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Plant-mycorrhizal interactions and their role in plant invasions in mountains

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Table of Contents

Summary

Non-native species invasions are one of the most impactful drivers of biodiversity and ecosystem services loss worldwide and their occurrence is increasing rapidly as a consequence of ever-growing anthropogenic activities. This makes a better understanding of how these invasions occur a priority for improving future conservation efforts. One aspect of plant species invasion, which is only recently starting to be recognized as a significant determinant of invasion success, is the symbiosis between plant and mycorrhizal fungi which is crucial in shaping plant distributions globally. Understanding how anthropogenic activities can impact these mycorrhizal associations and their relationship with non-native plants could therefore offer us great insights in predicting plant invasion trajectories.

Here, I focus on anthropogenic disturbance in mountain ecosystems and its impact on both plant communities and mycorrhizal fungi to answer how these communities are impacted by said disturbance and whether non-native plants can benefit from the altered conditions to establish and spread. To this end I used a combination of different approaches: 1) repeated surveys of plants and arbuscular mycorrhizal fungi, over respectively ten and four years, along disturbed roadsides in the mountains of Norway, 2) combining a global dataset of native and non-native plants along mountain roads with a comprehensive database associating plants with their mycorrhizal types, and 3) an in-situ seed addition experiment measuring non-native plant success and changes in fungal community following different types of disturbance treatments. Through these methods, I could assess the effects of anthropogenic disturbance on mycorrhizal symbiosis and non-native plant species, as well as the underlying mechanisms behind these effects, at multiple scales and resolutions.

Consequently, we found that road disturbance has a globally consistent effect on mycorrhizal types in mountain systems, as plants associated with arbuscular mycorrhizal (AM) fungi were more abundant following disturbance. Conversely, vegetation associated with either ectomycorrhizal fungi (EcM) or ericoid mycorrhizal fungi (ErM) was less abundant in disturbed sites. In the Norwegian regional study,

AM fungi were similarly more abundant and diverse in the roots of plant communities affected by road disturbance, compared to the undisturbed vegetation. Non-native plants were also restricted to these disturbed sites. Furthermore, the experimental results showed that both physical disturbance and nutrient addition have negative effects on EcM fungi and positive effects on fungal pathogens, and that they facilitate non-native plant success.

Overall, our results show that anthropogenic disturbance does have an effect on mycorrhizal fungi that in turn impacts the distribution of plant species in disturbed mountain systems. The resulting shift in mycorrhizal fungi being to the advantage of AM fungi and AM plant species could in turn have implications for non-native plant invasions. Indeed, we know from previous studies, and also observed this here, that non-native plants predominantly form associations with AM fungi. Therefore, anthropogenic disturbance can facilitate non-native plant success through disruption of the native fungal communities, and especially so in high elevation and cold climate regions which are naturally less dominated by AM plants. I believe that these conclusions highlight the importance of mycorrhizal symbiosis in understanding plant invasions trajectories and in turn emphasize the importance of closely monitoring sources of anthropogenic disturbance in mountain systems in order to prevent future establishment of non-native plants.

Dutch Summary

Invasieve uitheemse soorten zijn een van de hoofdoorzaken voor het verlies aan biodiversiteit en ecosysteemdiensten wereldwijd, en hun voorkomen neemt snel toe als gevolg van steeds groeiende menselijke activiteiten en bijhorende verstoringen. Een aspect van de invasie van plantensoorten dat pas recentelijk wordt erkend als een belangrijke bepalende factor voor het succes van invasies, is de symbiose tussen planten en mycorrhizale schimmels. In deze thesis onderzoek ik de impact van menselijke verstoring in bergecosystemen op plantengemeenschappen en mycorrhizale schimmels. Ik probeer te achterhalen hoe verstoring de relatie tussen beiden beïnvloedt en of niet-inheemse planten kunnen profiteren van die veranderde omstandigheden. Om deze onderzoeksvraag te beantwoorden heb ik drie verschillende onderzoeksmethoden gebruikt: 1) herhaalde metingen van planten en hun arbusculaire mycorrhizale schimmels in verstoorde wegbermen in de Noorse bergen, 2) de combinatie van een wereldwijde plantendataset met een focus op bergwegen met een database die planten koppelt aan hun mycorrhizale types, en 3) een in-situ experiment met plantenzaden waarbij het succes van niet-inheemse planten en veranderingen in de schimmelgemeenschap werden gemeten na verschillende soorten behandelingen. Met behulp van deze methoden kon ik de effecten van menselijke verstoring op mycorrhizale symbiose en niet-inheemse plantensoorten op meerdere schalen en resoluties beoordelen.

Als gevolg hiervan kon ik vaststellen dat de vegetatieverstoring langs bergwegen een wereldwijd consistent effect heeft op mycorrhizale types, aangezien planten die geassocieerd zijn met arbusculaire mycorrhizale (AM) schimmels telkenmale profiteerden van verstoring, terwijl vegetatie die geassocieerd is met ectomycorrhizale- of ericoïde-mycorrhizale schimmels juist in aantal afnam. In de regionale studie in Noorwegen waren AM-schimmels eveneens talrijker aanwezig, en diverser, in de wortels van plantengemeenschappen die door wegverstoring werden beïnvloed. De experimentele resultaten toonden aan dat fysieke verstoring en toevoeging van voedingsstoffen het succes van niet-inheemse planten bevorderden,

negatieve effecten hebben op EcM-schimmels en positieve effecten hebben op schimmelziekten.

Onze resultaten tonen aan dat menselijke verstoring effect heeft op mycorrhizale schimmels en dus ook op die manier de verspreiding van plantensoorten in verstoorde bergsystemen kan beïnvloeden. Bovendien kan de resulterende verschuiving in mycorrhizale schimmels naar AM het succes van niet-inheemse planten vergemakkelijken door verstoring van de inheemse schimmelgemeenschappen, vooral in hooggelegen en koude klimaatgebieden die van nature minder worden gedomineerd door AM-planten. Deze conclusies benadrukken het belang van mycorrhizale symbiose bij het begrijpen van trajecten van planteninvasies en tegelijkertijd de noodzaak om nauwlettend toezicht te houden op

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CHAPTER I

General introduction

1.1 Global change and non-native plant invasions

We are living in unprecedented times where humanity's actions have reached a scale so all-encompassing, that they are actively reshaping how our planet functions. Foremost in the collective consciousness, this is reflected through climate change, and specifically global warming, as the one issue at the center of our impact on the biosphere. However, we know that there is much more to the impact of human activities on ecosystems and natural processes. Humans are everywhere, and everywhere they alter their surroundings in different ways, be it through land-use change, through pollution or through the introduction of disruptive non-native species (Balvanera et al., 2019). If we want to tackle the challenges posed by global change, we will need to not only tackle the large, global, and systemic issues that are increasingly being recognized, but also address these issues at the regional and local levels (Baldos et al., 2023; Hertel et al., 2019). In this light, a good understanding of the mechanisms of anthropogenic change and their implications across diverse ecosystems is paramount in managing and ultimately addressing the consequences of the Anthropocene.

In this context of fast and intense change, one key factor in understanding the impact of anthropogenic activity on ecosystems is the unprecedented rate at which the distribution ranges of species are changing (Chen et al., 2011). With climate change resulting in globally rising temperatures (IPCC, 2022), species worldwide have been observed to shift their distribution towards cooler climatic conditions, i.e. higher latitude and elevations, to better match their temperature optimum (Lenoir & Svenning, 2015). Alongside these comparatively slower distribution shifts which follow gradual environmental changes, anthropogenic activity has also led to more radical shifts caused by the drastic modification of the environment through land use changes and by the introduction of species outside of their native range in ecosystems which they could previously not have reached (Inderjit et al., 2017; Sirami et al., 2017). These changes in species distribution have wide ranging implications as moving species form new biotic interactions with other species that they were previously not in contact with and have the potential to disrupt the balance, and in turn the functioning, of affected ecosystems (Gutiérrez et al., 2014). Being able to understand the mechanisms driving these shifts and to predict the consequences of the resulting novel interactions is therefore crucial if we want to be able to devise good preventive measures and management approaches to preserve ecosystems and ecosystem services (H. E. Roy et al., 2023).

Non-native movement specifically is known to be one of the leading drivers of biodiversity loss and highly disruptive for the functioning of native ecosystems (IPBES, 2019). Human activity, directly and indirectly, has led to the establishment of more than 37000 species outside of their native range, with this number increasing at a rate of more than 200 per year (Roy et al., 2023). Of these, roughly 10% go on to be invasive species, with negative impacts on ecosystems and ecosystem services (H. E. Roy et al., 2023). It is well known that these non-native species have increased chances of establishment and long-term success when coupled with other factors of global change such as rising temperature, disturbance from land use change or changes in nutrient availability (Jauni et al., 2015; Knauf et al., 2021; Zettlemoyer et al., 2019). Furthermore, non-native species also extend their distribution range at higher rates compared to native species once introduced outside of their native range (Montesinos, 2022; Wolf et al., 2016). Being able to predict the establishment and spread of non-native species is therefore as important as it is difficult, with numerous factors ranging from land use change to novel biotic interactions having to be taken into consideration for accurate prognostics to be possible.

Previous studies have investigated many of these individual mechanisms and their relations through recent years (Lembrechts et al., 2018; O'Loughlin & Green, 2017; Sinclair et al., 2020), leading to a greater understanding of the big picture of non-native movement and of invasions. Yet, some individual mechanisms and specific relationships between these mechanisms remain understudied (H. E. Roy et al., 2023). In this thesis I aim to investigate one such combination of drivers: the combined role of mycorrhizas and anthropogenic disturbance in facilitating non-native plant success with a focus on mountain ecosystems.

