures (100%).
4. Data analyses and interpretation:
   DM performed the statistical analyses (90%) with input from MB and ELN (in total 10%). **DM** interpreted results (75%), MB, IM, KBG and ELN contributed with the interpretation of the results (in total 25%).
5. Preparation of manuscript:
   DM 75%; MB, IM, KBG in total 10%, ELN 15%

Spatial patterns of pathogenic and mutualistic fungi across the elevational range of a host plant

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Abstract

1. Fungi are both agents of disease and mutualistic partners of plants. Previous studies have tested the effects of abiotic or biotic factors on plant-associated fungal communities in isolation. However, to better understand patterns of plant-fungal associations, the combined effects of abiotic and biotic drivers across environmental gradients may be important.

2. We investigated the effects of temperature, pH, soil moisture, vegetation cover and distance to host plant on the occurrence and abundance of fungi associated with Swiss stone pine (*Pinus cembra*). We did this by DNA metabarcoding 288 soil samples taken across and beyond the elevation range of *P. cembra* (i.e. 1850 – 2250 m a.s.l.) in two valleys in the Swiss Alps. We modeled the effects of abiotic and biotic factors on DNA read abundance of pathogenic and mutualistic fungal operational taxonomic units (OTUs) associated with *P. cembra*. We also tested whether abiotic and biotic factors differentially affected fungi of varying host specificity (i.e. host generalists, host specialists).

3. We found that the occurrences of both host generalist and specialist fungi exceeded the current elevational range of their host plant. Abiotic factors had only minor effects on the abundances of all fungal OTUs. However, we found positive effects of the host plant on the abundance of a host specialist pathogenic fungus, providing support for a Janzen-Connell effect of high pathogen accumulation close to conspecific host plants. We also found a positive response to the host plant in a specialist ectomycorrhizal fungus, suggesting an "inverse" Janzen-Connell effect.

4. *Synthesis*. Our findings imply that negative distance dependence shapes not only the distribution of host-specific fungal pathogens, but also host-specific fungal mutualists. We conclude that the occurrence of both pathogenic and mutualistic fungi beyond the current elevational range of host plants may determine their potential range shifts under projected climate warming.

Keywords: DNA metabarcoding, host specificity, Janzen-Connell hypothesis, *Pinus cembra*, plant-fungal interactions, plant-soil (below-ground) interactions, plant-soil feedback, Swiss stone pine

Introduction

Fungi are mutualistic partners of plants, but also agents of disease (Smith & Read 2008; Butin 2011; Martin et al. 2016). Plant-associated fungi have an important effect on the structure and diversity of plant communities through species-specific facilitative or inhibitive interactions (Barrett et al. 2009; Bever, Mangan & Alexander 2015) and yet the factors controlling their distribution and abundance remain poorly understood (Smith & Read 2008, Tedersoo et al. 2014). To date, a considerable work has shown that pathogenic and mutualistic fungal occurrences are determined by abiotic factors. For instance, fungal species richness declines across elevational gradients, probably due to changes in temperature and precipitation (Bahram et al. 2012, Tedersoo et al. 2014, Soudzilovskaia et al. 2015). Further, soil properties, such as pH, can affect fungal communities (Bahram et al. 2012, Tedersoo et al. 2014, Soudzilovskaia et al. 2015). In contrast, other studies have emphasized the importance of biotic factors, such as host availability and distance to host plants (Dickie & Reich 2005; Bell, Freckleton & Lewis 2006; Kohout et al. 2011; Liang et al. 2016).

The spatial occurrence of pathogenic and mutualistic fungi has important implications for plants (Smith & Read 2008; Butin 2011). For instance, according to the Janzen-Connell hypothesis, seedling establishment success is reduced close to adult conspecifics, with fungal pathogen accumulation being an important mechanism (Bell, Freckleton & Lewis 2006; Liang et al. 2016). As such, fungi can "create space" for heterospecific plant establishment, increasing species diversity in plant communities (Bever et al. 2015). On the other hand, recent studies demonstrated that seedlings of plants with ectomycorrhizal fungal mutualists (ECM) grow better under conspecific adults (Bennett et al. 2017; Teste et al. 2017). Until to date the relative importance of both accumulations of pathogenic and mutualistic fungi for plant establishment is still unclear.

The degree of host specificity is a good predictor of the spatial occurrence of plant-associated fungal species. Host generalists can potentially occupy large geographic areas,

because they do not depend on a single specific host (Hallenberg & Kúffer 2001; Jumpponen & Egerton-Warburton 2005). In contrast, host specialists are generally limited to the distribution of their host (Hallenberg & Kúffer 2001). Thus, host generalists and specialists might be affected differently by biotic factors, such as host availability and distance to host. The degree of host specificity has important implications for host plants (Bruns, Bidartondo & Taylor 2002; Bever et al. 2015). For example, specialist antagonistic fungi can have more negative effects on host fitness than generalists (Bever et al. 2015) and specialist mutualists can have more beneficial effects on host fitness than generalists (Smith & Read 2008). It is also hypothesized, that host generalists and specialists differ in their spatial occurrence and abundance across environmental gradients, with specialists potentially having a narrower distribution (Hallenberg & Kúffer 2001; Jumpponen & Egerton-Warburton 2005). However empirical evidence for such differences is lacking.

To evaluate the ecological impacts of global climate change on plant-fungal interactions, we need to understand the association between antagonistic and mutualistic fungi with their host plant, and the spatial patterns these three players generate. Many plants are migrating upwards in elevation or northwards in latitude to track their preferred climatic niche (Lenoir et al. 2008; Chen et al. 2011). The presence of plant-assocciated fungi within the plants' new ranges may affect the plants' migration capacities both positively, and negatively (Bardgett et al. 2013). Understanding the spatial pattern of fungi in relation to their host plants, can provide an initial insight into how such interactions may develop in the future.

We investigated how abiotic and biotic factors jointly determine the abundance of pathogenic and mutualistic fungi associated with an alpine tree species, the Swiss stone pine (*Pinus cembra* L.) across and beyond the tree's elevational range. We compared the spatial patterns of pathogenic and mutualistic fungal abundances with different levels of host specificity, from generalists to specialists. *Pinus cembra* has its main distribution in the European Alps, where it occurs at elevations ranging between 1500–2400 m a.s.l., and is the

dominant species forming the tree line (Ulber, Gugerli & Bozic 2004). *Pinus cembra* is negatively affected by several fungal pathogens (e.g. *Lophodermium* Chevall., *Gremmenia infestans* (P. Karsten) P.W. Crous) and also depends obligately on ECM fungal mutualists for survival (e.g. *Suillus* Gray, *Rhizopogon* Fr) under natural conditions (Moser 1967; Nierhaus-Wunderwald 1996).

We combined field measurements with DNA metabarcoding (Taberlet et al. 2012) 1) to quantify the abundance of pathogenic and ECM fungal operational taxonomic units (OTUs) across the elevational distribution of *P. cembra*, 2) to evaluate how abiotic (temperature and soil properties) and biotic factors (vegetation cover, distance to host) affected these abundances, and 3) to assess whether abundances of generalist and specialist fungal OTUs responded differently to abiotic and biotic factors. We expected that abundances of generalist fungal OTUs vary independently from *P. cembra*'s range, because alternative hosts can be colonized (Jumpponen & Egerton-Warburton 2005). In contrast, we expected that abundances of specialist fungal OTUs correspond to the elevational distribution of their host plant (Hallenberg & Küffer 2001). We also expected abiotic factors to strongly influence the abundances of all fungal OTUs, regardless of the degree of host specificity (Classen et al. 2003; Tedersoo et al. 2014; Carrino-Kyker et al. 2016). Finally, we expected that distance to host would not affect the abundance of generalist fungi (Jumpponen & Egerton-Warburton 2005), but that specialist pathogenic fungi would be most abundant close to their host in accordance with the Janzen-Connell hypothesis (Liang et al. 2016).

Material and Methods

Study area and sampling protocol

The study sites were located within the core distribution of *P. cembra* in the Central Alps in the eastern part of Switzerland (Figure 1), in two valleys near Davos: the Flüela valley (46°48′0.25″N 09°54′15.38″E) and the Sertig valley (46°44′0.76″N 9°51′3.5″E). At the valley

bottoms (about 1850 m a.s.l.) forests are dominated by Norway spruce (*Picea abies* (L.) H. Karst.) and European larch (*Larix decidua* Mill.), with low *P. cembra* abundance. *Pinus cembra* is found in a unimodal abundance distribution from 1850 m a.s.l. to 2150 m a.s.l., with the highest abundances at mid-elevations. The tree line is formed at 2150 m a.s.l., the highest elevation at which *P. cembra* trees (i.e. pines over three meters in height, Harsch et al. 2009) are found. Small *P. cembra* individuals (< 3m tall) persist up to 2200 m a.s.l. and none are found ≥ 2250 m a.s.l. (Neuschulz et al. 2017). Pine health is reduced at all life stages by needle cast disease, caused by generalist pathogenic *Lophodermium* Chevall. species (Nierhaus-Wunderwald 1996). The snow fungus *Gremmenia infestans* (P. Karsten) P.W. Crous, a specialist upon *P. cembra*, kills seedlings and saplings that are fully immersed in snow during winter (Nierhaus-Wunderwald 1996). *Pinus cembra* also depends on ECM fungal mutualists for survival. In addition to multiple generalist ECM species (Göbel & Ladruner 2000, Kernaghan & Harper 2001, Bacher et al. 2010, Dickie et al. 2010), there are several specialist ECM fungi associated with *P. cembra* (e.g. *Suillus placidus* [Bonord.] Singer, *S. plorans* [Rolland] Kuntze, *S. sibiricus* [Singer] Singer, *Rhizopogon salebrosus* A.H. Sm.; Bacher et al. 2010, Kohout et al. 2011).

For soil sampling of fungal communities, we sampled each valley at nine elevational levels with 50 m elevational intervals ranging from 1850 to 2250 m a.s.l. (Fig. 1). We collected soil samples at each elevational level in two microhabitat types (under ericaceous vegetation cover or close (0.05 - 1 m) to mature *P. cembra* individuals), following a random-stratified sampling design (Fig.1). Ericaceous plants do not support the specialist pathogens of *P. cembra* and are associated with ericoid mycorrhizae, which are not suitable for *P. cembra* mutualists (Kohout et al. 2011). Hence, we expected that vegetation cover would not affect the abundance of generalist pathogens, but would influence the abundance of specialist pathogenic and ECM fungi. Due to the absence of trees at high elevations (i.e. at 2250 m a.s.l.) the microhabitat "close to *P. cembra*" was replaced by matgrass (*Nardus stricta* L)

dominated sites at these elevations, to guarantee a balanced random stratified sampling design (i.e., same sample size at each elevational level). At each elevational level, we replicated each microhabitat four times, resulting in eight soil sampling sites per elevational level in each valley (total n = 144). We conducted two sampling rounds, one in May 2015, at the start of the growing season and one in September 2015, at the end of the season, resulting in a total of 288 soil samples. Soil samples were taken with a 1 cm soil core sampler (Ehlert & Partner). For each soil sample, we took five five-centimeter deep soil cores from a 15 x 15 cm^2 area that we pooled and stored in a Ziploc bag. Each soil sample was dried until it reached constant weight, which took 24 – 48 h depending on initial water content. We processed soil samples in batches, so that only samples of spatially close elevational levels were dried simultaneously. To exclude potential cross contamination among samples, we tested the effect of batch on the composition of the fungal community and found no significant effect (Supplement Table S1). Soil samples from May 2015 were stored for five months, and samples from September 2015 for one month with 5 g silica-gel each in a - 20 °C freezer until DNA extraction.

Additionally, we sampled ECM roots of *P. cembra* to create a reference database for associated ECM fungi. For this, we collected *P. cembra* roots in both valleys at 1850, 2050 and 2200 m a.s.l. in May 2015. We inspected the rinsed roots visually at 11.5 x magnification with a SZX2-ILLT stereomicroscope (Olympus Corporation) and selected 100 colonized (i.e. ectomycorrhizal) root tips per elevational level for DNA extraction.

Abiotic and biotic factors

A range of potential biotic and abiotic drivers of fungal abundance were recorded across the elevational gradients. We measured temperature at six out of eight soil sampling sites per elevational level per valley using iButton data loggers (Maxim) that recorded soil surface temperatures every four hours over the duration of the study. Mean summer temperature was

calculated as mean June-August period for each microhabitat type at each elevational level. Mean winter temperature was calculated as mean of the December-February period. We also considered maximum and minimum temperatures (Oberhuber 2004), by calculating mean daily maximum temperature for June-August, mean daily minimum temperature for December-February, mean temperature and mean daily maximum temperature of the hottest month (July), and mean temperature and mean daily minimum temperature of the coldest month (January). We measured soil pH of each soil sample in a 1 M solution of Potassium chloride (KCl) with a pH / conductivity meter CPC-401 (Elmetron). We collected soil moisture data in September under dry weather conditions by averaging five tensiometer (Theta-Kit version 3) measurements at each soil sampling site. Ericaceous vegetation cover for each soil sampling site was recorded by estimating the coverage of dominant species: alpine azalea (*Loiseleuria procumbens* (L.) Desv.), *Vaccinium* spp. L. and alpenrose (*Rhododendron ferrugineum* L.) within 1 m of each soil sampling site (Braun-Blanquet 1964). Distance to host plant was measured as the distance from each soil sampling site to the closest adult *P. cembra*, with the aid of a Laser Range Finder (Nikon 800S) for distances > 10 m.

Laboratory protocol

We randomized the order of all samples prior to DNA extraction to prevent sampling biases (Bálint et al. 2018). We used 300 mg of each root and soil sample for DNA extraction. DNA extraction followed the protocol by Cubero and Crespo (2002). We amplified the ITS2 region using the universal fungal primer ITS3_KYO2 5′-GATGAAGAACGYAGYRAA-3′ and ITS4_KYO3 5′-RBTTTCTTTTCCTCCGCT-3′ (Toju et al. 2012). Combinatorial primer labeling was used for sample identification after multiplexed sequencing (Gloor et al. 2010), i.e. both primers were tagged with 8 bp long tags (Kozarewa & Turner 2011). We used the PCR protocol described in Schmidt et al. (2013), except that two parallel PCR runs (1 x 52 °C, 1 x 55 °C annealing temperature) were conducted before pooling of PCR products.

Paired-end sequencing (2 x 150 bp) was executed on an Illumina MiSeq sequencer at Fasteris SA, Plan-les-Ouates, Switzerland. Fasteris SA uses a proprietary protocol that eliminates the PCR step from the library preparation of amplicons – this is essential to control for the most important source of tag switching (Schnell, Bohmann & Gilbert 2015). The library preparation followed a PCR-free ligation-based MetaFast protocol, with 1 µg DNA dissolved in 30 µl of water. We used a multiplexing control to detect erroneous read numbers resulting from tag-switching. Multiplexing controls consisted of empty reaction wells on PCR plates, i.e. wells that did not contain any reagents, DNA template or water. Consequently, it is expected that no DNA reads should contain multiplexing tag combinations which correspond with these empty wells. If there are indeed reads with such unexpected label combinations, these may originate only from label jumps (see a detailed description of these in Schnell, Bohmann & Gilbert et al 2015). The most abundant label combination resulting from tag switching had a total of 8 reads (i.e. 0.0002 % of total fungal reads). Thus, tag switching was extremely rare. To yet control for potential bias introduced by tag switching, we subtracted the erroneous reads from the final OTU table.

We used the default options for the pipeline described by Bálint et al. (2014). We filtered out all sequences containing unknown nucleotides (i.e. "N"s), and assembled paired-end reads using the "PANDASeq" program (Masella et al. 2012). In the program "fqgrep" we did not allow for mismatches (option = 0, https://github.com/indraniel/fqgrep). For extracting fungal ITS sequences, we used "ITSx" (Bengtsson-Palme et al. 2013). We performed initial denoising with a 97 % similarity clustering threshold using the heuristic clustering algorithm "UCLUST 2.1" (implemented in USEARCH v7, Edgar et al., 2010). Since the average intraspecific ITS variability is around 1.96 % (+/- 3.73 SD) for Ascomycota and 3.33 % (+ / - 5.62 SD) for Basidiomycota (Nilsson et al. 2008), we were confident that the canonical 3 % clustering threshold worked well for our purpose. Clustering was set to discard reads that appeared less than ten times. Chimera filtering was performed using "USEARCH v7" (Edgar

et al., 2010). For blasting, the e-value was set to 0.001 (Altschul et al. 1997). We used the program "MEGAN" to parse the blast results and select fungal OTUs (Huson et al. 2011). The LCA option were set to "min support = 1, min score = 170, top percent = 5".

We were not interested in the entire soil fungal community, but only in pathogenic and ECM fungi. These groups were identified by blasting (Altschul et al. 1997) the OTU representative sequences against the Gen-Bank nucleotide database (ftp://ftp.ncbi.nlm.nih.gov/blast/db/nt, downloaded on 3 March 2016) and UNITE Version no. 7.0 database (https://unite.ut.ee/repository.php; Koljalg et al. 2005) using a 97 % similarity threshold. To exclude rare OTUs derived from potential erroneous sequences, we followed the recommendation of Bokulich et al. (2013) to quality filter the OTU table by removing those OTUs that are represented by less than 0.005 % of the total read abundance.