1.2 Mycorrhizal symbiosis

The symbiosis between plants and mycorrhizal fungi is an extremely widespread phenomenon as it is known to occur in more than 90 % of terrestrial plant species (S. E. Smith & Read, 2010). While the existence of this mutualistic relationship between plants and fungi has been known since the end of the nineteenth century (Bonfante, 2018; Frank, 2005), the development in the last decades of new methods of observation, especially in the field of molecular biology, have led to a renewed consciousness of the importance of mycorrhizal symbiosis in shaping terrestrial ecosystems (Neuenkamp et al., 2018; van der Heijden et al., 2015; Cameron Wagg et al., 2014). Mycorrhizal fungi do indeed play crucial roles in many key ecosystem processes such as nutrient cycling or carbon cycling and strongly influence both productivity and diversity of plant species (Smith & Read, 2010; van der Heijden et al., 2015). Their role in nutrient uptake is especially important as it is estimated that up to 80% of all phosphate (P) and nitrate (N) present in terrestrial plants is acquired through their relationship with symbiotic mycorrhizal fungi (Azcón-Aguilar & Barea, 2015; Hobbie & Hobbie, 2008; Read & Perez-Moreno, 2003). Furthermore, mycorrhizal fungi are also known to be a complementary source of water intake for plants (Khalvati et al., 2005; Smith & Read, 2010)

There are four main mycorrhizal types currently defined which differ by their structure, fungi involved, and by their specific nutrient provision traits. Arbuscular mycorrhizas (AM) are the most evolutionary ancient and involve the most widespread type of mycorrhizal fungi which associate with 70% of all known terrestrial plants, including the majority of grasses and herbs, and are found across all regions of the world (Brundrett & Tedersoo, 2018). The fungi forming these associations are mostly from the Glomeromycotina division and form arbuscule structures inside of the cortical cells of vascular plants' roots. Ectomycorrhizal (EcM) fungi mostly belong to the Basidiomycota and Ascomycota phyla and associate with around 2% of terrestrial plants, mostly trees and shrubs, and are most common in the temperate and Arctic regions of the Northern Hemisphere (Tedersoo et al., 2010). Contrary to other mycorrhizal types they do not penetrate root cells, instead forming a dense intercellular structure known as the Hartig net (S. E. Smith & Read, 2010). Ericoid mycorrhizal (ErM) fungi also associate with around 2% of terrestrial plants, specifically with species of the Ericacea family, and are most often found in acidic and nutrient poor soils and mostly at higher latitudes or in mountain systems (Kohout, 2017). Fungi forming ErM associations mostly belong to the Ascomycota, with some exceptions from the Basidiomycota, and form intracellular coils in the epidermal cells of fine roots. Finally, orchid Mycorrhizal (OM) fungi associate exclusively with orchids which represent about 10% of known terrestrial plants. OM fungi belong typically to the Basidiomycota and are paramount to the success of their associated plants as most orchids cannot even germinate in the absence of their fungal symbionts (Sathiyadash et al., 2020).

Nevertheless, not all terrestrial plants form mycorrhizal associations with 20% of plants not forming any symbiosis with mycorrhizal fungi, which are referred to as being non-mycorrhizal (NM).A further 17% of plant species are facultative species, meaning that they are capable of forming mycorrhizal associations when beneficial but can also survive without (Moyano et al., 2020). Indeed, the degree of mycorrhizal colonisation is a continuum from intense to sparse, depending on individual species and across different environmental conditions. This is especially true for associations with AM fungi which as mentioned above can be entirely facultative for a certain subset of species (S. E. Smith & Read, 2010). Furthermore, not all plant species are neatly restricted to associating with a single type of mycorrhizal fungi as some species, such as for example poplars, can associate with both AM and EM fungi interchangeably or even at the same time (Teste et al., 2020). Ultimately, mycorrhizal fungi and mycorrhizal symbiosis are as varied as the plants they associate with and as the ecosystems they are part of, and we are increasingly realizing how important their role is in shaping plant communities (Tedersoo et al., 2020).

Given the ubiquity of mycorrhizal associations it is only logical that this mutualist relationship could also play an important role in non-native plant success and in the mechanisms behind plant invasions. In recent years, research has indeed shown that the interaction between mycorrhizal fungi, non-native plants and local plant communities is an important factor in understanding patterns of non-native plant establishment and spread (Aslani et al., 2019; Pringle et al., 2009). It is however a complicated and highly diverse topic, as the nature of these interactions is dependent, amongst other factors, on the type of mycorrhizal associations formed by the non-native plants, the types present or absent from the local ecosystems and the specific interactions between them (Dickie et al., 2017). Given this complexity, many different patterns have been theorized and observed (Dickie et al., 2017). The first and most logical pattern is that of reduced symbionts for the non-native species as a lack of appropriate mycorrhizal fungi in the novel environment would be a limiting factor for their success compared to their native range where they would rely at least partly on forming mycorrhizal symbiosis (Catford et al., 2009). A good example of such a limitation is that of invasive EcM-associated Pinaceae species which cannot establish outside of their native ranges in the absence of compatible EcM-symbionts, but become able to establish and thrive when co-introduced with said compatible EcM fungi (Dickie et al., 2010; Hayward et al., 2015). In the latter case, both fungi and pines can be considered co-invasive. This is an extreme example in which establishment is conditional on the presence of a specific and somewhat uncommon type of mycorrhizal fungi, however most non-native species are not this specific in their mycorrhizal requirements. Indeed, the vast majority of non-native plant species are AM-associated, and often facultative in their AM-associations, or non-mycorrhizal (Menzel et al., 2017).

With AM fungi being present across all continents and in most environments their absence is less of a clear barrier for the establishment of most non-native species (Pyšek et al., 2019; Wang & Qiu, 2006). Furthermore, the associations between plants and AM fungi are rather non-specific, as most AM plants can form symbiotic relationship with most AM fungi and AM fungi also generally show low levels of endemism (Davison et al., 2015). Therefore, non-native plants could maintain mycorrhizal associations outside of their native range either by forming novel interactions with local mycorrhizal fungi or due to co-occurrence of adequate mycorrhizal fungi in both their native range and in their invasive range (Dickie et al., 2017). This is reflected in the fact that contrary to initial beliefs, non-mycorrhizal non-native species do not seem to be more successful than their AM-associated counterparts (Bunn et al., 2015; Menzel et al., 2017). The effect of non-native plants on their surrounding mycorrhizal community once established outside of their native range is also an important factor in the impact of non-native plants on native plant communities. One possible such effect is that of symbiont disruption (Meinhardt & Gehring, 2012; Stinson et al., 2006) in which the presence of the non-native species leads to a reduced availability of mycorrhizal fungi for the native vegetation to associate with. This can occur either through a lower investment of resources in mycorrhizal mutualists by the non-native plants to the general detriment of the mycorrhizal community and in turn of the native plant species, or through active disruption by the non-native species. The latter is the case, for example, with garlic mustard (*Alliara petiolata* M.Bieb.), which is known to inhibit surrounding mycorrhizal fungi and therefore facilitates its own continued success (Callaway et al., 2008). Conversely, a non-native species could also have a positive effect on the native mycorrhizal fungi it is most suited to form associations with, to the detriment of other native mycorrhizal fungi. This great diversity of observed and theorized interactions between non-native plant and mycorrhizal fungi makes prediction of establishment and long-term trajectories difficult to assess. A clear understanding of the process behind how certain combinations of non-native plant mycorrhizal type and native fungal communities can interact is therefore paramount for future management of non-native plant species.

1.3 Anthropogenic disturbance

Disturbance has been defined in many different ways across studies as ‘any discrete event that can affect the structure of a community, changing its resource disposition, edaphic and physical environment’ (Luisa, 2012; Pickett et al., 1989). These disturbances can take numerous forms and be natural in origin, with events such as fires, grazing, erosion or floods, or can be consequences of anthropogenic origin such as construction works, agriculture, or resource exploitation (Liding & Bojie, 2000). These sources of disturbances will typically manifest as discreet events causing partial or total destruction of plant biomass, and in turn increase resource availability for example through increased nutrient, space, or light availability. We know that species richness in frequently disturbed areas reflects the ability of species to efficiently colonise and grow rather than their capacity to compete and tolerate low resource availability which are more beneficial under low disturbance regimes (Huston & Smith, 1987; Roxburgh et al., 2004). This is particularly relevant for plant invasions as non-native plants introduced along human activities typically possess traits that predispose them to exploit disturbance, such as high relative growth rate or small seed size (Catford et al., 2012). As such, non-native species tend to be disproportionally successful following disturbance, and especially anthropogenic disturbance as non-native plant species propagule pressure is highest in areas of human activity (Pyšek et al., 2010).. Furthermore, it is well established that most non-native species have ruderal strategies and can take advantage of the conditions brought about by disturbance and especially of the resulting reduction in competition (Catford et al., 2012). This effect has been observed repeatedly in the case of both natural and anthropogenic disturbances (Hunter et al., 2006; Kuhman et al., 2011; Skultety & Matthews, 2017; Waddell et al., 2020) with increased abundance and diversity of non-native plant species following disturbance events (Jauni et al., 2015).

In this thesis I focus mainly on cases of anthropogenic disturbance, as its impact on non-native success is typically higher since it is usually accompanied by increased non-native propagule pressure (González-Moreno et al., 2014) and native plant populations are less adapted to withstand these novel pressures than those of natural events which have been happening regularly over their evolutionary history (Chiuffo et al., 2018). While the effect of anthropogenic disturbance as a driver of non-native species is well established (Lembrechts et al., 2014), the mechanisms behind this effect are not yet fully understood. These mechanisms are numerous and studies on the subject have assessed such diverse factors as reduced competition, increased nutrient availability, increased propagule pressure or changes in temperature (Biswas & Mallik, 2010; Blumenthal, 2006; Vilà & Ibáñez, 2011). All these have been shown to combine to facilitate the success of non-native plant invasions. However, little attention has been offered to the impact of anthropogenic disturbance on mycorrhizal fungi and symbiosis in this context.

Historically, most of the research effort concerning the effect of anthropogenic disturbance on mycorrhizal fungi has been done in the context of agricultural settings (Jansa et al., 2006). In most cases, this means investigating the effect of tillage on AM fungi diversity and abundance. Results typically indicate that this type of physical disturbance in AM dominated fields leads to both diversity and abundance diminishing (Goss & De Varennes, 2002; Jansa et al., 2006; Kabir, 2005; Schnoor et al., 2011), however this pattern is not universal and seems to be context dependent (Moora et al., 2014). While less common, studies on the impact of disturbance on mycorrhiza in natural systems have also been conducted, especially in more recent years (Brundrett & Ashwath, 2013; García de León et al., 2018; Trejo et al., 2016). Patterns observed in this context are highly variable with both decreases (Brundrett & Ashwath, 2013; Sharmah & Jha, 2014), increases (Picone, 2000), and neutral results (Lekberg et al., 2012) in AM fungi diversity and/or abundance and diverse degrees of shift in mycorrhizal community composition (Rosendahl & Matzen, 2008; van der Heyde et al., 2017). The drivers behind this variability in responses are not yet fully understood, however it is clear that the combinations of a wide range of disturbance types and intensity with an equally wide range of affected ecosystems and fungal communities is bound to result in the diverse patterns observed (van der Heyde et al., 2017). It is also worth noting that the vast majority of surveys conducted on the impact of disturbance on mycorrhizal associations focus on AM fungi and very little research has been done on the impact of disturbance on other mycorrhizal types. Furthermore, as we know that anthropogenic disturbance has a sizeable impact on mycorrhizal fungi and is a strong driver of non-native plant invasions, it stands to reason that these two factors could be linked. However, to the best of our knowledge, no study has been conducted on the combined effect of disturbance on both mycorrhizal fungi and non-native species. Therefore, one of the main aims of this thesis is to fill that gap and investigate whether anthropogenic disturbance could be driving non-native success through disruption of the native mycorrhizal communities.

1.4 Mountain ecosystems

Mountain ecosystems are highly valuable, both due to their disproportionate share of the world's diversity (Rahbek, Borregaard, Antonelli, et al., 2019) and their crucial role in maintaining ecosystem services (A. A. Grêt-regamey et al., 2021). However, mountain systems are also under increasing pressure from global change as they are particularly sensible to climate warming (Pepin et al., 2015; Rangwala & Miller, 2012) and are seeing a steep increase in land-use change (Payne et al., 2020; Spehn et al., 2006), introducing novel disruptions in many ecosystems which had long remained pristine. Historically, land use practices in mountains have consisted mostly of pasturing, forestry, and occasional mining. However, human activities in mountains have seen a marked upward trend over recent decades, especially driven by increased industry (mining and hydroelectric power (Korner & Spehn, 2019)) and tourism (Debarbieux et al., 2014; Pickering & Barros, 2012).

Non-native plant invasions in particular strongly benefit from the combined effect of climate warming and novel anthropogenic disturbance in these systems (Alexander et al., 2016; Petitpierre et al., 2015). Until recently, mountain ecosystems were seen as relatively resistant to non-native plant invasions, as most research indicates that non-natives are regionally less common at higher elevations compared to lower elevations. This is assumed to be due to harsh alpine conditions forming a strong barrier to non-native plant success and comparatively lower human activity (Pauchard et al., 2009). Despite these limitations, non-native plant species have recently been observed to increase their elevational range and abundance in mountain systems globally (Iseli et al., 2023). Such patterns have been detected across a wide variety of regions and environments, from the Arctic (Lembrechts et al., 2014) to tropical mountains (Jakobs et al., 2010; Khuroo et al., 2011) and across all continents (Seipel et al., 2012). Furthermore, most non-native species are introduced at lower elevations, where human activities are the most common, before spreading upwards over time so as to fill in their climatic niche (Alexander et al., 2011). As many of these introductions are recent, it is to be expected that non-native presence at higher elevations will keep increasing in the future (Haider et al., 2010).

As upward shifts of non-native species are already having marked impacts on mountain ecosystems by leading to reduced diversity amongst native species, loss of ecosystem functions and biotic homogenization (Daehler, 2005; Haider et al., 2018; Pecl et al., 2017), being able to predict and manage further spread should be a priority for the protection of mountain ecosystems.

Anthropogenic disturbance in particular is known to be a crucial factor in the establishment and spread of non-native plants in mountain systems (Alexander et al., 2016; Fuentes-Lillo et al., 2021; McDougall et al., 2018). Events of physical disturbance leading to removal of native vegetation, typically associated with construction, are particularly key in the spread of non-native species in mountains as they create favorable conditions with low competition where the typically ruderal non-native species can establish (Lembrechts et al., 2016). These locally disturbed sites can then act as stepping stones for non-native species which can spread from one disturbed site to the next, given sufficient propagule pressure, to extend their distribution and once settled then may have the potential to spread towards the natural vegetation (McDougall et al., 2018).

In this regard, roads present ideal conditions for the spread of non-native species across mountain systems as they offer both constant disturbance in the roadside and high propagule pressure from vehicular traffic, letting them act as corridors for plant species movement (Ansong & Pickering, 2013; Haider et al., 2018; Lembrechts et al., 2017). The physical disturbance associated with mountain roads is not limited to the moment of their construction as most roads are actively maintained through mowing of the roadside vegetation and maintenance of road surface, be it gravel or asphalt, which generate repeated small scale disturbance events (Müllerová et al., 2011). Alongside this physical disturbance mountain roads are also known to locally affect soil conditions, including nutrient availability or changes in pH and soil moisture (Müllerová et al., 2011). These effects combined make roadsides ideal environments for non-native species which can typically benefit more from reduced competition, increased nutrient availability, and improved microclimate than native species (Averett et al., 2016; Müllerová et al., 2011). This is reflected by the fact that studies across numerous regions globally have consistently found non-native species to be more numerous in roadsides than in the adjacent vegetation (Haider et al., 2018). Furthermore non-native plant species have been observed to use road networks to access high elevation sites that were previously free of non-native species, for example in the Andes (A. Barros et al., 2020), Tenerife (Dickson et al., 1987) or Hawaii (Jakobs et al., 2010). This driving effect on non-native plant distribution combined with the clear juxtaposition of disturbed and undisturbed environments in otherwise relatively pristine environments makes mountain roads ideal systems in which to study the mechanisms linking disturbance and plant invasions.

1.5 Goals & Methods

Much of the work presented in this thesis was performed in the context of the Mountain Invasion Research Network, or MIREN (www.mountaininvasions.org). MIREN is an international network of researchers originally founded in 2005 with a focus on studying non-native plant invasions in mountains, and has over time expanded its scope to include general patterns of species redistribution in mountains under global change (Haider et al., 2021; Kueffer et al., 2014). The network's central aim is to understand how species distributions in mountains change over time, what the mechanisms and consequences of these changes are and ultimately to develop more effective ways to manage and protect mountain systems. To this end, screening and monitoring of native and non-native vegetation is by now being conducted in over 20 mountain regions globally, covering all continents and an extensive range of climatic zones, and for varying length of time with the initial regions of the project having been repeatedly surveyed since 2007 (Haider et al., 2021). At the center of MIREN's activities is the monitoring of native and non-native plants along mountains roads following a standardized protocol to assess changes in distribution and community turnover over both space and time under the combined effects of climate change and anthropogenic disturbance (Haider et al., 2021).

For each region participating in this survey, three mountain roads spanning large elevational gradients are selected and 20 sample sites are established per road, covering the whole elevational range in regular intervals. Each sample site is formed of three 2 x 50 m subplots organized in the shape of a “T”, with the first plot parallel to the road and the next two plots extending into the natural vegetation (Fig. 1.1). For each plot, all vascular species are recorded, and their abundance and cover are estimated by eye. Additionally, environmental metadata is collected. This process is then repeated at the same 60 sites every 5 years. This approach explicitly contrasts vegetation in anthropogenically disturbed conditions (roadside plots) with that in natural or semi-natural conditions (plots further away from the road’s effect) and across large elevational gradients (ranging from ca. 700 m to >4000 m, >1700 m on average across all regions, Haider et al., 2022) and as such covers a wide range of climatic conditions and vegetation types. Furthermore, many add-on projects have been developed over the years to supplement this core monitoring protocol including efforts to locally measure soil temperatures at high temporal resolutions (Lembrechts et al., 2020), determine plant functional traits (Kühn et al., 2021), assess pathogen distributions (Khaliq, 2019) or in the context of this thesis, measure mycorrhizal fungi and soil chemical properties. The resulting datasets consist of more than 2700 surveyed plots and over 100 000 observations of circa 5000 plant species as of 2021 (Haider et al., 2021). Both this existing dataset and the standardized MIREN protocol were crucial for the work carried out in this thesis.