Fungi classification

We carefully classified fungi into pathogens and mutualists, and by their host specificity, given the ambiguous life histories in fungi (e.g. switching from asymptomatic occurrence on many plant species to pathogenic behavior on a very few; Klironomos 2003, Kiers et al. 2011, Aguilar-Trigueros et al. 2014, Kia et al. 2016). Since the focus of our study was on fungi relevant for *P. cembra*, we retained exclusively fungal OTUs known to be associated with *Pinus*. We approached the classification differently for pine-associated pathogens and ECM. For pathogens, we conducted a systematic search of peer-reviewed journal articles including all records until December 2016 in Web of Science using the following keywords "((*Pinus cembra* OR Swiss stone pine) AND (pathogen OR pathogenic OR disease))". This search resulted in 13 articles, from which *P. cembra*-associated pathogens were extracted (Table S1). We classified pathogens as generalists if publications reported host plants other than pine (i.e. genus *Pinus*), whereas pathogens described to occur exclusively on pines (i.e. genus *Pinus*) were classified as specialists. For ECM, we first used our own ECM database (generated from

sequenced *P. cembra* ECM root samples) to obtain a list of candidate ECM OTUs. Second, we taxonomically assigned these OTUs and confirmed the taxon list by searching the assigned names in Web of Science for all taxa. This resulted in 34 species reported as ECM mutualists (Table S1). We then classified ECM fungi into three categories following Molina, Massicotte and Trappe (1992): 1) narrow host specificity (all plant hosts within the same genus), 2) intermediate host specificity (all plant hosts within the same family) and broad host specificity (plant hosts extending across multiple plant families or even orders; Table S1). Preliminary analysis found that fungi with intermediate and broad host specificity showed the same response to the tested variables; hence we grouped these two categories in subsequent analyses as host generalists.

Data analyses

We used DNA read abundance derived from the Illumina MiSeq sequencer as a proxy for real abundances, since abundance changes within a single OTU are approximately quantitative (Amend, Seifert & Bruns 2010). Therefore, fitting individual GLMs to each fungal OTU allowed us to test the effects of abiotic and biotic factors on abundances of fungi (Bálint et al. 2015).

First, we tested how pathogenic and ECM fungal OTUs changed in abundance across the elevational distribution of *P. cembra*. We modeled the abundances of generalist pathogenic and ECM OTUs separately as a function of elevation by fitting individual generalized linear models (GLMs) for each OTU using the manyglm function of the mvabund package in R v.3.2.3 (Wang et al. 2012; R Development Core Team 2013; Bálint et al. 2016). The multivariate manyglm function can handle multiple response variables and allows for the detection of individual significant effects of independent variables on each OTU abundance as well as the detection of community wide patterns. We modeled abundances of the specialist pathogenic snow fungus *G. infestans*, and the specialist mutualistic *R. salebrosus* OTUs in individual GLMs, since their low number (i.e. two OTUs assigned to the same taxon *G.*

infestans and one OTU assigned to *R. salebrosus* respectively) made a multivariate modeling approach unnecessary. Read numbers of *G. infestans* OTUs were pooled prior to analysis, since they were assigned to the same species (Amend, Seifert & Bruns 2010). To account for differential sequencing success (i.e. "sequencing bias"; Bálint et al. 2015), we included the square root of sequencing read numbers into all models. This method has been shown to be more adequate than using rarefied data to account for potential sequencing bias (McMurdie & Holmes 2014). We also included the valley and the sampling season to account for spatio-temporal autocorrelation. Models were fitted assuming a negative binomial error distribution, which accounts for overdispersion (O'Hara & Kotze 2010).

Second, we tested the effect of abiotic and biotic factors on the abundance of pathogenic and mutualistic OTUs in relation to host specificity (i.e. fitting separate models for generalists and specialists). We modeled the effects of abiotic (i.e. temperature, soil pH, soil moisture) and biotic factors (i.e. ericaceous vegetation cover, distance to host) as predictors of abundances of generalist pathogenic and ECM OTUs using the sequencing read numbers, the valley and the sampling season as control variables. We tested the effects of abiotic and biotic factors as predictors of the abundances of the specialist pathogenic snow fungus *G. infestans* and the specialist mutualistic *R. salebrosus* OTUs; again including the sequencing read numbers, the valley and the sampling season as control variables.

Prior to the analyses, we tested the abiotic and biotic factors for correlation and used only uncorrelated factors in the same models (Pearson's $r > 0.7$, Table S3). All temperature variables within the same season (i.e. summer or winter) were highly correlated and could not be used in the same model (Table S3). Therefore, we ran separate models for each uncorrelated pair of summer and winter temperature variables (Table S4-S6). We fitted full GLMs including all abiotic and biotic factors and performed model selection in a stepwise manner, were we removed the non-significant variables step by step until all variables within the model were significant ($p < 0.05$). Control variables were kept even when non-significant.

Selected models were tested against a null model, containing the square root of sequencing read numbers, valley and season as control variables, using ANOVA-based F-tests for nested models to evaluate if more variation is statistically significantly explained by the more complicated model (Bálint et al. 2015).

Results

We received a total of 9,587,848 raw reads from the Illumina MiSeq sequencer. After paired-end assembly we kept 6,788,986 reads. The de-multiplexing step reduced read number to 5,490,158. We could assign 3,315,494 quality-filtered read pairs to fungi. Fungal reads were clustered into 2218 unique OTUs. From these, we retained 1074 OTUs after quality filtering the final OTU table.

We found two generalist pathogenic OTUs (assigned to the genus *Lophodermium*) that occurred in 74 % of all samples (n = 213; Table S2) and two host-specific pathogenic OTUs (assigned to the snow fungus *G. infestans*) in 29 % of all soil samples (n = 83; Table S2). In the *P. cembra* root samples, we found 30 generalist ECM OTUs and one host-specific ECM OTU (assigned to *R. salebrosus*). The 30 generalist ECM OTUs occurred in 64 % of the soil samples (n = 184; Table S2), the host-specific ECM OTU occurred in 54 % of the soil samples (n = 156; Table S2).

First, we tested if the abundance of pathogenic and ECM fungal OTUs changed across the elevational distribution of *P. cembra*. Generalist and specialist fungi were modeled separately. Here, generalized linear models revealed that the abundance of pathogenic and ECM fungal OTUs did not change significantly across the elevational gradient (Fig. 2, Table 1). Abundances of both generalist and specialist OTUs above the tree line (> 2150 m a.s.l.) did not significantly decline beyond the upper range limit of *P. cembra*.

Second, we tested how abiotic (i.e. temperature, soil pH, soil moisture) and biotic factors (i.e. vegetation cover, distance to host) affected abundances of pathogenic and

mutualistic OTUs in relation to their host specificity (i.e. fitting separate models for generalists and specialists). Summer and winter temperatures, soil pH, soil moisture, vegetation cover or distances to host did not significantly affect abundances of generalist pathogenic and ECM fungal OTUs (Table 2). Individual GLM models revealed that the abundance of the host-specific pathogenic *G. infestans* was significantly positively affected by soil pH and significantly negatively affected by distance to host (Fig. 3a, Table 2 & 3). Predicted abundance of *G. infestans* OTUs declined by 2 % within 5 m of distance to the closest *P. cembra* tree, 4 % within 10 m, 37 % within 100 m and 80 % within 340 m (longest distance in this study). The abundance of *G. infestans* was significantly lower in the second sampling season in September than in May. The abundance of the host-specific ECM *R. salebrosus* OTU was significantly positively affected by summer temperature and significantly negatively affected by vegetation cover and by distance to the host (Fig. 3b, Tables 2 & 3). Predicted abundance of the OTU declined by 2 % within the first 5 m, 3 % at 10 m, 31 % at 100 m and 71 % at 340 m distance from the closest *P. cembra* tree. *Rhizopogon salebrosus* was found in significantly higher numbers in the Sertig valley as compared to the Flüela valley. All models using mean daily maximum and minimum temperatures instead of mean temperatures showed qualitatively similar results (Table S4-S6).

Discussion

We present a comprehensive study of the effects of abiotic and biotic factors on the abundances of pathogenic and mutualistic fungi associated with *P. cembra* in relation to their host specificity. We show that host generalist and specialist fungal OTUs are widely distributed within and beyond the current elevational range of their host plant. Abundances of generalist fungal associates were neither influenced by abiotic nor by biotic factors. Abundances of host specialist fungi were mainly determined by distance to the host (i.e. negative distance dependence). Our results show that, in line with the Janzen-Connell

hypothesis, specialist pathogens accumulate close to conspecific adult host plants. We found the same pattern also for a specialized mutualist. These results suggest that high abundances of mutualists close to the host could potentially cancel out the negative feedback of pathogens on plant establishment, suggesting a positive feedback by ECM mutualists.

In our study system, both generalist and specialist fungi were widely distributed within and beyond the current range of their host plant. The occurrence of specialist fungi outside the current elevational range of their host was surprising. There could be two, not mutually exclusive, explanations for this pattern. First, while fungal growth might be constrained by host availability, fungi can disperse over considerable distances (Read & Haselwanter 1981; Nunez et al. 2009; Urcelay et al. 2017). In our study, there appeared to be no dispersal limitation of fungi beyond the range limit of *P. cembra*. However, we might have recorded fungal spores at high elevations, and not established host specialist colonies. Persistent spore banks above the treeline could be present in particular for pine-associated fungal species as a result of a higher treeline in the past (Gehrig-Fasel et al. 2007, Pini et al. 2017). In many regions of the Alps, treelines have been lowered by past human disturbances and deforestation (Krpata et al. 2007, Carrer et al. 2018). Second, it is hypothesized that promiscuous host associations occur under harsh alpine and arctic conditions (Ryberg, Larsson & Molau 2009; Botnen et al. 2014; Mundra et al. 2015). Thus, specialist fungi might colonize alternative hosts. For instance, it has been shown for bearberry (*Arctostaphylos* spp.), a species belonging to the family of Ericaceae which usually form ericoid mycorrhiza, to interact under unfavourable environmental conditions with pine-associated ECM fungi (Tedersoo et al. 2006, Krpata et al. 2007). Harsh environmental conditions may, however, limit the growth and performance of fungi on these alternative hosts (Krpata et al. 2007).

In our study, pathogen occurrence above the current tree line suggests that there will be no escape from enemies for *P. cembra*, when dispersed into newly suitable habitats. On the other hand, ECM fungi occurrence beyond their hosts' current range could provide suitable

conditions for seedling establishment, allowing *P. cembra* to track its preferred climatic conditions to higher elevations under climate warming.

Abundances of generalist fungal OTUs revealed no significant response either to abiotic or to biotic factors. Generalist fungi (pathogenic or ECM) are able to colonize many plants and to occupy wide geographic ranges (Jumpponen and Egerton-Warburton 2005). Temperature, soil pH and soil moisture could be an important factor determining fungal occurrences mostly on a broader (i.e. regional or global) scale (Tedersoo et al. 2014), whereas abiotic and biotic gradients at local scales could be all within the tolerances of most species in local communities.

Abundances of host specialist fungal associates were mostly determined by distances to the host plant. In the specialist pathogenic *G. infestans*, we found that abiotic (i.e. soil pH) and biotic factors (i.e. distances to the host) are important determinants of abundances. The pH may potentially influence the pathogen through conditions favorable for spore germination (Magan & Lacey 1984; Land, Banhidi & Albertsson 1987). Negative distance dependence supports the Janzen-Connell hypothesis, implying that high pathogen abundance could cause high seedling mortality close to conspecific adults (Packer & Clay 2000; Liang et al. 2016; Liu et al. 2016). In the specialist ECM fungi, abiotic (i.e. summer temperatures) and biotic factors (i.e. vegetation cover, distances to the host) were important drivers of abundance: Temperature has been shown to favor fungal growth and ericaceous vegetation cover to inhibit ECM fungi (Kohout et al. 2011a; Tedersoo et al. 2014; Teste et al. 2017). The specialist mutualist was similarly abundant close to the host tree (i.e. negative distance dependence effect) as the specialist pathogen. High abundance of the specialist mutualist close to adult plants could imply positive responses of plant seedlings through beneficial effects, e.g. early colonization by the ECM fungus could alleviate pathogen-induced stress by mechanical protection against root parasites or enabling of enhanced nutrient uptake (Marx 1972; Mukerji, Chamola & Singh 2000). This “inverse” Janzen-Connell effect is supported by

additional evidence from Bennett et al. (2017) and Teste et al. (2017), who show that ECM plant seedlings receive positive soil feedback under conspecific adult trees. The high abundance of specialist ECM fungi close to conspecific adult host plants that we found in our study could mediate positive soil feedback on pine seedlings.

Conclusion

Examining the occurrence of fungi across environmental gradients has been a great challenge in past research. The combination of DNA metabarcoding, the ecologically relevant classification of barcoded ITS sequences, and suitable statistics allowed us to analyze the relationship of plant-associated fungi with their host plant along a steep elevational gradient. We show that negative distance dependence might be more important for determining specialist fungal occurrences than previously assumed. Further, our data indicate that a potential upward shift of *P. cembra* to higher elevations under warming climates could be inhibited by the presence of antagonistic fungi, but is not limited by the absence of mutualistic fungal partners. Fungi and plants interact along an antagonistic-mutualistic continuum and it is unclear how ongoing climate change alters these interactions. Understanding plant-fungal interactions is therefore key to project potential responses of plants to global change.

Authors Contribution

D.M., M.B., I.S., K.B.G. and E.L.N. conceived the ideas and designed methodology. D.M. and M.B. collected the data. D.M. performed the analyses with input from M.B. and E.L.N. D.M. and E.L.N. led the writing of the manuscript. All authors contributed to the various drafts and gave final approval for publication.

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Data accessibility

Data from this paper are deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.66rm7 (Merges, Bálint, Schmitt, Böhning-Gaese, Neuschulz, 2018).

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Figure 1. Map of study area and sampling design. a) Study sites located in the Flüela valley and the Sertig valley close to Davos, Switzerland. Elevational distribution of *Pinus cembra* is shown in brown colour. *Picea abies* and *Larix decidua* are shown in blue colour. b) Elevational levels (white rectangles), ranging from 1850 to 2250 m a.s.l., spaced by 50 m vertical height difference. c) Random-stratified sampling design: Soil samples were taken at eight soil sampling sites in two microhabitat types per elevational level. Background images: Google maps.

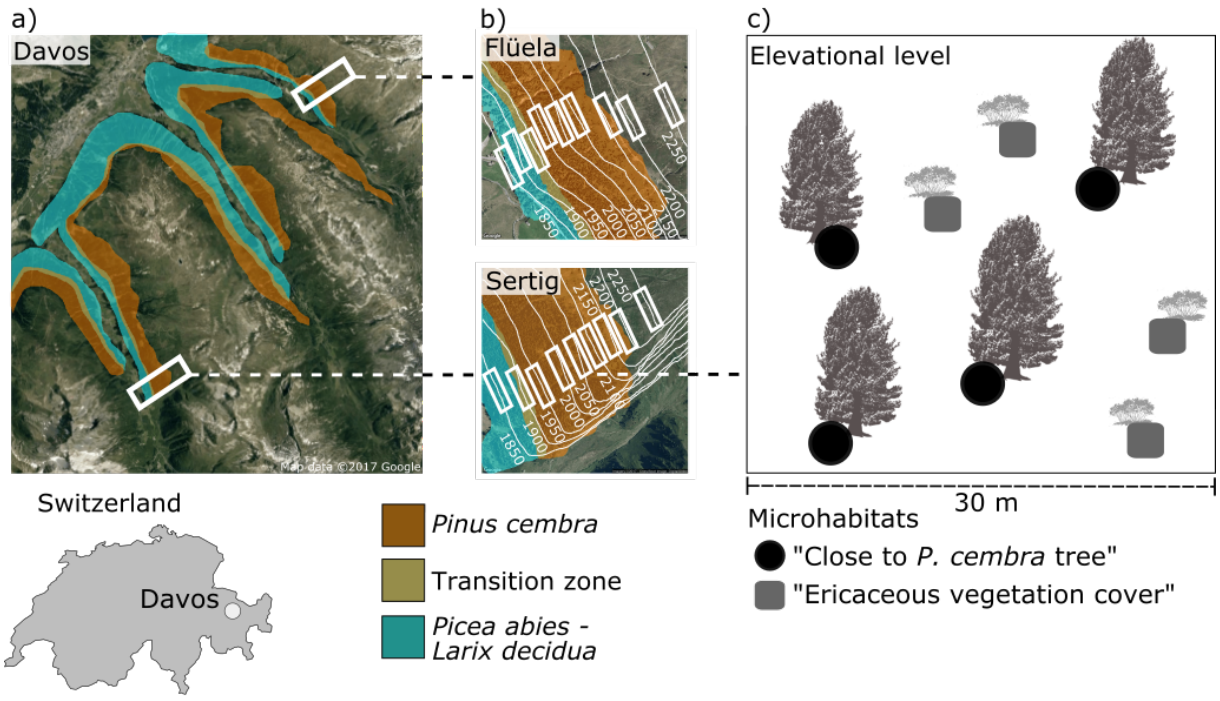

Figure 2. Abundances of OTUs of a) generalist (n = 2) and b) specialist pathogenic (n = 2) and c) generalist (n = 30) and d) specialist (n = 1) ectomycorrhizal fungi associated with Swiss stone pine (*Pinus cembra*) as a function of elevation. Generalized linear models of fungal OTU abundances were fitted assuming a negative binomial error distribution accounting for overdispersion. Coloured points indicate jittered raw data and represent different OTUs in each panel. Dashed lines show non-significant effect of elevation on fungal abundance ($p > 0.05$). *Pinus cembra* core distribution displayed as grey box.