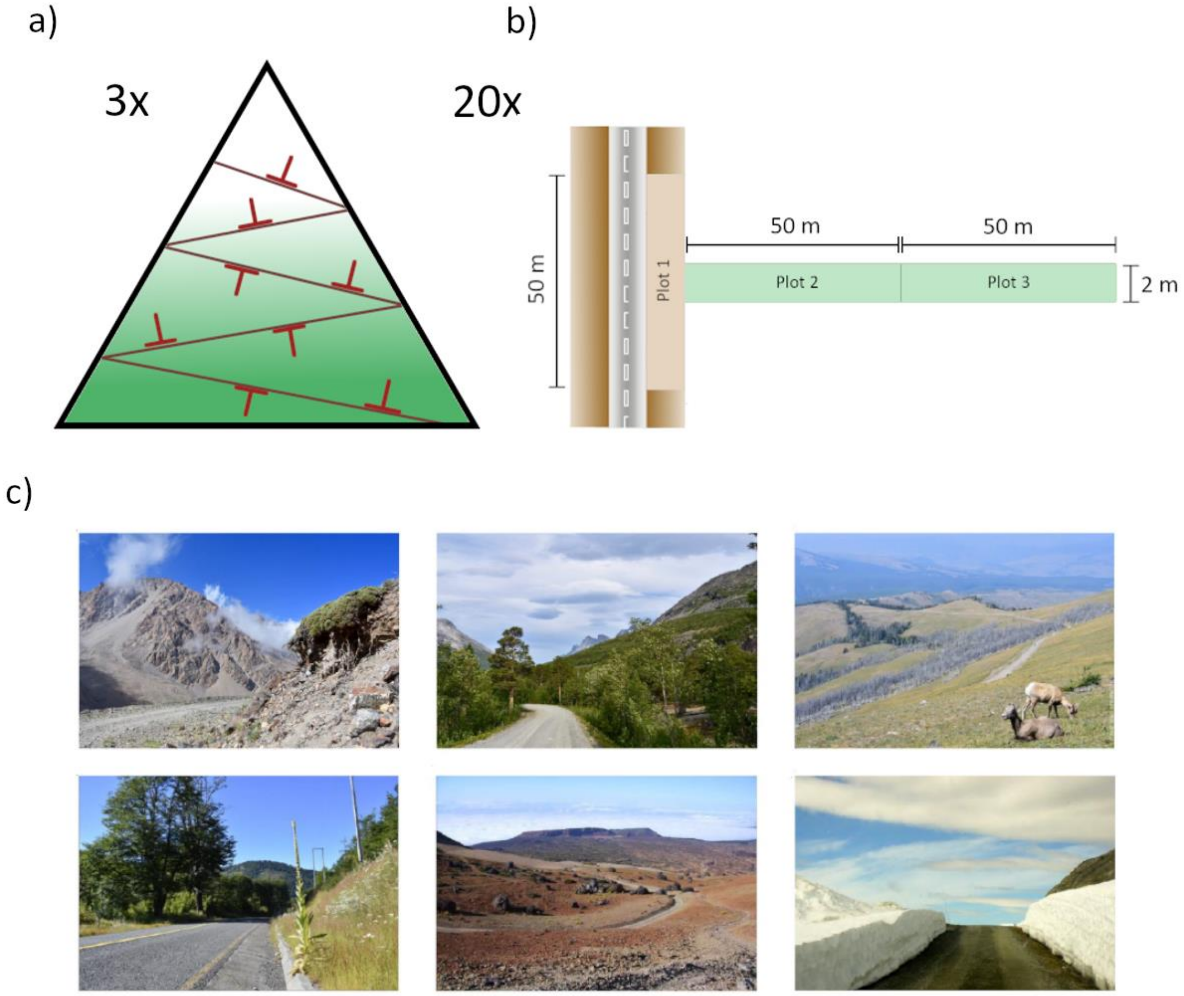

Figure 1.1: Layout of the MIREN survey design a) 20 sample sites are setup at equal elevational intervals along three mountain roads per survey region. b) Each sample site is composed of three 2 m x 50 m subplots, plot 1 is parallel to the road and plots 2 and 3 are perpendicular to the road to allow for comparison of the disturbed and undisturbed vegetation. c) Examples of roads from regions participating in the MIREN survey. From left to right: gravel roads in the Argentina, lowland service road in the Norway, high elevation asphalt road in Yellowstone, lowland asphalt road in Chile with strongly invaded roadside vegetation, high elevation gravel road and lowland snowed-in gravel road in Norway.

In terms of mycorrhizal associations, the tree species of the region as well as the very common dwarf shrub *Betula nana* are known to associate with EcM fungi and most of the other dwarf shrubs are known or expected to associate with ErM fungi (Wang & Qiu, 2006). AM fungi-associated plant species are also present in these vegetation types (e.g., *Solidago virgaurea* L. and *Deschampsia flexuosa* L., and ferns). However, the latter species are facultative mycorrhizal and overall less numerous than their EcM- and ErM-associated counterparts (Gardes & Dahlberg, 1996; Wang & Qiu, 2006). The Swedish side of the Scandes, near the research station of Abisko, is where the plots for the experimental part of my research were established. The climate there is more continental and less oceanic than on the Norwegian side with overall lower temperature and precipitation (approximately 0°C yearly average temperature and 400 mm of rainfall), however the vegetation types remain highly similar between the two sides of the mountain range with the main notable exception being the almost entire absence of pine trees in the lowland forests of the Swedish side.

Non-native plant species in the region are currently limited to the lowlands and to disturbed sites, but have been observed to possibly be in the process of extending their range (Lembrechts et al., 2014), making it an ideal system to study the early stages of the impact of anthropogenic disturbance on non-native species spread in mountains. We defined plants in this system as being non-native if they were introduced to the region after the end of the fifteenth century and capable to reproduce in the wild, more specifically for the Norway road survey we referred to the Norwegian generic ecological impact assessment of alien species (GEIAA) (Sandvik et al., 2019) which offers a comprehensive list of all non-native plants in Norway. It is worth noting that we started using this dataset as a reference after 2020 as we previously used the similar MIREN definition of non-native species (plant species introduced after 1492) combined with different sources including the Norwegian blacklist (Gederaas et al., 2007), DAISIE (Roy et al., 2020) and a study by Weidema, 2000 as explained in Lembrechts et al., 2014. However, the list from the GEIAA was retroactively applied to vegetation surveys of 2012 and 2017 to make comparison between years possible. The most common non-native species observed in the region during the initial survey in 2012 were *Achillea millefolium* L., *Trifolium pratense* L. and *Trifolium repens* L., all three ruderal AM fungi-associated species and currently there are no non-native plant invasions in the area (Lembrechts et al., 2014). It is worth noting that both *Trifolium* species are also known to be nitrogen fixer by forming additional symbiosis with rhizobia bacteria within root nodule (Sprent, 1996).

The goal of this thesis is to investigate how anthropogenic disturbance affects mycorrhizal symbiosis and the subsequent implications for non-native plant species invasions. To this end, I use a combination of repeated mycorrhizal and plant surveys in the mountains of northern Scandinavia, global datasets of plants along disturbed gradients and in situ seed-addition experiments in order to approach this core topic at different scales, both temporally and spatially. Through this process I hope to improve our knowledge of the mechanisms behind plant invasions following anthropogenic activities and in turn allow for the development of management approaches that take better account of the role of plant-fungi symbiosis.

1.6 Chapter overview

Chapter 2:

Most studies on the effect of disturbance on mycorrhizal associations have been done in agricultural settings (Jansa et al., 2006) and the few studies in natural settings were in lowland, temperate systems. To our knowledge, little is known about the impact of anthropogenic disturbance on mycorrhizal fungi in cold climates and mountain systems.

For chapter 2, we conducted a regional survey in the northern Scandes to investigate the impact of anthropogenic disturbance on AM fungi along mountain roads in an EcM and ErM dominated environment using a combination of plant surveys following the MIREN protocol, measures of AM fungi abundance and measures of AM fungi community composition. Previous studies have shown that mountain roads in this region (Lembrechts et al., 2014), as well as globally (Haider et al., 2018), promote the success ruderal native and non-native species. As these species are most commonly AM-associated we hypothesized that road disturbance has a positive effect on AM fungi which participates in the increased success of these plant species, which would be reflected by levels of AM fungi in the roadside vegetation compared to the adjacent undisturbed vegetation. Furthermore, we expected AM-abundance and diversity to diminish with elevation as non-native plant species were less common in higher parts of the regional elevational gradient and AM fungal abundance and diversity had previously been observed to diminish with elevation (Bueno et al., 2017). Finally we expected non-native plant presence to correlate with increased AM-abundance and diversity in the surrounding vegetation.

Chapter 3:

In chapter 3, we combined a global dataset of plant species distributions along mountain roads (Haider et al., 2021) and datasets linking plant species and their type of mycorrhizal associations (Soudzilovskaia et al., 2020) to answer whether the local observations of increased AM fungi in disturbed roadsides obtained from chapter 2 could be extrapolated to a global scale. To this end we estimated the total cover percentage of AM plants, EcM plants, ErM plants and non-mycorrhizal plants along the roads of 11 mountain regions from across the globe and compared these to their counterparts in the adjacent undisturbed vegetation.

Our expectations were that the patterns of increased AM fungi, and here AM plants, following road disturbance we observed in chapter 2 would be similar in other cold-climate mountain regions with many ErM and EcM-associated plant species but might not be observed in regions with milder climates and AM-dominated vegetation types, resulting in varying patterns of anthropogenic disturbance impact on the distribution of different mycorrhizal types.

Chapter 4:

Following the observational results of chapter 2 we aimed to disentangle the mechanisms behind non-native plant facilitation by anthropogenic disturbance in cold environments. To this end we set-up a seed-addition experiment which expended on a previously conducted in the region of northern Scandes near Abisko, Sweden (Lembrechts et al., 2016). The original study focused on the success of non-native plant species following disturbance in cold-climate mountain through plant measurements only, we aimed to use a similar approach but with the added dimension of fungal interactions by both measuring and altering the native fungal community. Eight experimental sites were selected along two elevation gradients where the seeds of four non-native species were treated with different combinations of three factors associated with anthropogenic disturbance: physical disturbance through top soil and vegetation removal, nutrient addition, and AM fungi inoculum. The germination rate and biomass of the non-native plant species was then measured one year later along with a survey of the associated fungal community. Our expectation from the results of previous studies was that physical disturbance would be the main driver of non-native success and have the strongest impact on the fungal community, unless this previously observed effect was itself a consequence of the mycorrhizal disruption, while nutrient addition would drive higher biomass production. We also thought AM inoculum addition would increase non-native success as we had observed strong correlations between increased AM fungi abundance and diversity and high abundance of non-native species in previous chapters.