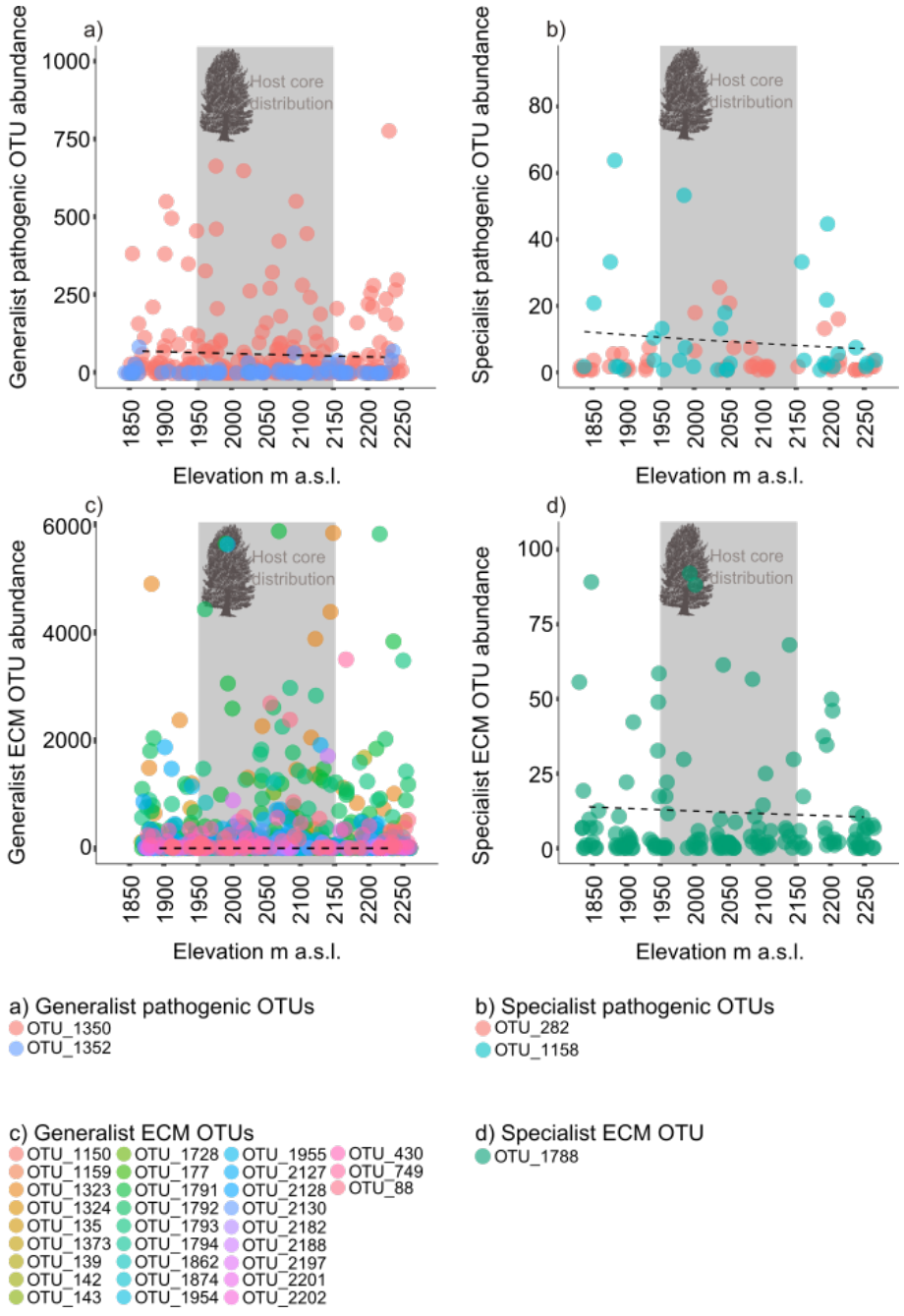

Figure 3. Predicted abundances of a) the specialist pathogenic fungus *Gremmenia infestans* (OTU 282 and 1198 combined) and b) the specialist ECM fungus *Rhizopogon salebrosus* as a function of distance to host (*P. cembra*). Generalized linear models were fitted assuming a negative binomial error distribution accounting for overdispersion. Points indicate jittered raw data. Black lines show the model fit ($p < 0.01$) with 95 % confidence intervals added as grey shadow.

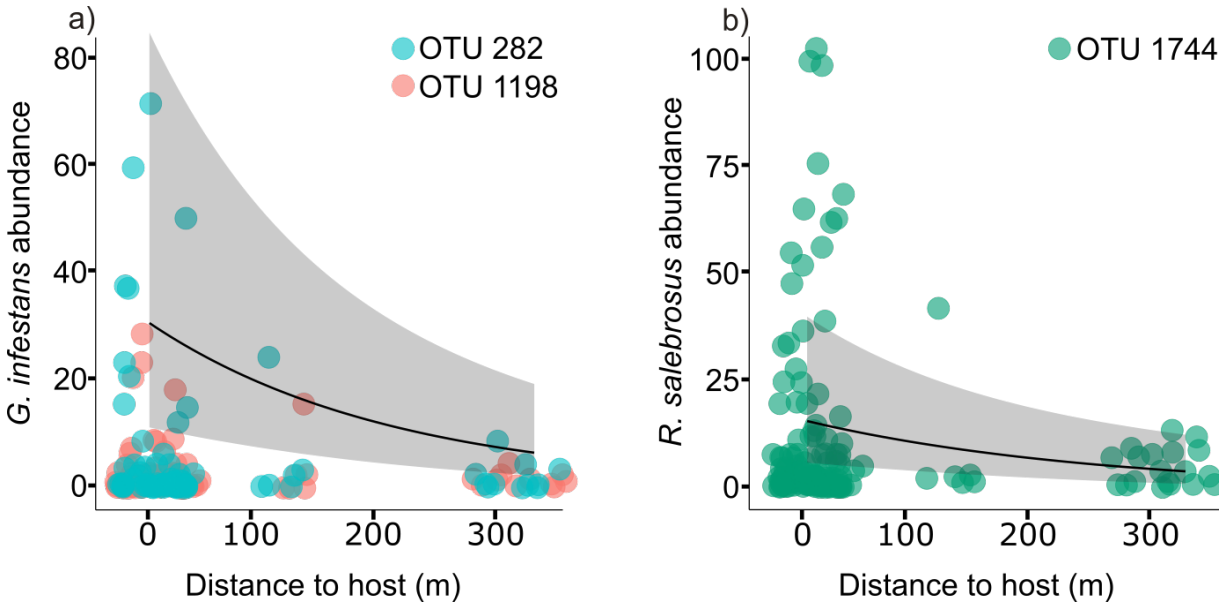

Table 1: Summary statistics of GLMs of generalist and specialist OTU abundances of *P. cembra*-associated pathogenic (a, c) and ectomycorrhizal (b, d) fungi including elevation as predictor variable. GLMs were tested against a null model, which contained the square root of read numbers, valley and season as control variables, using ANOVA-based F-tests for nested models to evaluate if more variation is statistically significantly explained by the more complex model.

Model response	**Coefficients**	***p* full model**
a) Generalist pathogens	√Read number	0.010
	Elevation	0.535
	Valley	0.426
	Season	0.228
	ANOVA	$p = 0.752$
b) Generalist ECM	√Read number	0.010
	Elevation	0.366
	Valley	0.386
	Season	0.119
	ANOVA	$p = 0.782$
c) Specialist pathogen (*G. infestans*)	√Read number	0.023
	Elevation	0.125
	Valley	0.386
	Season	0.256
	ANOVA	p = 0.266
c) Specialist ECM (*R. salebrosus*)	√Read number	0.003
	Elevation	0.513
	Valley	< 0.001
	Season	0.057

ANOVA $p = 0.591$

Table 2: Summary statistics of backwards stepwise regression from full to final GLMs of generalist and specialist OTU abundances of *P. cembra*-associated pathogenic (a, c) and ectomycorrhizal (b, d) fungi including all abiotic and biotic factors. Backwards stepwise regression was carried out by dropping step by step the least significant explanatory variables of the full model. Final models were tested against a null model, which contained the square root of read numbers, valley and season as control variables, using ANOVA-based F-tests for nested models (significant models highlighted in bold face). MST = mean summer temperature, MWT = mean winter temperature.

Model response	**Coefficients**	***p* value full model**	***p* value 1. model**	***p* value 2. model**	***p* value 3. model**	***p* value 4. model**	***p* value final model**
a) Generalist							
pathogens	√Read number	0.020	0.010	0.010	0.020	0.010	0.010
	Distance to host	0.832					
	pH	0.347	0.307				
	Vegetation cover	0.059	0.069	0.020	0.040	0.059	
	MST	0.139	0.218	0.426			
	MWT	0.158	0.158	0.376	0.257		
	Soil moisture	0.238	0.119	0.050	0.059	0.040	0.040
	Valley	0.436	0.465	0.584	0.455	0.406	0.475
	Season	0.406	0.347	0.079	0.149	0.119	0.109
					ANOVA		$p = 0.123$
b) Generalist							
ECM	√Read number	0.010	0.010	0.010	0.010	0.010	0.010
	Distance to host	0.614					
	pH	0.396	0.604				
	Vegetation cover	0.228	0.020	0.307			
	MST	0.010	0.228	0.079	0.366		
	MWT	0.040	0.257	0.228	0.287	0.218	0.287
	Soil moisture	0.059	0.178	0.158	0.010	0.396	

	Valley	0.059	0.238	0.030	0.069	0.139	0.218
	Season	0.040	0.554	0.010	0.050	0.010	0.059
					ANOVA		$p = 0.356$
c) Specialist pathogen (*G. infestans*)	√Read number	0.116	0.189	0.147	0.138	0.077	
	Distance to host	0.044	< 0.001	< 0.001	< 0.001	< 0.001	
	pH	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
	Vegetation cover	0.323	0.515				
	MST	0.230	0.367	0.500			
	MWT	0.162	0.274	0.192	0.224		
	Soil moisture	0.361					
	Valley	0.476	0.373	0.269	0.277	0.261	
	Season	0.016	0.011	0.013	0.012	0.012	
					ANOVA		**$p = < 0.001$**
d) Specialist ECM (*R. salebrosus*)	√Read number	0.012	0.016	0.013	0.013		
	Distance to host	0.040	0.031	0.001	< 0.001		
	pH	0.327					
	Vegetation cover	0.223	0.107	0.024	0.042		
	MST	0.121	0.043	0.024	0.035		
	MWT	0.293	0.369	0.598			
	Soil moisture	0.2961	0.391				
	Valley	< 0.001	< 0.001	< 0.001	< 0.001		
	Season	0.088	0.167	0.136	0.156		
					ANOVA		**$p = 0.003$**

Table 3: Significant final generalized linear models showing the effect of abiotic and biotic factors on abundances of (a) *Gremmenia infestans* and (b) *Rhizopogon salebrosus*. Read numbers, accounting for differences in sequencing depth, were square-root normalized. Valley (i.e. Sertig valley, Flüela valley) and season (i.e. May, September) were included as control variables. MST = Mean summer temperature.

Model response	**Coefficients**	**Estimate**	**SE**	***p* value**
a) *G. infestans*	Intercept	2.03	0.22	**< 0.001**
	√Read number	-0.21	0.12	0.077
	Distance to host	-0.51	0.13	**< 0.001**
	pH	0.77	0.15	**< 0.001**
	Valley	0.29	0.26	0.261
	Season	-0.68	0.27	**0.012**
b) *R. salebrosus*	Intercept	2.47	0.21	**< 0.001**
	√Read number	0.28	0.12	**0.013**
	Distance to host	-0.40	0.12	**< 0.001**
	Vegetation cover	-0.26	0.13	**0.042**
	MST	0.27	0.13	**0.035**
	Valley	0.97	0.24	**< 0.001**
	Season	-0.38	0.23	0.156

Supplementary material

Supplementary material for Merges *et al.* "Spatial patterns of pathogenic and mutualistic fungi across the elevational range of a host plant".

Table S1. Summary statistics of GLM testing the effect of soil moisture at sampling location on OTU read abundances. The model including batch and the square root of read numbers was tested against a null model containing the square root of read numbers only. ANOVA-based F-tests for nested models were used for model comparison.

Model response	**Coefficients**	***p* value full model**
OTU read abundance	√Read number	0.01
	Batch	0.01
	ANOVA	$p = 0.069$

Table S2. Taxonomic and functional assignment of *P. cembra*-associated OTUs. Taxonomic assignment was based on BLAST results from NCBI database. Functional assignment were conducted with all BLAST hits > 97 % similarity. References marked by an asterisk indicate sequences only published in NCBI database. Only mycorrhizal OTUs found on *P. cembra* root samples are included.

Function	Taxonomic assigment	OTU	Total read abundance	Score	Similarity	Accession	Accession reference	Classification	Host specificity (Molina, et al. 1992)	Hosts	Reference
Mycorrhizal	*Amanita submembranacea*	1862	3680	292	100	FJ705275.1	(Borovička *et al.* 2010)	Generalist	Intermediate	Conifers	(Contu 2003)
Mycorrhizal	*Amanita mortenii*	88	9057	287	100	KT317713.1	(Tulloss *et al.* 2015)	Generalist	Broad	Conifers, Angiosperms	(Tulloss & Roosevelten 1996)
Mycorrhizal	*Amphinema byssoides*	2127	10332	327	100	KP125585.1	(Hutter et al. 2014)*	Generalist	Intermediate	Conifers	(Erland & Taylor 1999)
Mycorrhizal	*Amphinema byssoides*	2128	5602	327	100	KP814522.1	(Rosenthal *et al.* 2017)	Generalist	Intermediate	Conifers	(Erland & Taylor 1999)

Mycorrhizal	*Amphinema byssoides*	2130	1266	311	98	JQ711820.1	(Jones *et al.* 2012)	Generalist	Intermediate	Conifers	(Erland & Taylor 1999)
Mycorrhizal	*Cortinarius caesiobrunneus*	1874	57	357	100	NR_121337.1	(Niskanen et al. 2009)	Generalist	Broad	Conifers, Angiosperms	(Niskanen, Kytövuori & Liimatainen 2009)
Mycorrhizal	*Cortinarius privignipallens*	2202	131	342	100	KP165569.1	(Liimatainen 2014)*	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	*Cortinarius cadi-aguirrei*	2201	864	375	100	KJ866953.1	(Garrido et al. 2014)*	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	*Cortinarius orasericeus*	2188	1685	357	100	KP013204.1	(Liimatainen *et al.* 2015)	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	*Cortinarius colus*	2197	902	346	99	HM240522.1	(Berbee et al. 2010)*	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De

											Miguel 2005)
Mycorrhizal	*Dermocybe cinnamomea*	2182	241	372	100	AY750159.1	(Cline, Ammirati & Edmonds 2005)	Generalist	Intermediate	Conifers	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	*Elaphomyces* cf. *granulatus*	1323	38303	357	99	EU597088.1	(Jones *et al.* 2008)	Generalist	Broad	Conifers, Angiosperms	(Molina, Massicotte & Trappe 1992)
Mycorrhizal	*Elaphomyces muricatus*	1324	1547	357	100	KR029732.1	(Larsson et al. 2015)*	Generalist	Broad	Conifers, Angiosperms	(Miller & Miller 1984; De Roman, Claveria & De Miguel 2005)
Mycorrhizal	*Lactarius rufus*	1373	1332	481	100	KX394300.1	(Barge et al. 2016)*	Generalist	Broad	Conifers, Angiosperms	(Giltrap 1979; De Roman, Claveria & De Miguel 2005))
Mycorrhizal	*Otidea leporina*	749	3843	327	100	KM010092.1	(Olariaga *et al.*	Generalist	Intermediate	Conifers	(Olariaga *et al.*

							2015)				2015)
Mycorrhizal	*Paxillus involutus*	1159	687	433	100	KP753338.1	(Klavina et al. 2017)*	Generalist	Broad	Conifers, Angiosperms	(Cairney & Chambers 1999)
Mycorrhizal	*Rhizopogon salebrosus*	1744	3797	451	99	KJ595008.1	(Garibay et al. 2014)*	Specialist	Narrow	*Pinus*	(Kennedy, Peay & Bruns 2009; Kennedy *et al.* 2010; Kohout *et al.* 2011)
Mycorrhizal	*Russula sapinea*	1150	440	474	100	KR019818.1	(Klavina *et al.* 2016)	Generalist	Intermediate	Conifers	(Ronikier & Adamčík 2009; Gaitnieks *et al.* 2016)
Mycorrhizal	*Tomentella stuposa*	135	465	401	100	JQ888213.1	(Pickles *et al.* 2012)	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	*Tylospora*	1791	40758	357	100	KT447180.1	(Klavina et al.	Generalist	Intermediate	Conifers	(Erland &

	asterophora						2015)*				Taylor 1999)
Mycorrhizal	*Tylospora fibrillosa*	1792	38733	357	100	KP783485.1	(Malysheva *et al.* 2016)	Generalist	Intermediate	Conifers	(Erland & Taylor 1999)
Mycorrhizal	*Tylospora fibrillosa*	1793	36954	355	100	KP753374.1	(Klavina et al. 2015)*	Generalist	Intermediate	Conifers	(Erland & Taylor 1999)
Mycorrhizal	*Tylospora fibrillosa*	1794	512	320	100	AF052561.1	(Eberhardt, Walter & Kottke 1999)	Generalist	Intermediate	Conifers	(Erland & Taylor 1999)
Mycorrhizal	Uncultured *Cortinarius* clone	1728	1052	377	100	KC412507.1	(Jarvis et al. 2012)*	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	Uncultured *Piloderma* clone	1954	604	359	100	KP125801.1	(Hutter et al. 2014)*	Generalist	Intermediate	Conifers	(Erland & Taylor 1999)
Mycorrhizal	Uncultured *Piloderma* clone	1955	6451	346	100	HM488495.1	(Kluber, Smith & Myrold 2011)	Generalist	Intermediate	Conifers	(Erland & Taylor 1999)

Mycorrhizal	Uncultered Tomentella clone	139	2231	403	100	JQ791166.1	(Teste, Lieffers & Strelkov 2012)	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	Uncultured *Tomentella* clone	142	394	401	100	JN544505.1	(Peitner et al. 2011)*	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	Uncultered Tomentella clone	143	201	394	99	FM992972.1	(Kjøller & Clemmensen 2009)	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	Uncultured *Tylospora* clone	430	5408	363	100	KP125725.1	(Hutter et al. 2014)*	Generalist	Broad	Conifers, Angiosperms	(De Roman, Claveria & De Miguel 2005)
Mycorrhizal	*Wilcoxina rehmii*	177	435	294	100	JX129137.1	(Huiying et al. 2012)*	Generalist	Intermediate	Conifers	(Bidartondo, Baar & Bruns 2001; Bingham & Simard 2012)

Pathogenic	*Gremmenia infestans*	1198	588	252	98	KM216393.1	(Crous *et al.* 2014)	Specialist	Narrow	*Pinus*	(Burdon *et al.* 1992; Barbeito *et al.* 2013)
Pathogenic	*Gremmenia infestans*	282	213	250	99	KU063958.1	(Gernandt, Camacho & Stone 1997)	Specialist	Narrow	*Pinus*	(Burdon *et al.* 1992; Barbeito *et al.* 2013)
Pathogenic, saprothrophic	*Lophodermium pinastri*	1350	18858	267	100	KF013542.1	(Prihatini *et al.* 2014)	Generalist	Intermediate	Conifers	(Nierhaus-Wunderwald 1996; Prihatini *et al.* 2015)
Pathogenic, saprothrophic	*Lophodermium conigenum*	1352	653	278	100	KF636501.1	(Maubane et al. 2013)*	Generalist	Intermediate	Conifers	(Nierhaus-Wunderwald 1996; Prihatini *et al.* 2015)

Table S3. Pearson correlation coefficients of abiotic and biotic factors. MST = mean daily temperature for the hottest three months (i.e. summer), MWT = mean daily temperature for the coldest three months (i.e. winter), MMaxST = mean daily maximum temperature for the hottest three months, MMinWT = mean daily minimum temperature for the coldest three months, MHM = mean daily temperature for the hottest month (i.e. July), MCM = mean daily temperature for the coldest month (i.e. January), MMaxHM = mean daily maximum temperature for the hottest month, MMinCM = mean daily minimum temperature for the coldest month

	Distance to host	Soil pH	Vegetation cover	Soil moisture	MST	MWT	MMaxST	MMinWT	MHM	MCM	MMaxHM	MMinCM
Distance to host												
Soil pH	0.15											
Vegetation cover	0.01	-0.12										
Soil moisture	0.50	-0.01	0.33									
MST	0.21	0.11	0.42	0.12								
MWT	-0.02	-0.18	0.23	0.51	-0.05							
MMaxST	0.28	0.11	0.45	0.11	0.97	-0.13						
MMinWT	0.15	-0.16	0.35	0.68	-0.02	0.89	-0.05					
MHM	0.26	0.13	0.41	0.13	0.99	-0.08	0.97	-0.03				
MCM	0.16	-0.16	0.44	0.69	0.18	0.88	0.15	0.93	0.16			
MMaxHM	0.28	0.11	0.45	0.11	0.97	-0.14	1.00	-0.05	0.97	0.14		
MMinCM	0.20	-0.15	0.46	0.72	0.17	0.82	0.16	0.95	0.16	1.00	0.16	

Table S4. Summary statistics of backwards stepwise regression from full to final GLMs of generalist and specialist OTU abundances of *P. cembra*-associated pathogenic (a, c) and ectomycorrhizal (b, d) fungi including all abiotic and biotic factors. Models included mean daily maximum summer temperature (MMaxST) and mean daily minimum winter temperature (MMinWT) instead of mean summer temperature (MST) and mean winter temperature (MWT).

Model response	Coefficients	*p* value full model	*p* value 1. model	*p* value 2. model	*p* value 3. model	*p* value 4. model	*p* value final model
a) Generalist							
pathogens	√Read number	0.010	0.010	0.010	0.010	0.010	
	Distance to host	0.743					
	pH	0.307	0.347				
	Vegetation cover	0.030	0.050	0.030	0.040	0.030	
	MMaxST	0.208	0.248	0.455			
	MMinWT	0.129	0.099	0.109	0.109	0.020	
	Soil moisture	0.436	0.248	0.277	0.327		
	Valley	0.406	0.436	0.624	0.465	0.584	
	Season	0.376	0.465	0.139	0.149	0.178	
					ANOVA		$p = 0.116$
b) Generalist							
ECM	√Read number	0.010	0.010	0.010	0.010	0.010	0.010
	Distance to host	0.673					
	pH	0.307	0.564				
	Vegetation cover	0.267	0.010	0.208			
	MMaxST	0.010	0.059	0.030	0.218	0.356	
	MMinWT	0.020	0.010	0.198	0.307		
	Soil moisture	0.079	0.198	0.069	0.010	0.099	0.030
	Valley	0.010	0.564	0.515	0.218	0.089	0.030
	Season	0.089	0.010	0.208	0.010	0.089	0.040

					ANOVA		$p = 0.158$
c) Specialist pathogen (*G. infestans*)	√Read number	0.089	0.076	0.091	0.144	0.077	
	Distance to host	0.050	0.016	0.003	< 0.001	< 0.001	
	pH	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
	Vegetation cover	0.407					
	MMaxST	0.220	0.3795				
	MMinWT	0.138	0.100	0.144	0.166		
	Soil moisture	0.2483	0.339	0.484			
	Valley	0.313	0,188	0.206	0.195	0.261	
	Season	0.014	0.018	0.014	0.011	0.012	
					ANOVA		**$p = < 0.001$**
d) Specialist ECM (*R. salebrosus*)	√Read number	0.014	0.013	0.018	0.014		
	Distance to host	0.005	0.001	0.001	< 0.001		
	pH	0.449	0.433				
	Vegetation cover	0.371	0.381	0.228	0.0401		
	MMaxST	0.240	0.255	0.122	0.050		
	MMinWT	0.426	0.402	0.436			
	Soil moisture	0.840					
	Valley	< 0.001	< 0.001	< 0.001	< 0.001		
	Season	0.060	0.062	0.101			
					ANOVA		**$p = 0.004$**

Table S5. Summary statistics of backwards stepwise regression from full to final GLMs of generalist and specialist OTU abundances of *P. cembra*-associated pathogenic (a, c) and ectomycorrhizal (b, d) fungi including all abiotic and biotic factors. Models included mean temperature of the hottest month (MHM) and mean temperature of the coldest month (MCM) instead of mean summer temperature (MST) and mean winter temperature (MWT).

Model response	**Coefficients**	***p* value full model**	***p* value 1. model**	***p* value 2. model**	***p* value 3. model**	***p* value 4. model**	***p* value final model**
a) Generalist							
pathogens	√Read number	0.010	0.020	0.020	0.010	0.010	0.020
	Distance to host	0.743					
	pH	0.297	0.317				
	Vegetation cover	0.050	0.030	0.050	0.059	0.040	0.050
	MHM	0.099	0.218	0.396			
	MCM	0.149	0.089	0.208	0.158	0.129	
	Soil moisture	0.485	0.218	0.188	0.178		
	Valley	0.485	0.455	0.614	0.406	0.624	0.446
	Season	0.366	0.396	0.129	0.139	0.149	0.139
					ANOVA		$p = 0.140$
b) Generalist							
ECM	√Read number	0.010	0.010	0.010	0.010	0.010	0.010
	Distance to host	0.624					
	pH	0.366	0.624				
	Vegetation cover	0.178	0.020	0.248			
	MHM	0.010	0.099	0.109	0.406		
	MCM	0.129	0.574	0.228	0.188	0.337	
	Soil moisture	0.020	0.317	0.149	0.089	0.089	0.208
	Valley	0.030	0.010	0.010	0.149	0.624	0.089
	Season	0.030	0.218	0.010	0.020	0.010	0.040
					ANOVA		$p = 0.168$

c) Specialist pathogen (*G. infestans*)	√Read number	0.112	0.096	0.141	0.124	0.077	
	Distance to host	0.022	0.010	< 0.001	< 0.001	< 0.001	
	pH	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
	Vegetation cover	0.511					
	MHM	0.258	0.362	0.470			
	MCM	0.293	0.231	0.281	0.345		
	Soil moisture	0.450	0.534				
	Valley	0.448	0.311	0.275	0.266	0.261	
	Season	0.016	0.018	0.013	0.012	0.012	
					ANOVA		***p* = < 0.001**
d) Specialist ECM (*R. salebrosus*)	√Read number	0.009	0.009	0.009	0.012	0.008	0.008
	Distance to host	0.007	0.001	< 0.001	< 0.001	0.002	0.001
	pH	0.391	0.384	0.378			
	Vegetation cover	0.416	0.405	0.119	0.057	0.319	
	MHM	0.519	0.213	0.140	0.062		
	MCM	0.220	0.341				
	Soil moisture	0.939					
	Valley	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Season	0.066	0.065	0.069	0.124	0.131	0.010
					ANOVA		***p* = 0.005**

Table S6. Summary statistics of backwards stepwise regression from full to final GLMs of generalist and specialist OTU abundances of *P. cembra*-associated pathogenic (a, c) and ectomycorrhizal (b, d) fungi including all abiotic and biotic factors. Models included mean daily maximum temperature of the hottest month (MMaxHM) and mean daily minimum temperature of the coldest month (MMinCM) instead of mean summer temperature (MST) and mean winter temperature (MWT).

Model response	Coefficients	*p* value full model	*p* value 1. model	*p* value 2. model	*p* value 3. model	*p* value 4. model	*p* value final model
a) Generalist							
pathogens	√Read number	0.010	0.010	0.010	0.030	0.010	0.020
	Distance to host	0.743					
	pH	0.277	0.307				
	Vegetation cover	0.059	0.089	0.059	0.059	0.020	0.020
	MMaxHM	0.129	0.178	0.327	0.376		
	MMinCM	0.139	0.069	0.139	0.149	0.099	
	Soil moisture	0.455	0.297	0.347			
	Valley	0.485	0.376	0.564	0.604	0.465	0.455
	Season	0.327	0.376	0.089	0.149	0.129	0.129
					ANOVA		$p = 0.133$
b) Generalist							
ECM	√Read number	0.010	0.020	0.020	0.010	0.010	0.010
	Distance to host	0.703					
	pH	0.257	0.752				
	Vegetation cover	0.248	0.010	0.218	0.317		
	MMaxHM	0.010	0.050	0.040	0.050	0.327	
	MMinCM	0.020	0.257	0.376			
	Soil moisture	0.010	0.198	0.030	0.050	0.079	0.238
	Valley	0.099	0.248	0.010	0.465	0.099	0.079
	Season	0.020	0.010	0.584	0.010	0.020	0.059

					ANOVA		$p = 0.228$
c) Specialist pathogen (*G. infestans*)	√Read number	0.099	0.082	0.135	0.123	0.077	
	Distance to host	0.041	0.012	< 0.001	< 0.001	< 0.001	
	pH	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
	Vegetation cover	0.371					
	MMaxHM	0.149	0.283	0.390			
	MMinCM	0.298	0.206	0.274	0.326		
	Soil moisture	0.358	0.468				
	Valley	0.424	0.247	0.228	0.233	0.261	
	Season	0.015	0.019	0.0138	0.012	0.012	
					ANOVA		**p = < 0.001**
d) Specialist ECM (*R. salebrosus*)	√Read number	0.011	0.011	0.010	0.008	0.013	0.008
	Distance to host	0.004	0.004	0.002	0.003	0.003	0.001
	pH	0.398	0.349	0.336	0.229		
	Vegetation cover	0.701					
	MMaxHM	0.237	0.267	0.347			
	MMinCM	0.108	0.076	0.071	0.092	0.074	
	Soil moisture	0.463	0.477				
	Valley	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Season	0.045	0.039	0.049	0.053	0.105	0.100
					ANOVA		**p = 0.005**

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Appendix 3: High throughput sequencing combined with null model tests reveals specific plant-fungi associations linked to seedling establishment and survival

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1. Development and planning:
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2. Field work/data collection:
   DM collected soil samples (95%) with help of MB (5%). **DM** conducted seed translocation experiment (100%).
3. Compilation of data sets and figures/tables:
   DM assembled the data sets and prepared the figures (100%).
4. Data analyses and interpretation:
   DM performed the statistical analyses (85%) with input from PM and ELN (in total 15%). **DM** interpreted results (80%), PM and ELN contributed with the interpretation of the results (in total 20%).
5. Preparation of manuscript:
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High throughput sequencing combined with null model tests reveals specific plant-fungi associations linked to seedling establishment and survival

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Abstract

1. Plant-fungal interactions are important for plant community assembly, but quantifying these relationships remains challenging. High throughput sequencing of fungal communities allows us to identify plant-fungal associations at a high level of resolution, but often fails to provide information on taxonomic and functional assignment of fungi.

2. We transplanted seeds of *Pinus cembra* across an elevational gradient (1850-2250 m a.s.l.) and identified environmental factors and known fungal associates important for seedling establishment and survival. We then applied null model tests to identify taxonomically unassigned fungi associated with pine recruitment.

3. Early seedling establishment was determined by abiotic environmental factors, while seedling survival was predominantly affected by biotic environmental factors (i.e., the abundance of a fungal pathogen known from literature and the distance to adult trees). Null model tests identified known mycorrhizal partners and a large number of unknown operational taxonomic units (OTUs) associated with seedling survival, including saprotrophic and pathogenic species. These results highlight that unknown fungal OTUs, which are usually discarded from analyses, could play a crucial role for plant survival.

4. *Synthesis*: We conclude that high throughput metabarcoding paired with null model tests, is a valuable approach for identifying hidden plant-fungal associations within large and complex DNA metabarcoding datasets. Such an approach can be an important tool in illuminating the black box of plant-microbe interactions, and thus understanding ecosystem dynamics.

Keywords: Elevational gradient, DNA metabarcoding, fungal pathogens, ITS, mycorrhiza, OTU, plant regeneration, plant-soil interaction, transplant experiment

Introduction

Plant-soil interactions play an important role in plant community assembly (Klironomos, 2002; Wardle *et al.*, 2004; Bever *et al.*, 2010; Mangan *et al.*, 2010). Positive or negative interactions between plants and soil microbiota can accelerate or retard community succession (e.g., Wardle *et al*,. 2004; Fukami, & Wardle, 2005), promote species co-existence (e.g., Janzen, 1970; Connell, 1971; Packer, & Clay, 2000; Hersh *et al.*, 2012) and influence the spread of alien plant species (e.g., Chun *et al.*, 2010; Urcelay *et al.*, 2017). Positive interactions, such as mutualisms between plants and fungi, promote the fitness of both partners (Smith, & Read, 2008; Lambers, & Teste, 2013; Peay 2018; Waller *et al.*, 2018). In contrast, negative plant-soil interactions, often associated with the accumulation of fungal pathogens, can have wide-ranging consequences in plant communities, such as preventing the dominance of species (Tucker, & Talbot, 2001; Malcolm *et al.*, 2013; Bever *et al.*, 2015). Fungal pathogens can cause seed decay, necrosis of roots and ultimately seedling mortality (Packer, & Clay, 2000; Bever *et al.*, 2015). Virulence of the pathogens may depend on environmental factors (Packer, & Clay, 2000; Bever *et al.*, 2015), including soil nutrient availability and temperature (Alexander, 2010; Bever *et al.*, 2015). Such effects can be further modified by mycorrhizal fungi, which can provide protection against disease, greater access to soil nutrients, and resistance to climate extremes (Jung *et al.*, 2012). It is also known that different aspects of plant-soil interactions affect different life stages of plants (van der Putten *et al.*, 2013). In the early stages of recruitment, e.g. during germination, plants are especially vulnerable to abiotic factors (e.g. water availability), whilst seedlings may be more affected by the abundance of fungal pathogens and mutualists, as well as other biotic factors (such as competition with neighbouring plants or herbivory; Packer, & Clay, 2000; Van Der Heijden, & Horton, 2009; Hersh *et al*,. 2012).

To date, most knowledge of plant-soil interactions has been obtained from lab and pot experiments, which have revealed the relative importance of positive and negative

interactions between entire microbial communities associated with a particular plant species or plant community (Kardol *et al.*, 2013). These experiments often only exhibit the overall net balance of positive and negative interactions (Klironomos, 2002; van der Putten *et al.*, 2013), and the role of individual soil microbial species is not identified. Accordingly, conclusions from these studies remain broad and lack mechanistic detail. Further, experimental settings often over-simplify the complexity of real ecosystems, although it is known that the environmental context affects the outcome of plant-soil interactions (Kardol *et al.*, 2006; Manning *et al.*, 2008; van der Putten *et al.*, 2013, 2016). The quantification of plant-soil interactions during different plant life history stages across 'real world' environmental gradients has been challenging (Kardol *et al.*, 2013). This is mainly due to methodological constraints, including difficulties in characterizing complex microbial communities, and difficulties in understanding the ecological roles of recorded species, given that only about 7% of an estimated 1.5 million fungal species are taxonomically and functionally assigned (Blackwell, 2011; Tedersoo *et al.*, 2014).

High throughput metabarcoding has advanced our understanding of fungal diversity (Tedersoo *et al.*, 2014), distribution (Bahram *et al.*, 2018), community composition (Mucha *et al.*, 2017), ecological roles of members of the community (de Vries *et al.*, 2018), and species interactions (Bogar *et al.*, 2018). This method allows microbial taxa to be grouped into ecologically meaningful molecular operational taxonomic units (OTUs; Taberlet *et al.*, 2012), and to assign putative ecological functions such as saprotrophs, pathogens, or mutualists (Nilsson *et al.*, 2018). However, the identification of fungal diversity relies on curated databases (e.g. UNITE; Koljalg *et al.*, 2005) and specific pipelines (e.g. FUNGuild; Nguyen *et al.*, 2016), and the sparsity of data in these databases severely limits the taxonomic and functional assignment of taxa detected by metabarcoding. As a result, a large fraction of OTU-level diversity is often excluded from downstream ecological analyses (Blackwell, 2011; Tedersoo *et al.*, 2014). For example, in a previous study on the spatial occurrences of

plant-associated fungi (Merges *et al.*, 2018) we obtained a dataset of over 1000 distinct fungal OTUs, indicating a highly diverse fungal community. However, BLAST searches of the acquired fungal OTUs against reference sequence databases (i.e. UNITE and GenBank; Koljalg *et al.*, 2005), allowed us to assign only 58 % of the OTUs to genus level. Only these taxa could be included in a literature research to identify candidate OTUs associated with our focal plant species, and the remaining 42 % of unassgined OTUs were omitted from further analysis (Merges *et al.*, 2018). This phenomenon has occurred in many similar studies where only a small fraction of the acquired OTUs were included in the final analyses (e.g. Mundra *et al.*, 2015; Glynou *et al.*, 2017; Schmidt *et al.*, 2017). It is unlikely that the problem of OTU identification due to incomplete databases will disappear soon given the sheer diversity of fungi (Blackwell, 2011; Tedersoo *et al.*, 2014) and our limited biological knowledge of many taxa, especially those which are only known by ITS sequences from environmental samples. Nevertheless, some unassigned OTUs could be major players in ecological dynamics (Toju *et al.*, 2016, 2017), and analysing all community members, regardless of taxonomic or functional assignment status, could greatly facilitate our understanding of ecosystem dynamics.