Chapter 5:

In this chapter we added a temporal dimension to our initial survey by repeating the MIREN survey of native and non-native plants along mountain roads that was initiated in the northern Scandes in 2012 (Lembrechts et al., 2014). These repeated measurements were done over intervals of five years in 2017 and 2022. Alongside these vegetation measurements, we also repeated the mycorrhizal survey initiated in 2017 (Chapter 2) twice, once in 2019 and once in 2021. Our aims were 1) to assess whether non-native plants are in the process of extending their upward range along these mountain roads as well as their horizontal range from roadsides into the natural vegetation as has been observed in other regions (McDougall et al., 2018; Pauchard et al., 2009); 2) to assess whether AM-fungal abundance had increased over time in the roadside and in the adjacent undisturbed vegetation; and 3) to combine measurements of non-native plants and of AM fungi in order to verify whether their changes over time were linked or independent. We found no increase in elevational range or spread into the natural vegetation of either non-native plants or AM fungi, however the abundance of both increased over time in low elevation disturbed plots.

Chapter 6:

In part 6 I will integrate the results obtained from the previous chapters to answer the following central questions:

- How does anthropogenic disturbance in general and road disturbance specifically impact mycorrhizal fungi and type of mycorrhizal association in the vegetation of mountain ecosystems?
- How are non-native plant species and mycorrhizal fungi interacting and changing over time along disturbed elevational gradients in the cold-climate mountainous region of the northern Scandes?
- What are the mechanisms behind the impact of anthropogenic change on non-native plants and their associated mycorrhizal fungi and, do said plant and fungi reinforce each other and what are the implications for plant invasion trajectories?

Finally, I will discuss the limitations of our studies and the implications and future research prospectives that follow from our results.

CHAPTER II

The role of arbuscular mycorrhizal fungi in non-native plant invasion along mountain roads

This Chapter was based on:

Clavel, J., Lembrechts, J., Alexander, J., Haider, S., Lenoir, J., Milbau, A., Nuñez, M., Pauchard, A., Nijs, I. & Verbruggen, E. (2021). The role of arbuscular mycorrhizal fungi in nonnative plant invasion along mountain roads. *New Phytologist*, *230*(3), 1156-1168.

Summary

- Plant associated mutualists can mediate invasion success by affecting the ecological niche of non-native plant species. Anthropogenic disturbance is also key in facilitating invasion success through changes in biotic and abiotic conditions, but the combined effect of these two factors in natural environments is understudied.
- To better understand this interaction, we investigated how disturbance and its interaction with mycorrhizas could impact range dynamics of non-native plant species in the mountains of Norway. Therefore, we studied the root colonisation and community composition of arbuscular mycorrhizal (AM) fungi in disturbed vs. undisturbed plots along mountain roads.
- We found that roadside disturbance strongly increases fungal diversity and richness while also promoting arbuscular mycorrhizal fungal root colonisation in an otherwise ecto- and ericoid-mycorrhiza dominated environment. Surprisingly, AM fungi associating with non-native plant species were present across the whole elevation gradient, even above the highest elevational limit of non-native plants, indicating that mycorrhizal fungi are not currently limiting the upward movement of non-native plants.
- We conclude that roadside disturbance has a positive effect on AM fungal colonisation and richness, possibly supporting spread of non-native plants, but that there is no absolute limitation of belowground mutualists, even at high elevation.

2.1 Introduction

The mutualistic association between non-native plants and mycorrhizal fungi, both native and introduced, is suspected to play a substantial role in the successful spread of non-native plant species (defined here as species which originated from outside the region and were introduced by anthropogenical means). A better understanding of this interaction could be crucial to improve our insight into invasion patterns (Bever et al., 2010; Dickie et al., 2017). Mycorrhizal associations occur in the majority of terrestrial plants (Wang & Qiu, 2006) and are increasingly recognized as fundamental determinants of plant community composition and ecosystem functioning (Klironomos et al., 2011; Neuenkamp et al., 2018; Wagg et al., 2014). Previous research on non-native plant invasion success has shown both mycorrhizal limitation and facilitation across a variety of ecosystems (Dickie et al., 2017; Policelli et al., 2019; Pringle et al., 2009). Furthermore, the impact of non-native plant species on the native mycorrhizal fungal community and subsequent effects on native flora also varies between studies from stimulation, through no observable effect, to disruption of mutualism (Dickie et al., 2017; Grove et al., 2017; Mummey & Rillig, 2006; Stinson et al., 2006; Urcelay et al., 2017). With such a diversity of possible responses, it is clear that a better understanding of the underlying processes is crucial to predict how mycorrhizal associations will affect the invasion process, and whether they may be drivers or passengers of non-native plant success (Zobel & Opik, 2014). Recent studies have moved in this direction, and identified mycorrhizal status (Menzel et al., 2017) and overlap in mycorrhizal associations with native vegetation (Bunn et al., 2015) as potential predictors of invasion success of non-native plant species.

Apart from mycorrhizal associations, anthropogenic disturbances are another important determinant of non-native plant invasion (Hobbs & Huenneke, 1992; Jauni et al., 2015; Lembrechts et al., 2016). The effects of disturbance on plant competition (Biswas & Mallik, 2010), nutrient availability (Blumenthal, 2006; Davis et al., 2000) and propagule and colonisation pressure (Blackburn et al., 2020; Vilà & Ibáñez, 2011) are all known to facilitate the invasion success of non-native plants. We hypothesize that changes in belowground mutualist interactions caused by disturbances could also play a significant role during the invasion process. Until now, the effect of physical disturbance per se on mycorrhizal fungal communities has been primarily studied in the context of tillage effects on arbuscular mycorrhizal (AM) fungi in lowland agricultural landscapes, showing reduced fungal diversity and root colonisation (Goss & De Varennes, 2002; Kabir, 2005; Schnoor et al., 2011). However, less is known about how disturbance in natural ecosystems influences mycorrhizas, where it may modulate AM fungi community and facilitate invasion success by AM plants. In this study, we aim to bridge this gap by investigating the effects of disturbance in an otherwise natural setting on belowground interactions and whether these could play a role in regulating non-native plant invasions.

To achieve this goal, we studied the abundance and diversity of mycorrhizal fungi along mountain roads in the northern Scandinavian mountains (the Scandes) in Norway. The crucial role of disturbance in facilitating invasion success in mountain systems is well known (Alexander et al., 2016; Lembrechts et al., 2016; Pauchard et al., 2009), making them ideal ecosystems in which to assess how mycorrhizas could mediate this role. Roads in particular offer a clear juxtaposition of disturbed and undisturbed conditions and have been shown globally to facilitate the upward expansion of non-native plant species (McDougall et al., 2018; Müllerová et al., 2011). The upward expansion dynamic of non-native plant species along roadsides has been repeatedly observed in local studies, for example in the Himalayas (Bhattarai et al., 2014), northern China (Zhang et al., 2015) and the Rocky Mountains in the USA (Pollnac et al., 2012). Furthermore, a global survey of non-native plant species in relation to mountain roads showed that the number of non-native plant species was found to be higher along roadsides than in the natural vegetation, leading in turn to a more homogenized flora along roadsides (Haider *et al.*, 2018). Similar patterns have been observed in the northern Scandes which are still in an early stage of invasion with non-native plant species increasing their elevation range along roads, yet currently remaining largely restricted to the roadsides, suggesting a crucial role of the disturbed environment in their range expansion (Lembrechts et al., 2014). Candidate causes for roadside affinity of non-native plants in high latitude acidic-soil ecosystems such as the northern Scandes, are the physical modification of the environment and alteration of chemical properties of soils, for example with alkaline building materials enhancing soil pH (Müllerová et al., 2011)

Non-native plants occurring in this system are typically associated with AM fungi, as opposed to the natural vegetation which mostly associates with ecto- and ericoid mycorrhizal fungi, especially at high elevations (Wang & Qiu, 2006; Newsham et al., 2009; Lembrechts et al., 2014). These native mycorrhizal fungi are better adapted to low temperatures, low soil pH and slow cycling of nutrients locked up in recalcitrant litter compared to AM fungi (Read, 1991; Soudzilovskaia et al., 2015). We therefore expect the previously mentioned changes caused by road disturbance, such as increased soil pH and nutrient availability, to lead to a more suitable environment for AM fungi and in turn for non-native AM-associated plant species which would benefit from the increased AM fungi availability. Conversely, a lack of appropriate AM fungi in the natural vegetation might constrain the expansion of non-native plants away from roadsides. Furthermore, we know from previous studies (Lembrechts et al., 2014) that non-native plant species richness in our study system decreases with increasing elevation, with no non-native species currently present above the tree line. This pattern coincides with the globally observed decline in non-native plant species richness along elevation gradients (Alexander et al., 2011). We hypothesize that this could be in part caused by a lack of adequate mycorrhizal fungal partners, as the harsher conditions at high elevations are likely to be less suitable for AM fungi (Bueno et al., 2017). Finally, the presence of the non-native plant species could lead, independently of the direct road effect, to a further increase in AM fungi colonisation in their surrounding vegetation, as observed in other systems (Stinson et al., 2006; Lekberg et al., 2013).

In this study, we assessed the distribution of AM fungi in the roots of three non-native AM plant species invading the northern Scandes: *Trifolium pratense* L.; *Trifolium repens* L.; and *Achillea millefolium*. L. These are the three most common non-native plant species in the region (Lembrechts et al., 2014), but have yet to reach a state in which they could be considered as invasive, as their impact on the ecosystem is currently minimal. We also assessed AM fungi in the roots of the surrounding native vegetation where the non-native species are mostly absent. Sampling was performed along three elevational gradients from sea-level to the alpine zone above the treeline at around 700 m a.s.l. to test the following hypotheses:

H1: There is a positive correlation between road disturbance and AM fungal abundance and diversity, which plays a role in the success of non-native plant species spread along roadsides.

H2: AM fungal abundance and diversity diminish towards higher elevations, which might limit the upward expansion of non-native plant species.

H3: The presence of non-native plant species along disturbed roadsides correlates with increased presence of AM fungi in their surrounding roadside environment.