Detecting influential species in a community, so-called keystone taxa, is important for understanding ecosystem function (Banerjee, Schlaeppi, & van der Heijden, 2018), and ecosystem responses to disturbance (Stinson *et al.*, 2006). Different approaches have been proposed to pinpoint species with putative key ecological functions in natural communities, including microbial network analysis (Berry, & Widder, 2014; Banerjee *et al.*, 2018). Network analysis has been applied to visualize co-occurrences between microbial species and statistically determine 'keystone' taxa (i.e. highly connected taxa, with a strong effect on the network structure (Berry, & Widder, 2014; Banerjee *et al.*, 2018). While network approaches identify possible interactions within soil fungal communities, we suggest a complementary null model approach, (Gotelli *et al.*, 2011), which can detect whether the presence, absence or

abundance of a 'species' is associated with a target variable, such as an ecosystem function or performance variable (Ulrich, 2010; Gotelli *et al.*, 2011; Ulrich *et al.*, 2012; Soliveres *et al.*, 2016). Null model randomization tests provide the opportunity to link a functional response to the abundance of species, functional groups, or OTUs (Gotelli *et al.*, 2011; Ulrich *et al.*, 2012; Soliveres *et al.*, 2016). Further advantages are that 1), randomization tests are distribution free and well suited to the non-normal distributions of OTU data; 2) potential species interactions are taken into account by maintaining the community structure during modelling (Gotelli *et al.*, 2011; Ulrich *et al.*, 2012); 3) null model randomization tests have a clear cause-and-effect hypothesis, where species affect the functional response. This underlying hypothesis is advantageous where a directional hypothesis is reasonable, e.g. for investigating effects of species on ecosystem functions (Gotelli *et al.*, 2011; Soliveres *et al.*, 2016). Null model approaches may allow the identification of species that influence other species or ecosystem functions (Ulrich, 2010; Gotelli *et al.*, 2011; Soliveres *et al.*, 2016), but their application to OTU-based microbial community data remains to be tested.

In this study, we used DNA metabarcoding to assess which fungal species were linked to the performance of Swiss stone pine (*Pinus cembra*) at two life stages, establishment and survival, across the pines' natural distribution. The elevational distribution of the pine, ranging from 1850 to 2250 metres above sea level (m a.s.l.), exhibits pronounced changes in both abiotic and biotic conditions, such as microbial community composition (Roll-Hansen, 1989; Neuschulz *et al.*, 2018; Merges *et al.*, 2018). Seedling establishment of *P. cembra* is affected by abiotic factors, such as light and water availability (Neuschulz *et al.*, 2015), but also by biotic factors, such as competition or plant-soil interactions (Barbeito *et al.*, 2012; Neuschulz *et al.*, 2015). While *P. cembra* seedlings are thought to need several mycorrhizal species to efficiently acquire nutrients and water, seedlings are also severely affected by needle pests, such as the snow blight fungus *Gremmenia infestans* (Roll-Hansen, 1989; Smith, & Read, 2008).

We hypothesized that seedlings experience different feedback effects from fungal communities during a) seedling establishment (0-4 months) than b) later stage survival (5-15 months). We predicted that initial seedling establishment is primarily vulnerable to abiotic environmental stresses (Walck *et al.*, 2011; Kueppers *et al.*, 2016), while seedling survival will be more strongly affected by the availability of mycorrhizal partners and the presence of known fungal pathogens (Bardgett *et al.*, 2005). Furthermore, we predicted that a null model approach could help to identify fungal associates important for seedling establishment and survival that are so far taxonomically unassigned. We tested these hypotheses by conducting seed translocation experiments and DNA metabarcoding of fungal communities along replicated altitudinal gradients that span the entire elevational distribution of *P. cembra* and reach beyond the tree's upper distribution limit. We build this study on that of Merges *et al.* (2018), which explored the occurrences of a *P. cembra*-associated subset of taxonomically assigned fungi in relation to environmental factors and adult conspecific trees. First, we used the full DNA metabarcoding dataset containing known plant associates (Merges *et al.*, 2018), and tested the association of known plant associates with seedling establishment and survival of *P. cembra*. Second, we applied null model randomization tests to a) reveal whether this approach can detect the same known fungal associates as used in the first analysis and b) identify previously taxonomically unassigned fungi that are associated with seedling establishment and survival.

Materials and Methods

Study area and design

We conducted this study in the Central Alps within the core of *P. cembra*'s distributional range. We selected two elevational gradients near Davos, Switzerland, one in the Flüela valley (46°48′0.25″N 09°54′15.38″E) and one in the Sertig valley (46°44′0.76″N 9°51′3.5″E).

In both valleys, the lowest elevational belts (about 1850 m a.s.l.) are covered by mixed coniferous forests, mainly comprised of European larch (*Larix decidua* Mill.) and Norway spruce (*Picea abies* (L.) H. Karst). *Pinus cembra* has a unimodal abundance distribution from 1850 m a.s.l. up to 2150 m a.s.l., where *P. cembra* trees (> 3m tall, Harsch *et al.*, 2009) form the tree line. Smaller *P. cembra* individuals can be found up to 2200 m a.s.l., but none are present at and over 2250 m a.s.l. (Neuschulz *et al.*, 2018).

Seed translocation experiments

To study the effects of plant-fungal associations on plant establishment and survival in an environmental context, we conducted seed translocation experiments in both valleys in 2014 and 2015. We divided each valley into nine elevational belts with 50 m elevational intervals ranging from 1850 to 2250 m a.s.l. Following a random-stratified sampling design we installed ten seed bags in 2014 and 20 seed bags in 2015 at each elevational belt (Figure 1). Each seed bag was made of 1.5 mm wire-mesh (to prevent loss of seeds) and contained five *P. cembra* seeds. Seed bags were placed 4 cm deep in the soil and fixed by metal pins. To break dormancy the seeds were placed in a wet clay-sand mixture and exposed to temperature shifts between 5-25°C for 22 weeks (simulated seasonal variation, G. Reiss, pers. comm.). Seed bags were distributed over five microhabitat types (1. under ericaceous vegetation cover, 2. close (0.05 - 1 m) to adult *P. cembra* individuals, 3. open soil, 4. rocky habitat, 5. microsite covered by snow [i.e. late snow lie areas]) at each elevational belt (Fig. 1). At 2250 m a.s.l. (treeless, high-elevational belt) the microhabitat "close to *P. cembra*" was substituted by matgrass (*Nardus stricta* L) dominated sites, to guarantee a balanced sampling design (i.e., equal sample size at each elevational belt). In total, we installed 180 seed bags in 2014 (i.e. two replicates per microhabitat per elevational belt) and 360 in 2015 (i.e. four replicates per microhabitat per elevational belt). We installed the experiment at the beginning of the growing season end of May and evaluated whether seedlings had established before the end of

the growing season at the end of September (Fig. S1). We further monitored the survival of seedlings until the end of their second growing season in the following year (Fig. S1).

Environmental factors

We tested the effects of environmental factors (i.e. light availability, temperature, soil moisture, vegetation cover, and distance to conspecific adults) and fungal occurrences on the establishment and survival of *P. cembra* seedlings. We focused on these early life stages, since they are most vulnerable, and thereby the bottleneck of plant regeneration (Vitasse *et al.*, 2012). Light availability was measured as canopy openness above each seed bag with a spherical densitometer. Ericaceous vegetation cover was recorded by estimating the percentage cover of dominant ground flora species: *Loiseleuria procumbens* (L.) Desv., *Vaccinium* spp. L. and *Rhododendron ferrugineum* L. within 1 m² of each seed bag (Braun-Blanquet, 1964). Ericaceous plants compete for resources with *P. cembra* seedlings but also act as nurse plants by ameliorating harsh environmental conditions (Castro *et al.*, 2002; Bardgett, & Wardle, 2010). Distance to conspecific adults was measured as the distance from each seed bag to the closest adult *P. cembra* by estimation and using a laser range finder (Nikon 800S) for distances over 10 m. The distance to conspecific adults can be an important factor in plant recruitment, as species-specific pathogens and herbivores accumulate close to conspecific adult plants and negatively affect seedling survival (Janzen-Connell Hypothesis; Janzen, 1970; Connell, 1971). Temperature was measured at 16 of the 30 seed bags per elevational belt per valley using iButton data loggers (Maxim). Soil surface temperatures were recorded every four hours over the duration of the study. We calculated the mean of daily maximum temperatures of the hottest three months (MMaxST), since extreme maximum temperatures can induce rapid drying of soils, and thereby induce desiccation of seedlings (Tingstad *et al.*, 2015; Kueppers *et al.*, 2016; Andrus *et al.*, 2018). We also calculated the mean daily minimum temperatures of the coldest three months (MMinWT), since extreme frost events can lead to high seedling mortality in high-elevation ecosystems (Lenoir *et al.*,

2008; Kueppers *et al.*, 2016). We measured soil moisture under dry weather conditions in September by averaging five tensiometer (Theta-Kit version 3) measurements at each seed bag. Water availability is especially important during early establishment, where root systems are barely developed and desiccation can rapidly occur (Kueppers *et al.*, 2016).

Soil fungal communities

Pinus cembra is obligately mycorrhizal, i.e. it requires an ECM mutualist for survival in field conditions (e.g. to acquire soil nutrients; Smith, & Read, 2008). In contrast, the presence of a known pathogen, the snow blight fungus (*G. infestans*), severely limits *P. cembra* survival (Roll-Hansen, 1989; Barbeito *et al.*, 2013), mostly by infecting needles covered by snow in winter. To assess the impact of these and other fungi on seedling performance we re-analysed a DNA metabarcoding dataset of the soil fungal communities that have been recorded at each elevational belt (Merges *et al.*, 2018). Occurrence data for pine-associated fungi obtained from this dataset are published in Merges *et al.*, (2018). This previous study focussed on occurrence patterns of pine-associated fungi in relation to environmental factors and their host, but the effect of fungal occurrences on seedling establishment and survival were not addressed (Merges *et al.*, 2018). Soil fungal communities were recorded by collecting soil samples near the eight seed bags planted per elevational belt following the stratified-random sampling design, focusing on the two most distinct microhabitats (i.e. ericaceous vegetation cover and close to adult *P. cembra* individuals, Fig. 1; Merges *et al.* 2018). Although soil microbial communities are known to be very stable across years, they often show high within-year seasonality (Schadt *et al.*, 2003; Lipson, & Schmidt, 2004; Rudolph *et al.*, 2018). Therefore, soil was sampled twice (May and September 2015) around each seed bag, to account for seasonality, resulting in a total of 288 soil samples (Merges *et al.*, 2018). ECM roots of *P. cembra* were collected to establish a reference database for *P. cembra*-relevant ECM fungi (Merges *et al.*, 2018). Roots of saplings and adult trees were sampled at 1850, 2050 and 2200 m a.s.l. in both valleys in May 2015, of which 100 ectomycorrhizal root tips

per elevation were collected for DNA extraction. DNA extraction, amplification, and sequencing are described in Merges *et al.* (2018). In brief, 300 mg of each root and soil sample were used for DNA extraction (Cubero, & Crespo, 2002). During PCR the ITS2 region was amplified. An Illumina MiSeq was used for paired-end sequencing (2 x 300 bp) at Fasteris SA, Plan-les-Ouates, Switzerland (Merges *et al.*, 2018). Default options for the Illumina pipeline developed by Bálint *et al.* (2014) were applied. Fungi were identified by blasting the OTU representative sequences against UNITE database and GenBank nucleotide database using a 97 % similarity threshold (Merges *et al.*, 2018). Rare OTUs from potential erroneous sequences were excluded, following the recommendation of Bokulich *et al.* (2013). Where possible, the OTU reads were classified into pathogens and mutualists (Merges *et al*., 2018). For pathogens, a systematic search of peer-reviewed journal articles was conducted (Merges *et al*., 2018). For ECM, the *P. cembra* ECM root samples taken at the study site were used to identify a list of candidate ECM OTUs, which resulted in 35 species known to be ECM mutualists (Table S1, Merges *et al*., 2018). For further details on the classification of pathogens and mutualists of *P. cembra* see Merges *et al*. (2018).

Statistical analyses

We fitted two models describing the determinants of establishment (i.e. seed germination and seedling establishment within the first growing season), and survival (i.e. survival from the end of the first growing season to the end of the second growing season). First, we used generalized linear mixed models with a binomial error distribution in the R package 'lme4' (Bates *et al.*, 2015). The predictor variables were environmental factors (i.e. light availability, temperature, soil moisture, vegetation cover, and distance to conspecific adults), the diversity of *P. cembra*-associated mycorrhiza (OTU antilogarithm of the Shannon diversity) and the abundance of pathogenic fungal OTUs, as identified by literature research (see Merges *et al.*, 2018). Abundance data was calculated at the plot level based on the number of soil cores in

which an OTU was detected (i.e. between 0-8, Fig. 1). We assume that OTUs with high plot abundances have a higher likelihood to interact with seedlings growing on the plots. Shannon diversity was chosen to represent the frequency of potential positive interactions since *P. cembra* is known to be associated with a large number of mutualist, whereas abundance was chosen for pathogens, since there are only few fungal pathogens reported to be explicitly associated with *P. cembra* (Barbeito *et al.*, 2012; Rainer *et al.*, 2015). We also included plot ID, region and year as random effects to account for spatial and temporal autocorrelation. Observational level random factors were included in the seedling establishment model to account for overdispersion (Albrecht *et al.*, 2015; Bates *et al.*, 2015). We selected the models by adding explanatory variables in a stepwise manner based on a hypothesized 'hierarchy of controls' using Akaike information criterion correcting for small sample sizes (AICc; Burnham, & Anderson, 2002) and likelihood ratio deletion tests to find the most parsimonious models explaining either establishment or survival (Fig. S2, Table S1; Diaz *et al.*, 2007; Manning *et al.*, 2015). The first step consisted of a model only containing abiotic environmental factors (i.e. maximum summer temperature, minimum winter temperature, mean soil moisture, light availability), which we hypothesized to be the underlying ultimate cause for distribution of all subsequently added explanatory variables in the following steps. In the second step, we added biotic factors, such as vegetation cover and distance to conspecific adults, since these proximate causes are potentially shaping the fungal community variables added in the following step. Finally, the third set of terms was fungal community data: Antilogarithm of Shannon diversity of ectomycorrhizal communities and the abundance of the two pathogenic OTUs (282, 1198). We calculated marginal and conditional R^2 to explore the variance explained by the models (Nakagawa, & Schielzeth, 2013). Marginal R^2 describes the variance explained by the fixed effects, whereas conditional R^2 is the variance explained by both fixed and random effects (Nakagawa, & Schielzeth, 2013).

Second, to detect associations of potential mycorrhizal and pathogenic fungal OTUs with seedling establishment and survival that are not described in the existing literature, we applied null model randomization tests on the full OTU table (Gotelli, Ulrich, & Maestre, 2011; Soliveres *et al.*, 2016). These tests are based on a null model approach where one linear regression is performed between the response and each given species (Ulrich *et al.*, 2012; Soliveres *et al.*, 2016). The observed regression slope is then compared to 1000 random permutations of the species' values and a standardized effect size (SES) is calculated for each species according to: $SES = S_{obs} - S_{sim})/SD$; where S_{obs} is the observed regression slope, S_{sim} is the average of the 1000 simulated regression slopes and SD is the standardized deviation of the slopes of these 1000 randomizations (Gotelli *et al.*, 2011; Ulrich *et al.*, 2012; Soliveres *et al.*, 2016). Significant relationships between the response variable and the abundance of each species is assumed when SES values are higher or lower than a SES of 2 and -2, respectively (Gotelli *et al.*, 2011; Ulrich *et al.*, 2012; Soliveres *et al.*, 2016). As the association between seedling performance and an OTU may be driven by shared responses to environmental drivers, we also conducted a more conservative test of association that accounted for the effects of environmental factors. To do this, we extracted the unexplained residual variances of models that included environmental factors and conducted a second round of null model randomization tests. Residuals were extracted from GLM models with the identical structure as the GLMM models to allow standardization of residuals with the "rstudent" command in R to compensate for difference in leverage (i.e. obtaining studentized residuals; Kutner *et al.*, 2004). Based on the increased risk of type II errors from multiple testing, we expected 5% of the OTUs to have a significant relationship with each response variable by chance (Gotelli & Ellison, 2013, Soliveres *et al.*, 2016). All OTUs that were significantly associated with seedling establishment and survival were parsed against the fungal community database FUNguild (Nguyen *et al.*, 2016). We only retained matches with over 97% similarity and

highly probable confidence rankings to examine which ecological guilds OTUs were assigned to.

Results

In the seed translocation experiments, 411 (15 %) of the 2700 planted seeds established as seedlings within the first growing season (i.e. the period between May and September in 2014 and 2015; Fig. S1). Of these seedling cohorts 68 (17 %) survived to the end of the following growing season (i.e. the period from September to September of the following year; Fig. S1).

We identified a total of 1074 OTUs across the whole study (Merges *et al.*, 2018). Of these, we found two pathogenic OTUs (assigned to the snow blight fungus *G. infestans*) in 83 (29 %) soil samples (Table S2). In *P. cembra* root samples, we found 35 ECM OTUs and 184 (64 %) of the soil samples contained at least one of these (Table S2).