2.2 Materials and methods

Study region

The study sites are located in the northern Scandes, 220 km north of the Arctic Circle in the vicinity of the city of Narvik, Norway (68°26’N, 17°25’E). Three mountain roads were selected, hereafter called R1, R2 and R3 (see Fig. **2.1a**), reaching respectively from sea level up to 609, 697 and 633 m a.s.l. across lengths of 7.1, 26.4 and 20 km. The roads are made of asphalt at lower elevations, turn to gravel upon reaching higher elevations and are flanked by drainage systems (see pictures Fig. **S2.1**). These roads were built in the 1980s, are maintained through yearly mowing and gravel addition approximately every three year and are used regularly in summer by cars and trucks for tourism and to access high elevation hydropower plants for two of the roads. The elevational gradients crossed by these roads allow us to observe the impact of roadside disturbances on mycorrhizal fungal communities across a wide climatic range. Vascular plant communities along these roads have been monitored since 2012 in the framework of a global long-term study on native and non-native plant species distributions in mountain ecosystems (The Mountain Invasion Research Network, MIREN, www.mountaininvasions.org), which revealed the communities to be in an early stage of colonisation by non-natives species (Lembrechts et al., 2014). The vegetation along the roads transitions from birch dominated forests with pines and willows at low elevations, with an understory of *Vaccinium spp.* and *Empetrum hermaphroditum* Hagerup, towards alpine shrublands at higher elevation mainly composed of a range of ericaceous dwarf shrubs (Lembrechts et al., 2014). These vegetation types are dominated by ecto- and ericoid mycorrhizal plant types (Bueno *et al.*, 2017). However, AM fungi are still likely to be present in association with native forbs and grasses such as *Solidago virgaurea* L. or *Calamagrostis purpurea* Trin., and various mosses, which occur along the whole elevational gradient in the study system.

We studied the distribution and mycorrhizal associations of the three most common non-native plant species spreading towards higher elevations along the mountain roads in the region (Lembrechts et al., 2014). These are *Achillea Millefolium* L.*,* *Trifolium repens* L.*,* and *Trifolium pratense* L., all three being AM-associated plant species (Wang & Qiu, 2006) with the *Trifolium* species additionally also being nitrogen fixers (Sprent, 1996). As a native reference species, we included *Solidago virgaurea* L.*,* which is the most common native AM plant species found along the whole studied elevational gradient, both along the disturbed roadsides and inside the undisturbed natural vegetation. The native and non-native status of these species was previously assessed in the study by Lembrechts *et al.*, 2014, with non-native plant species being defined as species having been introduced into the northern third of Norway from another region after 1492. *A. millefolium* is known as "facultative" AM plant species (known to have non-mycorrhized occurrences), while *T. repens, T. pratense* and *S. virgaurea* are known to be an “obligate” AM plant species (Wang & Qiu, 2006), although these delimitations should be interpreted with caution (Brundrett & Tedersoo 2019).

Sampling design

The three studied roads were each divided into segments with intervals of, on average, 111 m of elevation. One transect was established at each segment junction, resulting in 7 transects along roads R2 and R3, and 5 transects along the shorter road R1, for a total of 19 transects covering the three elevational gradients (Fig. 2.1**b**). Each of those transects was then further divided into two 2 m × 50 m plots organized in a T-shape, with one plot following the road and the other perpendicular to the first plot, extending from the road to 50 m into the undisturbed vegetation (Fig. 2.1**c**). This specific T-shaped set-up follows the MIREN design, aimed at the long-term survey of plant species composition along mountain roads as initiated in the region in 2012 (Lembrechts et al., 2014; Seipel et al., 2012). The presence or absence and estimated cover of each of the focal plant species was recorded in each of these plots in parallel with the sampling for mycorrhizal analysis. The sampling was done over a period of a month from July to early August 2017. To reduce the potential confounding effect of the difference in phenology between the start and the end of our field season, uneven numbered transects were surveyed and sampled at the start of the fieldwork period, while even transects were sampled at the end.

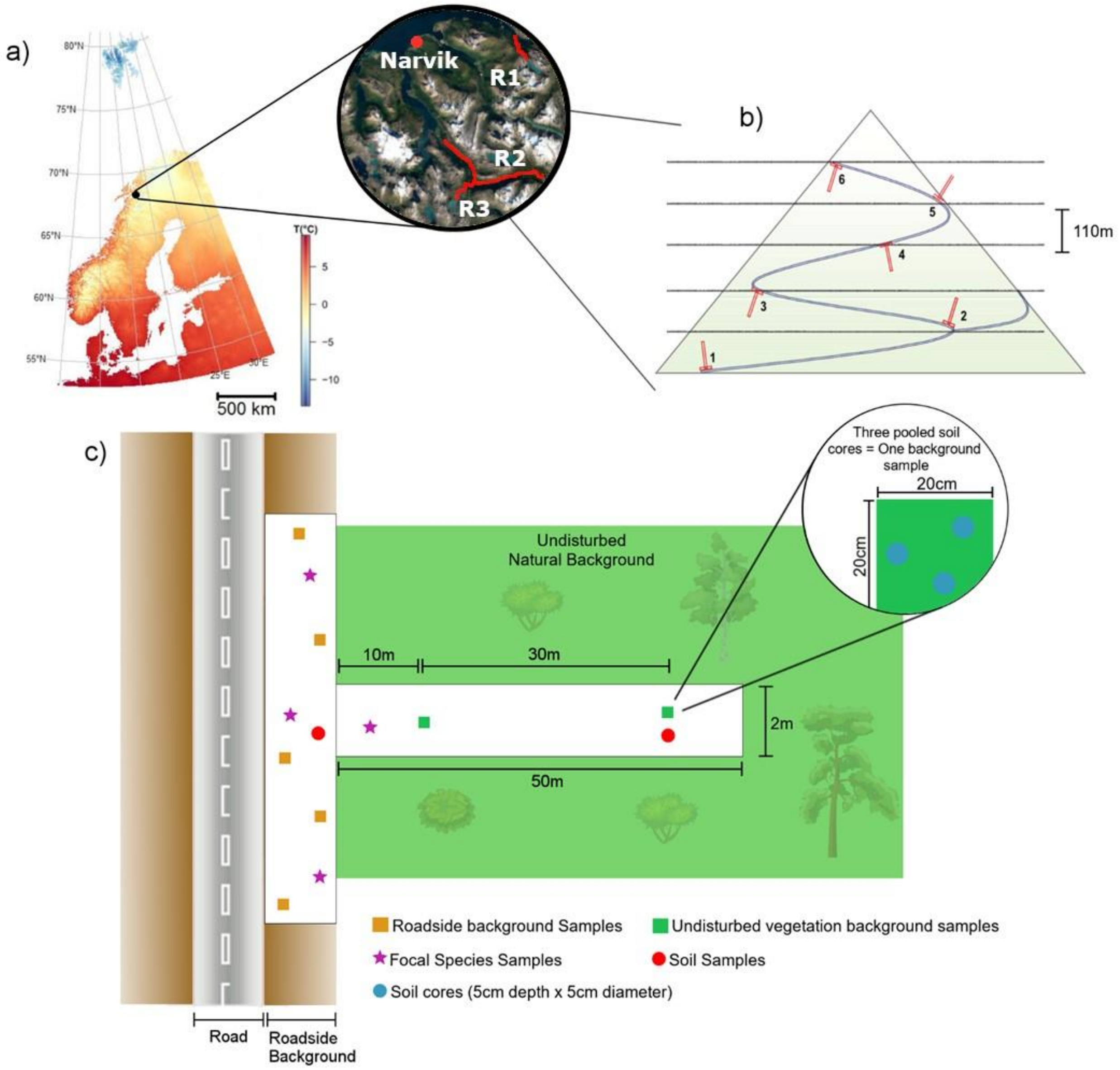

Figure 2.1: Sampling design along three mountain roads in the northern Scandinavian mountains. a) Location of the three studied mountain roads (R1, R2 and R3) in the northern Scandinavian mountains, near Narvik, Norway, with map showing mean annual temperature from CHELSA (Karger et al., 2017) for Scandinavia **b)** Transects were spread with fixed elevation steps along the whole elevation gradient covered by each road. **c)** Each transect was subdivided into 2 plots (white rectangles) following the MIREN protocol (Seipel et al., 2012). Each plot was 2 x 50 m, the first plot following the road covered the area impacted by the road disturbance while the second plot extended into the undisturbed natural vegetation perpendicular to the road. For each plot, the presence and total cover of focal plant species (*Achillea millefolium*, *Trifolium repens*, *Trifolium pratense* and *Solidago virgaurea)* was measured. Five disturbed vegetation background root samples (orange squares) were taken in the roadside plot in randomly chosen locations and two undisturbed vegetation background samples (green squares) were taken 10 m and 40 m away from the road. Each background sample was composed of three pooled soil cores of 5 cm diameter by 5 cm depth (blue circle) taken in a 20 x 20 cm square. Roadside samples were further pooled (see Methods section). When present, up to four root samples of focal plant species were taken in the roadside (purple stars) for each focal plant species present. Two additional soil samples were taken in the roadside and at 40 m into the undisturbed vegetation for soil pH, P and N analysis (red circles). Figure adapted from Lembrechts et al. (2014).

Four root samples (henceforth referred to as background samples) were taken for AM fungal measurement (see below) in each of the 19 transects. These four background samples were split between 2 disturbed vegetation samples and 2 undisturbed vegetation samples. For all background samples three topsoil cores of 5 cm diameter and 5 cm depth were taken inside a 20 cm × 20 cm square including a random assortment of roots from the surrounding vegetation, both the plant species present in each square and their estimated cover were also recorded. (See Fig. **2.1**). The roots in these soil cores were later removed from the soil and pooled to form one final background root samples. This approach was chosen to best represent the overall root make-up of the local vegetation without biases towards certain species. The two natural vegetation samples were taken at medium (10 m) and far (40 m) distances from the road to verify if there was a difference caused by proximity to the disturbance as Lembrechts et al. (2014) showed that the roadside disturbance effect on vegetation did not extent further than 25 m from the road, yet personal observations indicated that any roadside effect on the community was no longer observable around 5 m. Initially, five background samples were taken regularly along each roadside, although practical constraints kept us from individually processing all five samples. We kept one of those samples and pooled the remaining four, resulting in two disturbed vegetation samples per transect. We found no difference in colonisation or diversity between the pooled and non-pooled samples and thus decided to keep this pooling approach in the analysis.

In addition to these background samples, up to four root samples per transect were taken for each of the focal plant species, when present in one of the plots, and subjected to AM fungal measurements. For those focal plant species, a sample consisted of the roots of one individual excavated from inside the transect (See Table **S2.1** for the list of all background and focal plant species samples). Among the focal plant species, only *S. virgaurea* and *A. millefolium* (rarely) were found and sampled in the undisturbed vegetation and therefore the majority (89 % of non-native species, and 75 % for *S. virgaurea*) of focal plant species' root samples originated from the disturbed vegetation plots.

All root samples from both background and focal plant species samples were cleaned in demineralized water over a 2 mm mesh size sieve to remove the soil material, after which fine roots were cut into 1 cm pieces for further analysis of AM fungal colonisation and community composition. Finally, two soil samples were taken in each transect in the same way as the root background samples, one taken in the disturbed vegetation and the other in the undisturbed vegetation at 40 m from the road. With these two samples, we measured soil pH (using KCl-extractions), available P (using P Olsen (Olsen et al., 1954)) and mineral N (NH_4^+ and NO_3^-, using KCl extractions) to assess the abiotic differences between disturbed and undisturbed vegetation (Table **S2.2**).

Arbuscular Mycorrhizal fungal root colonisation and molecular analysis

Both AM fungal root colonisation rate and community composition were measured for all background and focal plant species samples. The root colonisation rate of AM fungi was measured by counting mycorrhizal structures (aggregating hyphae, arbuscules and vesicles) under the microscope using the gridline and intersection method described in McGonigle et al. (1990). This method obtains the proportion (%) of root length colonised by AM, henceforth referred to as “AM root colonisation rate”. For this purpose, root samples were cleared using a 5 % KOH solution and cut into on average twenty pieces of 1 cm before being stained using a solution of 10 % Schaeffer black ink and 10 % acetic acid, as described by Vierheilig et al. (2005).

For DNA-based barcoding of the AM fungi community of each sample, a subset of ten randomly selected 1 cm root pieces was lyophilised and pulverized with sterile tungsten beads in a grinder that holds Eppendorf tubes, vigorously shaken for 60 s, after which the DNA was extracted using DNeasy PowerSoil Kit following the standard protocol (Qiagen, Venlo, the Netherlands). We targeted the AM fungi 18S rDNA using the primer pair AMV4.5NF/AMDG (Sato et al., 2005; Maarten Van Geel et al., 2014), augmented with multiplexing barcodes and sequencing adapters in a second polymerase chain reaction (PCR) step. The first PCR was performed in 25 µl volumes using 1 µl of template, 400 nM of both primers, 1X PCR buffer, 200 µM of each dNTP and 1 unit of polymerase from the Phusion High-Fidelity DNA Polymerase kit (New England Biolabs, Ipswich, USA). The PCR conditions were: initial denaturation at 98 °C for 30 s; 30 cycles of denaturation at 98 °C for 30 s; annealing at 65 °C for 30 s and extension at 72 °C for 30 s; and a final step at 72 °C for 10 min. Successful amplification was confirmed using agarose gel electrophoresis and samples that failed to produce PCR products were run again for 40 cycles. Samples that did not successfully produce PCR products after the second attempt (about 19 % of the samples) were excluded. The second PCR used 1 µl of a 1:100 dilution of product of the first PCR, 200 nM for both forward and reverse barcoded primers and was otherwise identical to the former PCR mix. The PCR conditions were: initial denaturation at 98 °C for 30 s; 10 cycles of denaturation at 98 °C for 10 s; annealing at 63 °C for 30 s and extension at 72 °C for 30 s; and a final step of 72 °C for 10 min. Again, successful amplification was confirmed using agarose gel electrophoresis. The resulting 163 PCR products were purified and equalized using sequalprep plates (Thermo Fisher Scientific, Waltham, USA) before being pooled into a single library. A gel extraction was performed on the pooled library to ensure absence of primer-dimers, and further purified using QIAquick Gel Extraction Kit (Qiagen, Venlo, Netherlands). The library was then quantified using real-time PCR (KAPA Library Quantification Kit, Kapa Biosystems, Wilmington, MA, USA) and sequenced using the Illumina MiSeq platform (Illumina Inc; San Diego, CA, USA) with 300 cycles for forward and reverse reads and double indexing. The raw sequences were deposited in the National Center for Biotechnology Information's (NCBI's) Sequence Read Archive database under the accession no. PRJNA663438sky.

Note that AM fungi were studied in roots only, and not in soil samples. While some additional AMF taxa absent from root samples could have been picked up in soil samples, these would be non-associative and inactive AMF taxa which are not relevant to our study. We accounted for the possibility that different individuals and species would not include all AMF taxa present in the background vegetation by pooling roots from multiple individuals and species whenever present.

Bioinformatics

The USEARCH software was used following the UPARSE pipeline (Edgar, 2013) for the first steps of the bioinformatic analysis. Sequences were trimmed to 200 bp, paired end reads were merged, and primer sequences were removed. After quality filtering with a maximum expected error of 0.5, about 418,439 high quality sequences were kept. These reads were dereplicated and clustered into Operational Taxonomic Units (OTUs) using a threshold of 97 % similarity (Öpik et al. 2010; Lekberg et al. 2014). Chimera filtering resulted in the removal of 4.2 % of reads, leaving a total of 432 distinct OTUs. The resulting OTUs were then aligned against the AM fungi specialised MaarjAM database (Öpik et al., 2011). Out of the resulting hits, only the ones with an identity score higher than 90 % were retained. Those sequences were then aligned against the Silva database, specialised in small and large subunits of ribosomal RNA (Yilmaz et al., 2014) as well as against the full NCBI database (O'Leary et al., 2016). Sequences which had lower E-values for non-AM sequences in SILVA or NCBI compared to their AM fungi alignment in MaarjAM were discarded as likely not being AM fungal sequences. The remaining 43 AM fungal OTUs (Table **S2.3**) were then rarefied to 200 reads per sample which has previously been shown to adequately cover AM fungal communities in roots (Marteen Van Geel et al., 2017).

Statistical analyses

1) Arbuscular mycorrhizal fungal root colonisation

Models were made to test for the effects of both elevation, road disturbance and their interaction on the AM fungal root colonisation rate of background samples (N = 69). Since AM root colonisation was measured as a proportion of discreet counts, we used beta regressions, following transformation of the response variable (i.e. proportion data) to avoid extreme values of 0 and 1 (Cribari-Neto & Zeileis, 2010) and using the glmmTMB package (Brooks et al., 2017). As explanatory variables, we used elevation and disturbance (a two-level factor including disturbed vs undisturbed vegetation backgrounds) as well as their interaction term. The two-leveled disturbance variable was preferred over the three leveled variable including road, medium (10 m) and far (40 m) distance from the road as we tested for the effect of medium vs far amongst undisturbed vegetation and found no difference (GLMM, N=35, R^2=-0.004, P=0.65) between the two distances. Undisturbed samples taken at 10 and 40 m were henceforth treated as repeated samples in the same plot. A random intercept term of plot nested in transect nested in road was included to account for our hierarchical sampling design. Model analysis was performed through model selection by comparing candidate models with all possible combinations of fixed effects derived from the full model and retaining only candidate models with a ΔAICc (Akaike Information Criterion, corrected for small sample sizes) of less than 2 units compared to the best candidate model (Zuur et al., 2009).

A similar approach was used to test for the effect of elevation and species identity on focal species' AM root colonisation rate (N = 92). In this case, we used focal species AM root colonisation rate as a response variable, with species identity and elevation and their interaction as explanatory variables, with the same random intercept term as above. Disturbance was not included here due to the low number of observations of non-native species in the undisturbed natural vegetation (11 %).

To explore how disturbance (disturbed vs undisturbed) and elevation influence abiotic soil conditions, we ran linear mixed-effects models (lmer, Bates *et al.*, 2015) with soil pH, N and P as response variables, as a function of disturbance and elevation (N = 69). Additionally, we tested the residuals of the background sample AM root colonisation models against soil pH, N and P to investigate whether these factors had an additional impact separate from the direct disturbance effect. Residual normality and homoscedasticity was first tested using the DHARMa package (Hartig, 2020) and all models showed residual normality and homoscedasticity. Then the aggregate residuals were obtained by weighted averaging of the residuals of each independent retained model (ΔAICc < 2). The latter residuals were then tested with linear mixed-effects models (lmer, Bates *et al.*, 2015) against soil pH, N and P, with the same random structure as before. Similarly to the AM fungal root colonisation model, model selection was done by selecting all models with a ΔAICc smaller than 2 from the best model.

2) Root fungal community composition

To test for the effects of elevation and disturbance on the OTU community composition of the background samples, PERMANOVAs were performed (N = 144) using the adonis function from the R package vegan (Oksanen et al., 2019). To consider the nested nature of our design and avoid pseudo-replication we then ran this PERMANOVA one thousand times, each time randomly dropping one of two replicates from our dataset (i.e. one of the disturbed vegetation samples and one of the undisturbed vegetation samples). We then assessed the distribution of R^2's and P values across the thousand replicates to infer trends in the OTU community composition.

3) Relationship between non-native plant species presence and AM fungal root colonisation

To further disentangle whether non-native plant species presence influences rates of AM root colonisation independently of the direct effect of disturbance, we tested for the effect of non-native plant species presence/absence and soil pH, as a proxy for abiotic soil factors, on AM root colonisation rate of disturbed vegetation background samples, using the same approach as described in section 1 (N = 38).