Seedling establishment was negatively associated with maximum summer temperature and positively associated with light availability and soil moisture (Fig. 2 a, Table 1). However soil moisture was negatively associated with the establishment of seedlings at sites with high light availability (significant soil moisture x light availability interaction; Fig. 2 a, Table 1). In contrast, ectomycorrhizal diversity (i.e. antilogarithm of Shannon diversity) and pathogen abundance did not affect the establishment of seedlings, i.e. these variables were not selected for the most parsimonious model (i.e. Table 1). Seedling survival was significantly positively associated with light availability and light availability at long distances to conspecific adults (interaction between light availability and distance to conspecific adult; Fig. 2b, Table 2). In contrast to seedling establishment, survival of seedlings was negatively associated with the abundance of the pathogenic snow blight fungus, *G. infestans,* and the distance to conspecific adults (Fig. 2b & 3, Table 2). Survival of seedlings was estimated to be 16 % lower when *G. infestans* (OTU 282) was present in one soil core per plot and 69 % lower when present in six

soil cores per plot (i.e. highest abundance) relative to seedling survival at plots without *G. infestans* (Fig. 3).

For seedling establishment, the null model randomization test identified 29 (3 %) of the 1074 OTUs as significantly positively associated with established seedlings and 32 (3 %) as negatively associated (Fig. 4, Table S2). Here, 82 % of the OTUs were unassigned, 11 % could be assigned to ectomycorrhizal fungi, 2 % to (predominately) animal pathogen-saprotroph taxa, 3 % could be assigned as lichenized, and 2 % as root endophyte. For seedling survival, the null model randomization test showed 296 (28 %) of the 1074 OTUs as significantly positively associated with survival of seedlings and 217 (20 %) as negatively associated (Fig. 4, Table S2). Here, 87 % of the OTUs were unassigned, 10 % were ectomycorrhizal fungi, 1 % belonged to (predominately) animal pathogen-saprotroph taxa, 1 % was assigned as lichenized, and 1 % as root endophyte. Twenty-two of 35 ECM OTUs (63%) present in our ECM reference database were identified to be significantly associated with the survival of *P. cembra* seedlings. For seedling establishment, the null model randomization test in which environmental factors had been accounted for revealed that, of the 1074 OTUs, two (0.2 %) were positively, and eight (0.7 %) were negatively associated with established seedlings. Here, 80 % were unassigned, 10 % were assigned as lichenized, and 10 % as root endophytes. (Fig. 4, Table S2). For seedling survival, the environment-controlled null model randomization test yielded 247 of the 1074 OTUs (23 %) as significantly positively associated with survival of seedlings and 185 as negatively associated (17 %) (Fig. 4, Table S2). Here, 91 % of the OTUs were unassigned, 6 % were ectomycorrhizal fungi, less than 1 % belonged to (predominately) animal pathogen-saprotroph taxa, 2 % were lichenized and less than 1 % were root endophytes. Eight of 35 ECM OTUs (23 %), which were present in our ECM reference database, were significantly associated with the survival of *P. cembra* seedlings.

The number of OTUs that were significantly associated with seedling establishment in the randomization tests was close to or lower than that expected by chance. The null expectation was 54 out of 1074 OTUs. Tests on data uncorrected for the environment found 61 significant OTU-establishment relationships, and the tests on data corrected for the environment found 10 significant OTU-establishment relationships. In contrast, for seedling survival, the number of significant associations with OTUs was eight to ten times higher than what was expected by chance. Tests on uncorrected data found 513 significant OTU-survival relationships, and the tests on data corrected for the environment found 432 significant OTU-survival relationships. These results are consistent with our other results in indicating a weak role of soil fungi in establishment, but a strong role in determining survival.

Discussion

We found clear evidence that the very early life stages of juvenile trees (i.e. establishment of seedlings during the first four months after translocation) were determined by abiotic environmental factors, whereas the later stages of recruitment (i.e. survival of seedlings until the age of 15 months) were, predominantly affected by biotic environmental factors (i.e., the abundance of a known fungal pathogen and the distance to adult trees). Using null model randomization tests, we revealed patterns of association between unassigned fungi and the establishment and survival of seedlings. This demonstrates the general potential of this method for identifying microbial species involved in the plant-microbe interactions, which drive plant community assembly.

Our results concur with previous studies demonstrating that young seedlings are especially vulnerable to abiotic factors, but that the later stages of recruitment depend more on the biotic environment (Packer, & Clay, 2000; van der Heijden, & Horton, 2009; Hersh *et al.*, 2012). The early establishment of *P. cembra* was fostered by high light availability and soil moisture and limited by high maximum temperature. These findings are in accordance with a

previous study on coniferous subalpine tree species in North American that showed similar positive responses to light availability (Kroiss, Hillerislambers, & D'Amato, 2015). Several studies have shown that warm and dry conditions during seedling establishment limit seedling survival rates (Tingstad *et al.*, 2015; Kueppers *et al.*, 2016; Andrus *et al.*, 2018). Extreme temperature events are often related to summer drought stress, as the soil surface dries out rapidly and establishing seedlings with poorly developed root systems could fail to assimilate sufficient water for physiological processes (Andrus *et al.*, 2018; Brodersen, Germinot, & Smith, 2018). The survival of *P. cembra* seedlings was reduced where there was a high abundance of the pathogenic snow blight fungus *G. infestans*, and by increasing distance to conspecific adults. Snow blight fungi, such as *G. infestans*, are known to infect pine needles mostly under winter snow cover, where infected needles are killed (Roll-Hansen, 1989; Burdon *et al.*, 1992). Negative distance dependency of seedling survival is a well-known mechanism in the Janzen-Connell framework (Janzen, 1970; Connell, 1971) in which pathogens and herbivores accumulate close to adult plants, thereby creating negative above and belowground feedbacks for conspecific seedlings (Packer, & Clay, 2000; Bell *et al.*, 2006; Liang *et al.*, 2016; Merges *et al.*, 2018). However, in this study there was a decrease of seedling survival with increasing distances to conspecific adults. This could be explained by more favourable conditions for snow blight fungus *G. infestans* above the tree line (i.e. higher density snowpack), harsher abiotic conditions away from adult trees, and missing positive interactions with the mycelia of suitable ectomycorrhizal partners.

We could not detect any effects of ground vegetation cover on the establishment and survival of seedlings, although several previous studies found significant relationships between these factors (e.g. Castro *et al.*, 2002; Bardgett, & Wardle, 2010; Tingstad *et al.*, 2015; Kueppers *et al.*, 2016; Andrus *et al.*, 2018). Ectomycorrhizal diversity was also not associated with the establishment and survival of seedlings. This could be explained by the fact that mycorrhizal diversity was generally high, with a minimum of 16 ECM species being

present at each elevational belt. This high ECM diversity could have provided ample inoculum, as well as a suitable mutualistic partner, should the species identity of the fungal partner matter. Potential benefits for recruitment of seedlings mediated through only a few suitable mutualistic partners were indicated by the results from the environment-controlled null model approach, where only twenty-three percent of the ECM OTUs in our ECM reference database where shown as significantly associated with seedling survival.

The null model approach revealed that fungi, which are currently taxonomically and functionally unassigned, might be associated with the establishment and survival of plant seedlings. Interestingly, when we compared the results of the model that linked known fungal associates with the associations detected by the null model randomization tests, we found evidence from both approaches that early seedling establishment was less affected by microbial interactions. First, the number of fungal OTUs associated with seedlings during establishment was eight to 43 times lower than the number of fungal OTUs associated with the survival of seedlings. Second, the number of OTUs significantly associated with seedling establishment was comparable to or lower than what we had expected by chance, whereas the number of OTUs significantly associated with seedling survival was eight to ten times higher than expected. Such variation in the strength and number of associations between different seedling life stages could be linked to variability in potential benefits gained from mutualists such as mycorrhizal fungi (Bardgett *et al.*, 2005). For example, the very early life stages of large seeded plants like *P. cembra* (i.e. germination and early establishment) are independent from nutrient supply by fungal mutualist, whereas for survival the formation of mycorrhiza is obligate (Smith, & Read, 2008). In the second null model randomization test, where we used the residual variance of the models to account for environmental factors, less than one percent of fungal OTUs were significantly associated with establishment and 40 % with survival of seedlings. Fungal OTUs filtered out by this analysis may share the same environmental niche

as the establishing and surviving seedlings, whereas those retained fungal OTUs may be more intimately linked to the establishment and survival of seedlings.

Apart from the ECM OTUs present in our reference database that were yielded in the null model tests as significantly associated with seedling survival, the majority of fungi identified were not present in the UNITE database (Koljalg *et al.*, 2005) or the fungal community dataset FUNGuild (Nguyen *et al.*, 2016). However, those few fungal species that were assignable belonged mostly to the ecological guild of mycorrhiza, thereby representing a group of fungi known to interact with plants (Table S1). The identification of additional mycorrhizal fungi, so far unknown to be associated with *P. cembra*, supports the validity of the null model randomization tests for identifying plant-fungal associations. Accordingly, our results suggest that a null model approach can be a valuable technique for reducing the complexity of DNA metabarcoding datasets (e.g., the reduction of very large number of OTUs into a far smaller number of candidate OTUs), as it allows identification of potential plant-fungal associations with OTUs, independent from their functional and taxonomical assignment. Ideally, results of null model randomization tests are confirmed by checking whether at least some of the candidate taxa are known to be associated with the function or plant species of interest, thus demonstrating credibility. We are aware that by linking plot level abundance to seedling performance we might have missed some interactions with fungi that are only found in the rhizosphere (Genney, Anderson, & Alexander, 2006; Lindahl *et al.*, 2007). Nevertheless, we were able to identify the effect of a known pathogen, *G. infestans*, on seedling survival, which illustrates that associations can be detected at this scale. The null model approach suggested here can be an important tool in illuminating the black box of plant-microbe interactions, e.g. by generating a shortlist of microbial species that may be important in driving plant performance and thus should be investigated in further detail. For example, the unassigned OTUs identified here could potentially be located on our field site, cultured and used in more controlled studies of seedling recruitment. Previous studies have

demonstrated a scattered distribution of fungi across elevational (Van Nuland *et al.*, 2017; Merges *et al.*, 2018) and latitudinal (van der Putten, 2012; Tedersoo *et al.*, 2014) gradients and have predicted consequences for the movement of tree lines through plant seedling survival in response to climate change. Here we provide evidence that the abundance of certain fungal OTUs can be significantly associated with survival rates of plant seedlings. Our findings suggest that if fungi have a lower capacity to disperse and migrate under climate change than plants, potential tree line advances in response to altered climatic conditions could be slowed down or accelerated depending on the balance of pathogens to mutualists and their presence and dispersal beyond the current tree line. Such limitation may be even stronger in Arctic ecosystems, where dispersal limitation of microorganisms could be far greater due to the greater distances involved.

Conclusion

Identifying microbial species involved in plant-soil interactions is a major challenge in ecology, particularly in field-based studies. Here we present one of the first field studies to identify a subset of functionally distinct members of the soil fungal community that are likely to affect plant establishment and survival. Our results show that the proportion of taxonomically and functionally unknown fungi that are associated with seedling performance is particularly high during early plant establishment, which may hint at previously unknown roles of fungi in seed germination and survival. The results presented here outline future research directions in above and below ground species interactions, for instance testing whether individual soil microbial species can accelerate or retard plant community succession (e.g., Wardle *et al*,. 2004; Fukami, & Wardle, 2005), or promote species co-existence (e.g., Janzen, 1970; Connell, 1971; Packer, & Clay, 2000; Hersh *et al.*, 2012). We demonstrate that DNA metabarcoding coupled with an ecologically relevant classification of ITS sequences and experimental linkage to plant life stages is a promising approach to unravel potential

plant-fungal associations at a previously unattainable resolution. Furthermore, this approach may also be applied to a wide range of other poorly understood soil taxa, which determine plant performance (e.g. oomycetes and bacteria). In the past, incomplete reference databases and limitations in taxonomically assigning fungal OTUs have led researchers to disregard a substantial part of the observed biodiversity. Thus, only a small fraction of plant-fungal interactions are known, and ecologically important relationships remain hidden. Our study reveals that the combination of community barcoding and a null model approach has potential to overcome some of these constrains. For example, applying our approach in other systems could help to identify 'keystone' OTUs with an important role in structuring plant communities over a wide range of habitats and ecosystem types.

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Authors Contribution

D.M., M.B., I.S and E.L.N. conceived and designed the project. D.M. and M.B. collected the data. D.M. performed the analyses with input from M.B., P.M. and E.L.N. D.M. and E.L.N. led the writing of the manuscript. All authors contributed to the various drafts and gave final approval for publication.

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Data accessibility

Data from this paper are deposited in the Dryad Digital Repository: https://doi.org/10.5061/dryad.qh5js47 (Merges, Bálint, Schmitt, Manning, & Neuschulz, 2019)

Table 1: Final generalized linear mixed model showing the effect of environmental factors (i.e., temperature, soil moisture, light availability and their interactions on Swiss stone pine (*P. cembra*) seedling establishment. Observational level, plot ID, region and sampling season were included as random effects. Significance ($p < .05$) is indicated in bold. MMaxST = mean daily maximum temperature for the hottest three months. SM = soil moisture, LA = light availability.

Variable	Parameter Estimate	Std. Error	ΔAICc [b]	*P* value [b]
Intercept	-1.568	0.432		
Mean summer maximum temperature (MMaxST)	-0.384	0.111	-19.3	**<0.001**
Soil moisture (SM)	0.257	0.089	-10.4	**<0.001**
Light availability (LA)	0.285	0.092	-17.5	**<0.001**
MMaxST×SM	0.305	0.096	-7.9	**0.002**
MMaxST×LA	-0.240	0.097	-3.8	**0.017**
SM×LA	-0.270	0.076	-10.4	**<0.001**

N = 539, explained variance = 6.0% (marginal R^2) / 22% (conditional R^2)

[b] Assessed with a likelihood ratio deletion test.

Table 2: Final generalized linear mixed model showing the effect of environmental factors (i.e., light availability, distance to conspecific adult and their interactions) and fungal pathogen abundance (i.e. OTU 282) on Swiss stone pine (*P. cembra*) seedling survival. Plot ID, region and sampling season were included as random effects. Significance ($p < .05$) is indicated in bold. LA = light availability, DA = distance to conspecific adult *P. cembra*.

Variable	Parameter Estimate	Std. Error	ΔAICc [b]	*P* value [b]
Intercept	-2.60	0.85		
Light availability (LA)	1.44	0.73	-2.42	**0.041**
Distance to adult (DA)	-2.05	1.51	-10.24	**<0.001**
Fungal pathogen abundance (OTU 282)	-0.44	0.16	-7.63	**0.003**
LA×DA	2.55	1.41	-2.97	**0.026**

N = 302, explained variance = 49% (marginal R^2) / 53% (conditional R^2)

[b] Assessed with a likelihood ratio deletion test.

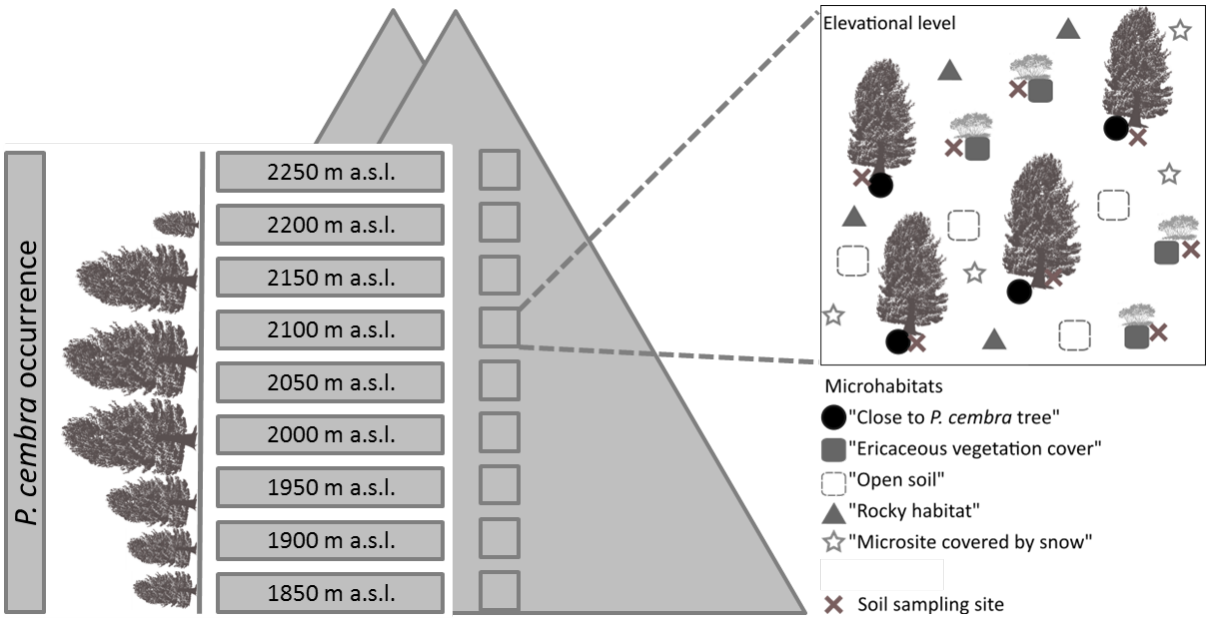

Figure 1: Random stratified sampling design (insert box on the right) along nine elevational levels (ranging from 1,850 to 2,250 m a.s.l.) covering the elevational distribution of Swiss stone pine (*P. cembra*).

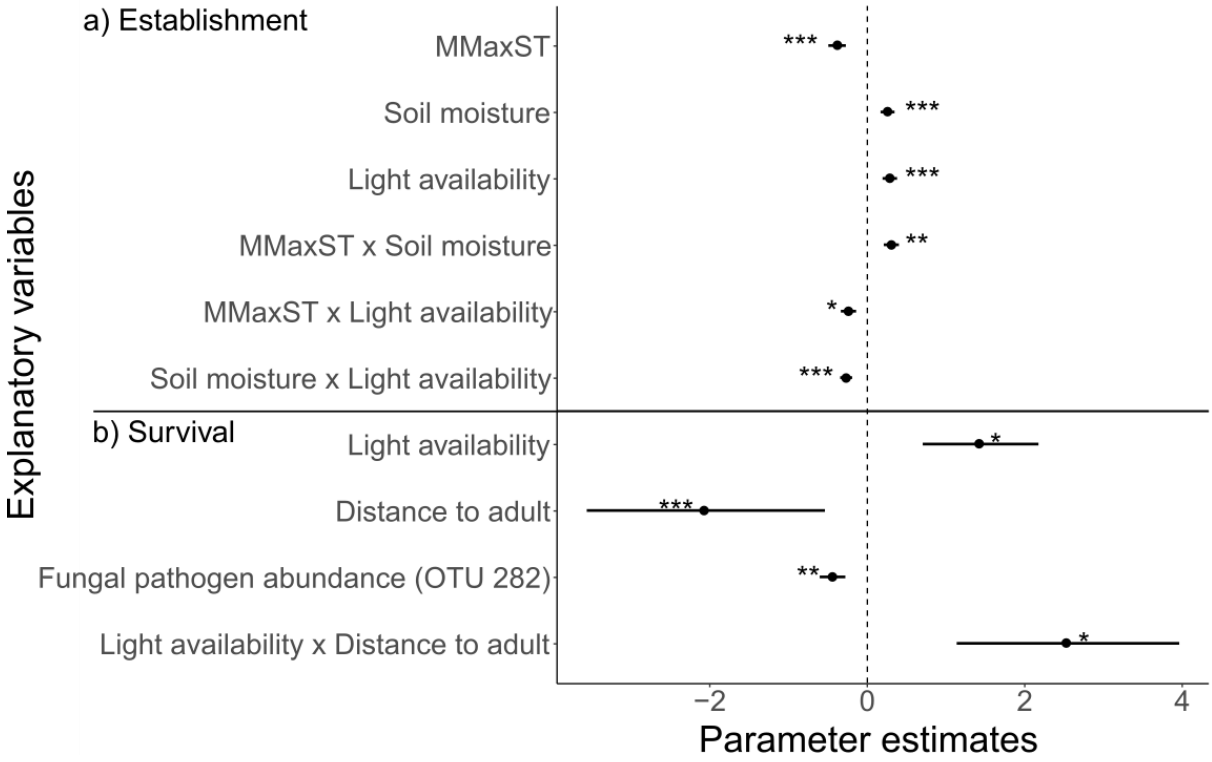

Figure 2: Generalized linear mixed effects model showing the effect of mean daily maximum temperature of the hottest three months, soil moisture, light availability, fungal pathogen abundance (OTU 282) abundance and the distance to an adult conspecific as well as their interacting effects on the probability of (a) seedling establishment and (b) survival of Swiss stone pine (*P. cembra*). Models were fitted assuming a binomial error distribution. Black points show the model fit ($p < 0.01$) with standard error added as black lines. Asterisks indicate level of significance obtained from likelihood ratio deletion tests.

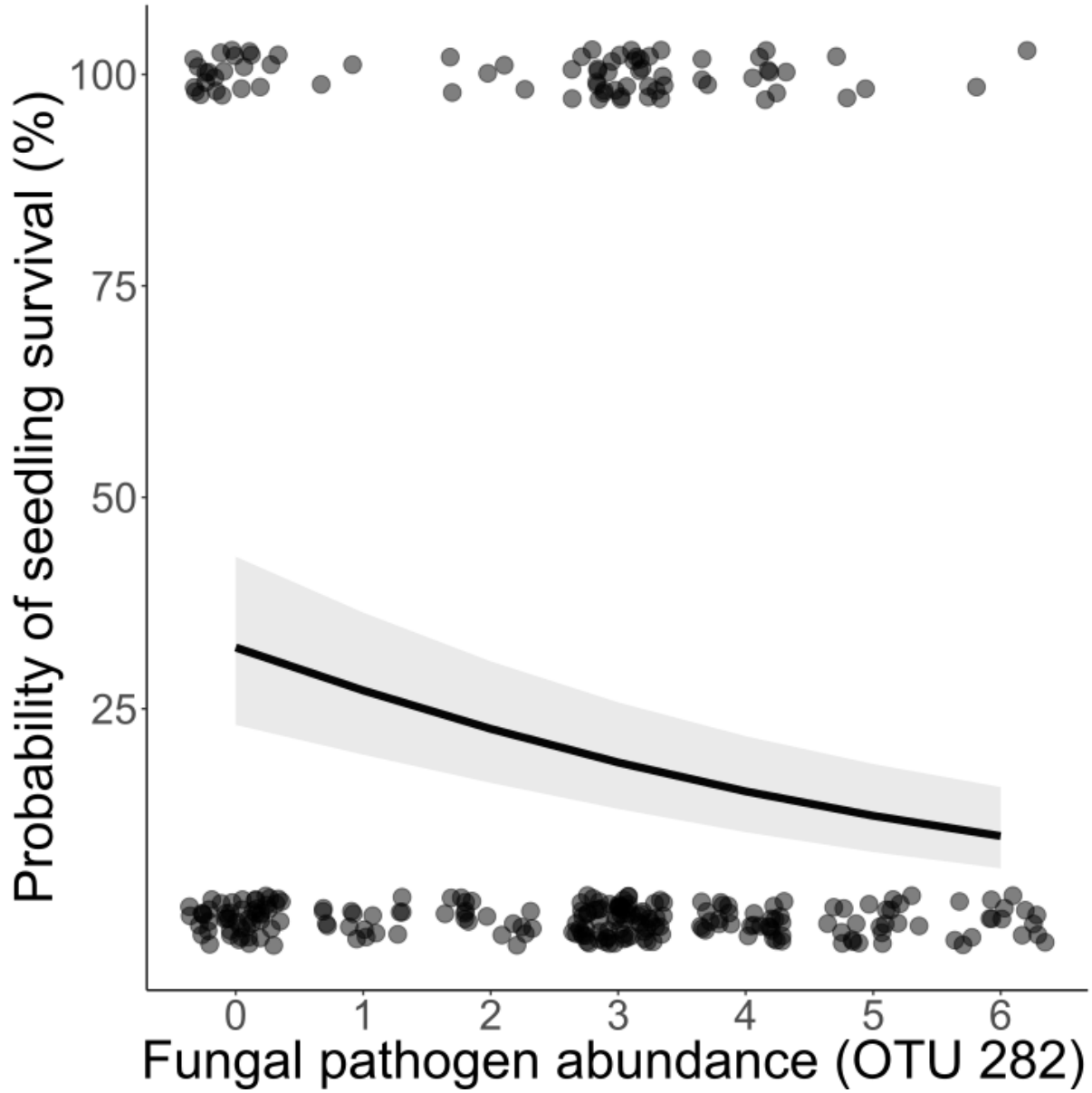

Figure 3: Generalized linear mixed effects model showing the effects of fungal pathogen abundance (OTU 282) (min = 0 soil cores per plot, max = 6 soil cores per plot) on the probability of Swiss stone pine (*P. cembra*) seedling survival. Models were fitted with binomial error distribution. Points indicate jittered raw data. Lines show the model fit ($p < 0.01$) with standard error added as grey shadow. Predictions for OTU 282 are plotted for the mean values of the other significant factors included in the model.

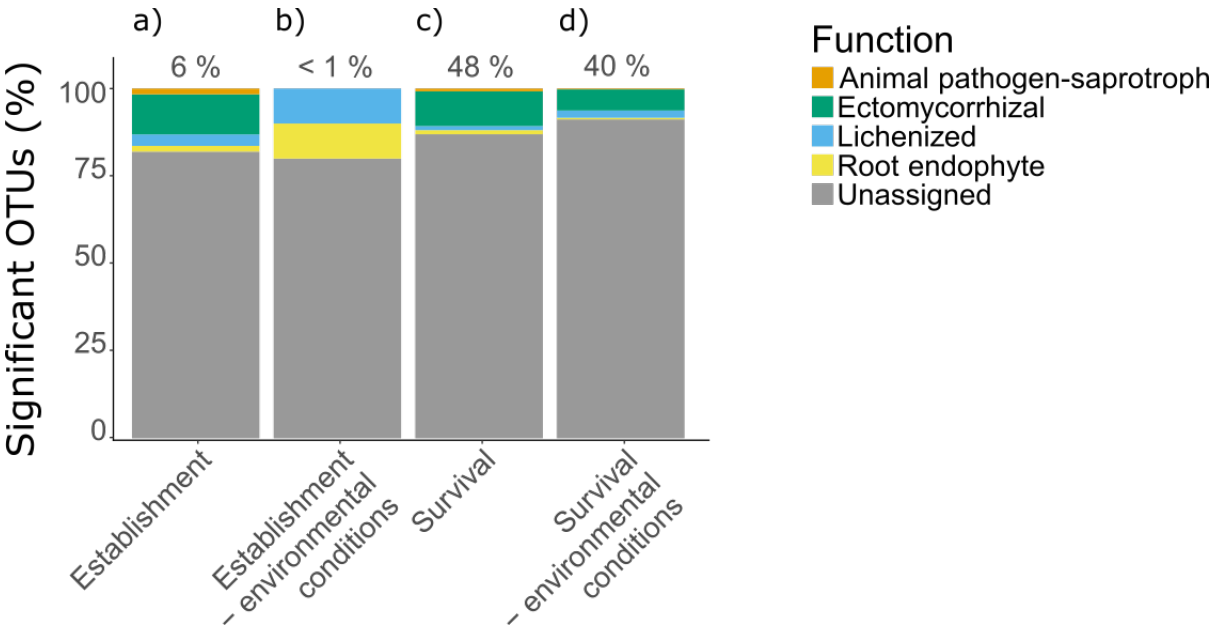

Figure 4: OTUs identified by null model randomization tests as being significantly associated with a) seedling establishment, b) seedling establishment after accounting for environmental factors, c) seedling survival and d) seedling survival after accounting for environmental factors. Percentage of the total of OTUs identified presented at the top of each bar. Functional assignment was done with FUNguild (Nguyen *et al.*, 2016). Taxonomic assignments and effect sizes are presented in Table S2.

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Supplementary material

Supplementary material 2 for Merges *et al.* "High throughput sequencing combined with null model tests reveals specific plant-fungi associations linked to seedling establishment and survival".

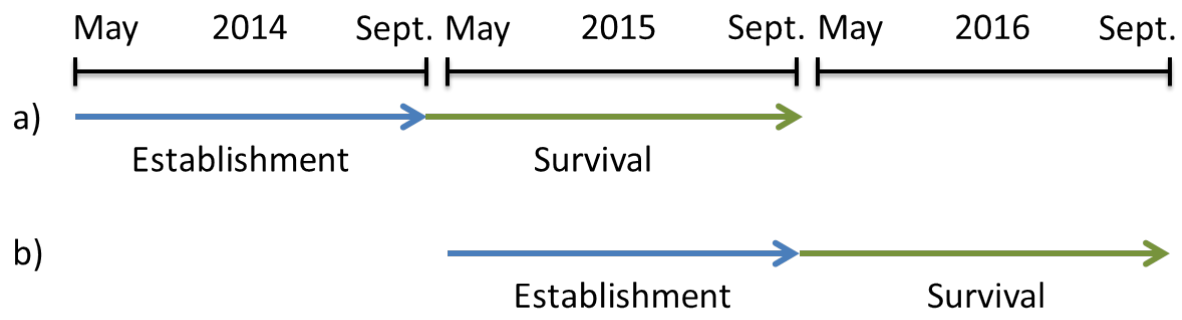

Figure S1: Seed translocation experiments conducted in a) 2014 and b) 2015. In each year, experimental replicates were installed at the beginning of the growing season end of May and seedling establishment of Swiss stone pine (*Pinus cembra*) was monitored before the end of the growing season end of September (blue arrows). Survival of each seedling was recorded at the end of the second growing season in the following year (green arrows).

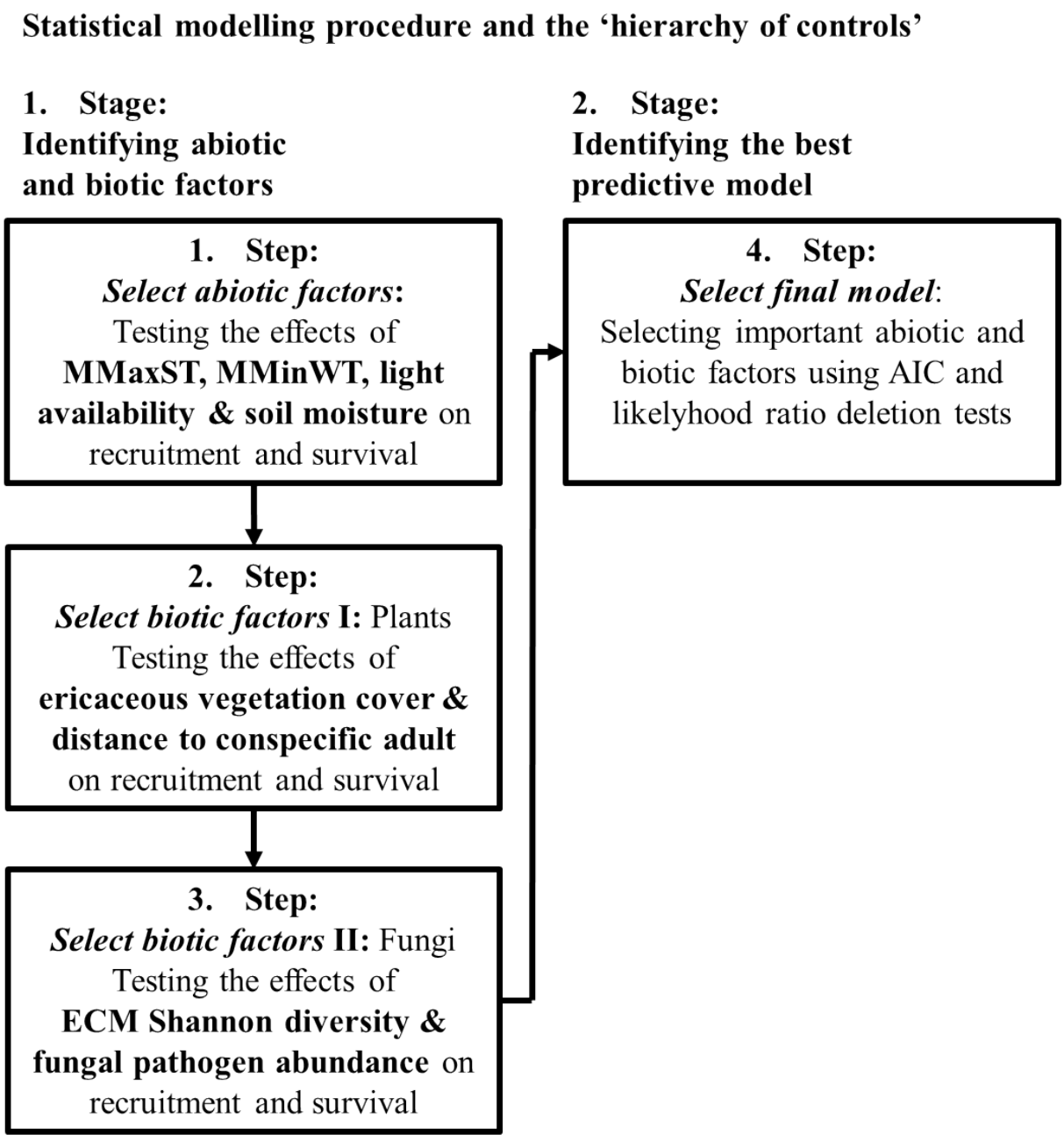

Figure S2: Schematic representation of the steps in the statistical modelling procedure and the 'hierarchy of controls' hypothesized to reduce uncertainty in modelling the establishment and survival of seedlings. The first stage serve to identify the important abiotic and biotic factors, which are tested for their importance in the establishment and survival process in the second stage using AICc and likelihood ratio deletion tests (according to Diaz *et al.*, 2007; *Manning et al.*, 2015). MMaxST = mean daily maximum temperature for the hottest three months, MMinWT = mean daily minimum temperature for the coldest three months (MMinWT was included in the survival models).

Table S1: Variable combinations fitted in the statistical modelling process. MMaxST = mean daily maximum temperature for the hottest three months, MMinWT = mean daily minimum temperature for the coldest three months (only included in the survival models), SM = soil moisture, LA = light availability, EVC = ericaceous vegetation cover.

Step	Parameter combinations tested
1. Abiotic factors	a) MMaxST, MMinWT, SM, LA b) MMaxST+MMaxST^2, MMinWT+MMinWT^2, SM+SM^2, LA+LA^2, MMaxST×MMinWT, MMaxST×SM, MMaxST×LA, MMinWT×SM, MMinWT×LA, SM×LA c) All combinations of parameters found to improve AICc from a) and b).
2. Biotic factors I: Plants	a) EVC, distance to adult b) EVC, distance to adult, EVC×distance to adult
3. Biotic factors II: Fungi	a) ECM Shannon, OTU 282, OTU 1192 b) ECM Shannon+ECM Shannon^2, OTU 282+OTU 282^2, OTU 1198+OTU 1198^2 c) All combinations of parameters found to improve AICc from a) and b).

[2] indicates the fitting of quadratic terms.

Table S2: List of candidate OTUs identified by literature research and null model randomization tests. Taxonomic assignments were based on BLAST results from the NCBI database. Assignment of functions were conducted with all BLAST hits > 97 % similarity. Column „Literature" indicates whether the assigned taxonomic name of an OTU was found in the literature research as being pine-associated („yes") or not („no"). Column "Test" shows if an OTU was identified as significantly associated with pine establishment or survival in a null model randomization test ("yes") or not ("no"). Column "Response" lists the effect of an OTU on the respective response variable in a null model randomization test (E = establishment, S = survival, (-e) = without environmental factors, n. s. = not significant). "Effect size" shows the effect sizes obtain from the null model randomization test.

OTU	Function	Taxonomic assigment	Similarity	Accession	Literature	Test	Response	Effect size	Reference
1894	Animal Pathogen-Saprotroph	*Cryptococcus diffluens*	100	AF145330	no	yes	E, S	0.10	(Kurtzman et al. 2011)
1890	Animal Pathogen-Saprotroph	*Cryptococcus wieringae*	100	AF444373	no	yes	S, S (-e)	-0.17	(Kurtzman et al. 2011)
1891	Animal Pathogen-Saprotroph	*Cryptococcus sp*	100	EF159211	no	yes	S	0.16	(Kurtzman et al. 2011)
1893	Animal Pathogen-Saprotroph	*Cryptococcus gastricus*	100	AF145323	no	yes	S	0.25	(Kurtzman et al. 2011)
139	Ectomycorrhizal	Uncultered *Tomentella* clone	100	JQ791166.1	yes	no	n. s.	/	(De Roman, Claveria & De Miguel 2005)
143	Ectomycorrhizal	Uncultered *Tomentella* clone	99	FM992972.1	yes	no	n. s.	/	(De Roman, Claveria & De Miguel 2005)
177	Ectomycorrhizal	*Wilcoxina rehmii*	100	JX129137.1	yes	no	n. s.	/	(Bidartondo, Baar & Bruns 2001; Bingham & Simard 2012)
749	Ectomycorrhizal	*Otidea leporina*	100	KM010092.1	yes	no	n. s.	/	(Olariaga et al. 2015)
1159	Ectomycorrhizal	*Paxillus involutus*	100	KP753338.1	yes	no	n. s.	/	(Cairney & Chambers 1999)

1324	Ectomycorrhizal	*Elaphomyces muricatus*	100	KR029732.1	yes	no	n. s.	/	(Miller & Miller 1984; De Roman, Claveria & De Miguel 2005)
1373	Ectomycorrhizal	*Lactarius rufus*	100	KX394300.1	yes	no	n. s.	/	(Giltrap 1979)
1728	Ectomycorrhizal	Uncultured *Cortinarius* clone	100	KC412507.1	yes	yes	S	/	(De Roman, Claveria & De Miguel 2005)
1791	Ectomycorrhizal	*Tylospora asterophora*	100	KT447180.1	yes	no	n. s.	/	(Erland & Taylor 1999)
1793	Ectomycorrhizal	*Tylospora fibrillosa*	100	KP753374.1	yes	no	n. s.	/	(Erland & Taylor 1999)
1862	Ectomycorrhizal	*Amanita submembranacea*	100	FJ705275.1	yes	no	n. s.	/	(Contu 2003)
2130	Ectomycorrhizal	*Amphinema byssoides*	98	JQ711820.1	yes	no	n. s.	/	(Erland & Taylor 1999)
1745	Ectomycorrhizal	Rhizopogon sp	100	JN544495	yes	yes	E, S, S (-e)	-0.09	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1792	Ectomycorrhizal	Tylospora fibrillosa	97.9	AB254392	yes	yes	E, S	0.09	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1955	Ectomycorrhizal	*Piloderma* sp	100	UDB001726	yes	yes	E, S	-0.09	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2128	Ectomycorrhizal	*Amphinema byssoides*	100	EF433987	yes	yes	E	0.11	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2151	Ectomycorrhizal	*Suillus cavipes*	99.2	UDB003222	no	yes	E	0.09	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2194	Ectomycorrhizal	*Cortinarius brunneus*	99	UDB017794	no	yes	E, S, S (-e)	0.10	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2209	Ectomycorrhizal	*Cortinarius rigens*	97.9	JF907880	no	yes	E, S	0.10	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1125	Ectomycorrhizal	*Tricholoma inamoenum*	100	UDB011572	no	yes	S	-0.20	(Rinaldi et al. 2008; Tedersoo et al. 2010)

1147	Ectomycorrhizal	*Russula puellaris*	100	UDB017168	no	yes	S	0.30	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1149	Ectomycorrhizal	*Russula emetica*	100	UDB000300	no	yes	S	0.30	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1150	Ectomycorrhizal	*Russula sapinea*	99.6	UDB015996	yes	yes	S	-0.12	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1151	Ectomycorrhizal	*Russula mustelina*	99.2	UDB016021	no	yes	S	0.23	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1152	Ectomycorrhizal	*Russula clavipes*	99.3	UDB011088	no	yes	S	-0.21	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1154	Ectomycorrhizal	*Russula postiana*	100	UDB000897	no	yes	S	0.23	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1302	Ectomycorrhizal	*Russula aquosa*	100	UDB011293	no	yes	S, S (-e)	-0.13	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1323	Ectomycorrhizal	*Elaphomyces muricatus*	99	KF359559	yes	yes	S	0.19	(Rinaldi et al. 2008; Tedersoo et al. 2010)
135	Ectomycorrhizal	*Tomentella* sp	99.1	JQ791170	yes	yes	S	-0.13	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1382	Ectomycorrhizal	*Cortinarius* sp	100	UDB019886	no	yes	S	0.23	(Rinaldi et al. 2008; Tedersoo et al. 2010)
142	Ectomycorrhizal	*Tomentella* sp	98.6	KF514672	yes	yes	S	0.24	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1759	Ectomycorrhizal	*Hygrophorus albicastaneus*	98.2	DQ097873	no	yes	S	0.12	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1794	Ectomycorrhizal	*Tylospora fibrillosa*	97.4	AB254392	yes	yes	S	0.15	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1874	Ectomycorrhizal	*Cortinarius caesiobrunneus*	100	UDB017795	yes	yes	S, S (-e)	0.28	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1954	Ectomycorrhizal	*Piloderma* sp	100	UDB001733	yes	yes	S	0.35	(Rinaldi et al. 2008; Tedersoo et al. 2010)

2127	Ectomycorrhizal	*Amphinema byssoides*	100	UDB008257	yes	yes	S	-0.23	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2129	Ectomycorrhizal	*Amphinema* sp	100	UDB001719	no	yes	S	-0.14	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2160	Ectomycorrhizal	*Lactarius necator*	99.6	EU711629	no	yes	S, S (-e)	-0.21	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2181	Ectomycorrhizal	*Cortinarius sanguineus*	99.5	JN114099	no	yes	S	0.24	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2182	Ectomycorrhizal	*Cortinarius croceus*	99.5	UDB021419	yes	yes	S, S (-e)	0.23	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2183	Ectomycorrhizal	*Cortinarius transatlanticus*	100	UDB021507	no	yes	S	-0.14	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2188	Ectomycorrhizal	*Cortinarius disjungendulus*	97.4	KM273090	yes	yes	S	0.24	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2190	Ectomycorrhizal	*Cortinarius integerrimus*	99	JF907926	yes	yes	S	0.25	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2191	Ectomycorrhizal	*Cortinarius acutus*	98	FJ769529	no	yes	S	0.13	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2192	Ectomycorrhizal	*Cortinarius obtusus*	99	HQ604666	no	yes	S	0.26	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2197	Ectomycorrhizal	*Cortinarius colus*	99.5	UDB002224	yes	yes	S	0.18	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2198	Ectomycorrhizal	*Cortinarius fillionii*	100	HQ845171	no	yes	S	0.21	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2199	Ectomycorrhizal	*Cortinarius acutovelatus*	99.5	UDB001000	no	yes	S	-0.13	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2200	Ectomycorrhizal	*Cortinarius biformis*	98.9	DQ481700	no	yes	S	0.12	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2201	Ectomycorrhizal	*Cortinarius* sp	97.5	JQ975956	yes	yes	S, S (-e)	0.19	(Rinaldi et al. 2008; Tedersoo et al. 2010)

2202	Ectomycorrhizal	*Cortinarius* sp	100	UDB018310	yes	yes	S	0.17	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2204	Ectomycorrhizal	*Cortinarius sobrius*	100	KF732429	yes	yes	S, S (-e)	-0.12	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2205	Ectomycorrhizal	*Cortinarius testaceofolius*	100	EU693242	no	yes	S	-0.26	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2206	Ectomycorrhizal	*Cortinarius anomalus*	100	UDB001008	no	yes	S	0.11	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2210	Ectomycorrhizal	*Cortinarius caperatus*	99.5	UDB001079	no	yes	S	0.28	(Rinaldi et al. 2008; Tedersoo et al. 2010)
430	Ectomycorrhizal	*Tylospora asterophora*	98	UDB002638	yes	yes	S, S (-e)	0.13	(Rinaldi et al. 2008; Tedersoo et al. 2010)
713	Ectomycorrhizal	*Wilcoxina* sp	97.5	EF619913	no	yes	S, S (-e)	0.30	(Rinaldi et al. 2008; Tedersoo et al. 2010)
753	Ectomycorrhizal	*Inocybe fuscidula*	100	AM882887	no	yes	S	-0.12	(Rinaldi et al. 2008; Tedersoo et al. 2010)
754	Ectomycorrhizal	*Inocybe lanuginosa*	99.5	HQ604311	no	yes	S	0.33	(Rinaldi et al. 2008; Tedersoo et al. 2010)
783	Ectomycorrhizal	*Hygrophorus olivaceoalbus*	98.9	UDB000558	no	yes	S	0.15	(Rinaldi et al. 2008; Tedersoo et al. 2010)
789	Ectomycorrhizal	*Piloderma byssinum*	97.5	EF619739	no	yes	S, S (-e)	0.13	(Rinaldi et al. 2008; Tedersoo et al. 2010)
852	Ectomycorrhizal	*Hygrophorus speciosus*	98.9	DQ097884	no	yes	S	0.13	(Rinaldi et al. 2008; Tedersoo et al. 2010)
88	Ectomycorrhizal	*Amanita olivaceogrisea*	99.4	UDB015459	yes	yes	S	0.23	(Rinaldi et al. 2008; Tedersoo et al. 2010)
894	Ectomycorrhizal	*Wilcoxina* sp	100	AM999663	yes	yes	S, S (-e)	0.18	(Rinaldi et al. 2008; Tedersoo et al. 2010)
897	Ectomycorrhizal	*Cortinarius acutus*	100	UDB001002	no	yes	S	0.31	(Rinaldi et al. 2008; Tedersoo et al. 2010)

1146	Ectomycorrhizal	*Russula paludosa*	99.6	JX029923	no	yes	S (-e)	0.15	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1153	Ectomycorrhizal	*Russula vinosa*	100	UDB000350	no	yes	S (-e)	0.23	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1286	Ectomycorrhizal	*Cenococcum geophilum*	98	FJ440882	no	yes	S (-e)	0.26	(Tedersoo et al. 2014)
1309	Ectomycorrhizal	*Russula cessans*	97.3	UDB001716	no	yes	S (-e)	0.23	(Rinaldi et al. 2008; Tedersoo et al. 2010)
138	Ectomycorrhizal	*Tomentella badia*	98.2	JQ711987	no	yes	S (-e)	0.15	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1744	Ectomycorrhizal	*Rhizopogon salebrosus*	98.8	AF377152	yes	yes	S (-e)	0.18	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1819	Ectomycorrhizal	*Hydnotrya michaelis*	99.1	EU784274	no	yes	S (-e)	-0.26	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1876	Ectomycorrhizal	*Piloderma bicolor*	100	UDB001740	no	yes	S (-e)	0.18	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1958	Ectomycorrhizal	*Piloderma olivaceum*	98.9	UDB001747	no	yes	S (-e)	-0.19	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1993	Ectomycorrhizal	*Cenococcum geophilum*	98.6	AY394919	no	yes	S (-e)	0.27	(Tedersoo et al. 2014)
2180	Ectomycorrhizal	*Cortinarius semisanguineus*	98	JQ711941	no	yes	S (-e)	0.20	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2189	Ectomycorrhizal	*Cortinarius uraceus*	100	KJ206522	no	yes	S (-e)	0.25	(Rinaldi et al. 2008; Tedersoo et al. 2010)
2196	Ectomycorrhizal	*Cortinarius malachius*	100	KF617653	no	yes	S (-e)	-0.33	(Rinaldi et al. 2008; Tedersoo et al. 2010)
96	Ectomycorrhizal	*Suillus tomentosus*	100	JN544503	no	yes	S (-e)	-0.18	(Rinaldi et al. 2008; Tedersoo et al. 2010)
1335	Lichenized	*Cladonia cornuta*	98.8	FJ536352	no	yes	E	-0.10	(James et al. 2006)

925	Lichenized	*Melanohalea exasperatula*	99.3	AY611090	no	yes	E	0.09	(Esslinger 2014)
1333	Lichenized	*Cladonia arbuscula*	98.8	AY170775	no	yes	E (-e), S	-0.10	(James et al. 2006)
1331	Lichenized	*Cladonia rangiferina*	98.8	JQ695918	no	yes	S	-0.15	(James et al. 2006)
1337	Lichenized	*Cladonia rangiferina*	98.1	AF458306	no	yes	S, S (-e)	-0.26	(James et al. 2006)
1749	Lichenized	*Trapeliopsis granulosa*	99.3	AF353569	no	yes	S	0.18	(Esslinger 2014)
1870	Lichenized	*Cladonia arbuscula*	97.5	GU169267	no	yes	S	0.23	(James et al. 2006)
410	Lichenized	*Cetraria islandica*	100	JQ301699	no	yes	S, S (-e)	-0.17	(Esslinger 2014)
132	Lichenized	*Cladonia arbuscula*	97.6	AY170775	no	yes	S (-e)	0.14	(James et al. 2006)
1338	Lichenized	*Cladonia novochlorophaea*	99.4	GU188414	no	yes	S (-e)	-0.12	(James et al. 2006)
1340	Lichenized	*Cladonia fimbriata*	100	GU188404	no	yes	S (-e)	0.21	(James et al. 2006)
1342	Lichenized	*Cladonia cenotea*	98.2	AF457900	no	yes	S (-e)	-0.15	(James et al. 2006)
211	Lichenized	*Cladonia humilis*	97.5	KC415933	no	yes	S (-e)	-0.13	(James et al. 2006)
312	Lichenized	*Pseudevernia furfuracea*	100	FR799280	no	yes	S (-e)	0.14	(Esslinger 2014)
498	Lichenized	*Pseudevernia cladonia*	100	AF297736	no	yes	S (-e)	-0.28	(Esslinger 2014)
282	Pathogenic	*Gremmenia infestans*	99	KU063958.1	yes	no	n. s.	/	(Burdon et al. 1992; Barbeito et al. 2013)

1198	Pathogenic	*Gremmenia infestans*	98	KM216393.1	yes	no	n. s.	/	(Burdon et al. 1992; Barbeito et al. 2013)
258	Undefined Root Endophyte	*Cadophora* sp	100	FJ553685	no	yes	E, S	-0.11	(Tedersoo et al. 2010)
968	Undefined Root Endophyte	*Phialocephala glacialis*	98.6	EU434843	no	yes	E (-e), S	-0.09	(Newsham 2011)
1209	Undefined Root Endophyte	*Cadophora finlandica*	98.6	AF486119	no	yes	S	0.26	(Tedersoo et al. 2010)
2069	Undefined Root Endophyte	*Phialocephala fortinii*	97.1	AY033087	no	yes	S	0.20	(Newsham 2011)
538	Undefined Root Endophyte	*Phialocephala sphaeroides*	97.9	AY524845	no	yes	S	-0.18	(Newsham 2011)
733	Undefined Root Endophyte	*Cadophora luteo-olivacea*	97.3	AY249066	no	yes	S	-0.17	(Tedersoo et al. 2010)
1481	Undefined Root Endophyte	*Leptodontidium* sp	100	FJ552955	no	yes	S (-e)	0.15	(Jumpponen & Trappe 1998)
1851	Undefined Root Endophyte	*Leptodontidium* sp	99.3	JF300526	no	yes	S (-e)	0.16	(Jumpponen & Trappe 1998)
1375	Wood Saprotroph	*Trechispora byssinella*	98	AY969779	no	yes	S	-0.12	(Gilbertson & Ryvarden 1987; Rinaldi et al. 2008. Tedersoo et al. 2010)
337	Wood Saprotroph	*Trechispora* sp	99.5	KJ140560	no	yes	S	-0.14	(Gilbertson & Ryvarden 1987; Rinaldi et al. 2008. Tedersoo et al. 2010)
700	Wood Saprotroph	*Trechispora* sp	100	JF300723	no	yes	S	-0.25	(Gilbertson & Ryvarden 1987; Rinaldi et al. 2008. Tedersoo et al. 2010)

Following OTUs significantly associated with establishment and survival, but were not present in databases: 3, 5, 8, 16, 20-24, 31, 32, 34, 35, 37, 38, 43, 44, 46-48, 51, 55, 57, 62, 64, 74, 81, 92, 96, 97, 106, 107, 115, 124, 132, 136, 138, 140, 141, 147, 149, 150, 152, 162, 168, 170, 172-183, 190, 196, 203, 206, 210- 212, 214-217, 220, 223-227, 236, 237, 240-246, 256, 257, 259, 261-269, 272-274, 276, 310, 312, 314-316, 318, 320, 321, 323, 325, 327, 333, 338, 339, 348, 351, 355-360, 363-366, 372, 375, 377-379, 381, 382, 387, 389, 390-392, 394, 395, 397, 407, 411, 412, 419-424, 432-436, 441, 443, 444, 448, 449, 455-457, 459, 461-470, 472- 475, 477-482, 484, 488, 492-494, 496-507, 509-513, 515-518, 524, 525, 529, 535-537, 609, 623, 643, 646, 653, 656-658, 669, 671, 674, 678, 679, 685, 686, 688-692, 694, 701, 705, 712, 720, 726, 728, 731, 732, 738, 742, 744, 747, 748, 750, 752, 760-762, 764, 767, 775-777, 780, 787, 790, 795, 805, 812-815, 822, 823, 826, 827, 831, 834, 840-843, 855, 856, 858-861, 865, 877, 885, 887, 888, 890, 892, 893, 900, 902, 904, 905, 907, 908, 910, 918, 920, 926-928, 932, 933, 935, 941, 944, 946-948, 950-967, 970, 972-974, 976- 980, 982, 984, 986, 988, 990-992, 994, 996, 997, 1001, 1002, 1007, 1017, 1051, 1053, 1057, 1058, 1060, 1061, 1070, 1074-1076, 1078, 1084, 1087, 1088, 1090, 1092-1094, 1097, 1101, 1110, 1113, 1116, 1118, 1123, 1126, 1137, 1146, 1153, 1161, 1165, 1173, 1176, 1177, 1179, 1180, 1184, 1185, 1187, 1191, 1192, 1194, 1195, 1197, 1199-1201, 1203-1205, 1208, 1213,

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