Additionally, we applied a variance partitioning procedure to determine the proportion of variance in disturbed vegetation background AM fungal root colonisation explained by both soil pH and the absence/presence of non-native plant species. To achieve this, we fitted LMMs with each explanatory variable (log-transformed) independently and one model including both together but without their interaction, using these to calculate the independent explained variance (R^2 calculated using the method described in Nakagawa & Schielzeth, 2013) for both factors (variance explained by factor A = variance of the full model – variance of the model with only factor B), as well as their shared explained variance. We could not use the above-mentioned beta regression models for this variance partitioning approach, as calculating R^2-values for beta regression mixed models is not supported. Results from the variance partitioning procedure thus have to be interpreted with caution.

2.3 Results

Arbuscular mycorrhizal fungal root colonisation

We found disturbance to be the strongest predictor of AM fungal root colonisation rate in the background samples (Table **2.1a**), with a higher colonisation rate in the disturbed plots. This pattern was reinforced by the higher proportion of background samples in which AM fungi were found by visual examination of stained roots in the disturbed vegetation compared to the adjacent undisturbed vegetation (76 % vs. 50 %). There was also a small decrease in the rate of AM fungal root colonisation with increasing elevation (Table **2.1a,** Fig. **2.2a**), as well as an interaction between elevation and disturbance, showing AM fungal root colonisation rate to diminish less strongly with elevation in the undisturbed vegetation (maintained in one of the three best models only, however). Similarly, there was a slight decrease in AM fungal root colonisation rate with elevation amongst focal plant species samples (Table **2.1**, Fig. **2.2b**). The *Trifolium* species had much higher colonisation rates than the other two focal plant species (Fig. **2.2b**). In line with their obligatory mycorrhizal status, the *Trifolium* species had much higher colonisation rates (100 %) than the other two focal plant species (Fig. **2.2b**), as opposed to only 78 % in *A. millefolium* and we observed a higher variability in the degree of AM fungal root colonisation rate between samples of *A.millefolium* than for the two *Trifolium* species.Contrary to expectation from the literature, we found *S. virgaurea* was a facultative species for AM fungi colonisation as only 66 % of its samples were colonised by AM fungi (Table **S2.1**). *T. pratense* was found in 17% of the disturbed vegetation plots and *T.repens* in 39% but neither of the *Trifolium* species was ever observed in the undisturbed vegetation, while *A. millefolium* occurred in 5 % of all the undisturbed vegetation plots, compared to 37 % of the disturbed vegetation plots, and *S. virgaurea* was found in 70 % of the undisturbed vegetation plots and 97 % of the disturbed vegetation plots.

Table 2.1: Selected models explaining percentage root length colonised by Arbuscular Mycorrhizal Fungi: Coefficients (and their p-values, * *P* ≤ 0.05, ** *P* ≤ 0.01, *** *P* ≤ 0.001) for **a)** background samples, **b)** focal plant species, **c)** abiotic factors (in this case the response factor was the residuals from **a)** instead of AM fungal root colonisation), **d)** the effect of non-native plant species presence. Model selection was performed by selecting all models with a delta AICc < 2 from the best model (i.e. Model 1). Blank spaces represent explanatory variables that were not retained in a given model. The factor level that serves as intercept is alphabetically assigned; other factor levels are compared to this baseline effect.

a) Background samples

Model	Intercept (Undisturbed vegetation)	Elevation	Disturbed vegetation	Elevation* Disturbed vegetation	AICc	Δ AICc
1	-2.376*** (P<0.001)		0.689** (P=0.002)		-164.6	0
2	-2.393*** (P<0.001)	-0.132 (P=0.231)	0.704** (P=0.002)		-164	0.6
3	-2.396*** (P<0.001)	-0.014 (P=0.936)	0.700** (P=0.002)	-0.193 (P=0.392)	-162.7	1.9

b) Focal plant species

Model	Intercept (*Achillea millefolium*)	Elevation	*Solidago virgaurea*	*Trifolium pratense*	*Trifolium repense*	AICc	Δ AICc
1	-1.417*** (P<0.001)		-0.386 (P=0.149)	1.597*** (P<0.001)	1.998*** (P<0.001)	-108.2	0
2	-1.486*** (P<0.001)	-0.131 (P=0.323)	-0.216 (P=0.499)	1.583*** (P<0.001)	2.019*** (P<0.001)	-107.2	1

c) Abiotic factors

Model	Intercept	pH	N	P	AICc	Δ AICc
1	-0.209*** (P<0.001)	0.052** (P=0.003)			-90.2	0
2	-0.238*** (P<0.001)	0.057** (P=0.002)	0.002 (P=0.250)		-89.6	0.6
3	-0.215*** (P<0.001)	0.053** (P=0.005)		0.0001 (P=0.877)	-88.3	1.9

d) Non-native plant species

Model	Intercept (Absence of non-natives)	pH	Presence of non-natives	pH*Presence of non-natives	AICc	Δ AICc
1	-5.450*** (P<0.001)	0.600** (P=0.001)	1.320*** (P<0.001)		-83.9	0
2	-4.503*** (P<0.001)	0.454** (P=0.002)	1.116*** (P<0.001)		-82.5	1.4
3	-5.663*** (P<0.001)	0.645** (P=0.001)	2.397 (P=0.242)	-0.223 (P=0.595)	-82.2	1.7

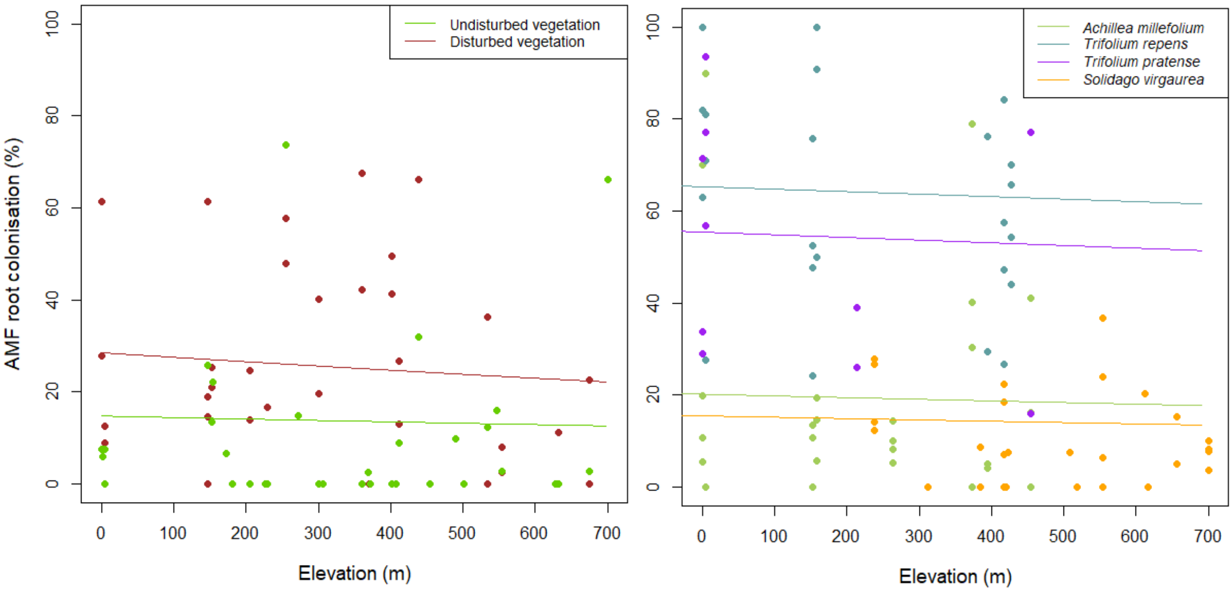

Figure 2.2: Elevation effect on the percentage of arbuscular mycorrhizal (AM) fungal root colonisation along mountain roads in the northern Scandinavian mountains across a 700 m elevation gradient for background samples (**a**) including undisturbed vegetation background (green) and disturbed vegetation background (brown), as well as for four focal plant species (**b**): two obligatorily mycorrhizal non-native plant species *Trifolium repens* (blue) and *Trifolium pratense* (purple), one facultative mycorrhizal non-native plant species *Achillea millefolium* (green) and one facultative mycorrhizal native species *Solidago virgaurea* (orange). See Table 1 for the coefficients of the relationships.

Soil pH was higher along the disturbed roadside compared to the undisturbed vegetation, while we found no difference for soil P and N (Table **S2.4**). Variation in soil pH was also the strongest abiotic predictor of remaining variation in the residuals of the background samples models, while soil P and N had a much weaker correlation with the residuals (Table **2.1c**).

Root AM fungal community composition

Disturbed vegetation background samples showed a higher total richness of AM fungal OTUs than the undisturbed vegetation. A total of 34 OTUs was found in the disturbed vegetation, of which 23 were exclusive to this habitat type, as opposed to only 14 in the undisturbed vegetation backgrounds (3 unique, Fig. **2.3b**). We found that OTU specificity was low across the focal plant species, with only 1 of the 15 most frequent OTUs across all samples (focal species and background samples) not present in each of the focal plant species (Fig. **2.5a**). Six additional OTUs were found in the focal plant species roots that did not previously occur in the background samples, bringing the total to 43. The few OTUs restricted to one focal plant species were all rare, with the most common one occurring in only 20 % of its associated species samples and thus unlikely to be critical for that plant species' establishment.

The results of the whole dataset PERMANOVA showed AM fungal community composition to not change with elevation across all background samples combined (PERMANOVA, R^2 = 0.018, F1,55 = 0.98, P=0.46), or when considering road backgrounds (PERMANOVA, R^2 = 0.036, F1,29 = 1.05, P=0.41) or undisturbed vegetation backgrounds (PERMANOVA, R^2 = 0.044, F1,25 = 1.09, P=0.36) separately. It also showed a significant difference in AM fungal community composition between the disturbed and undisturbed vegetation backgrounds (PERMANOVA, R^2 = 0.346, F1,55 = 1.94, P=0.016).

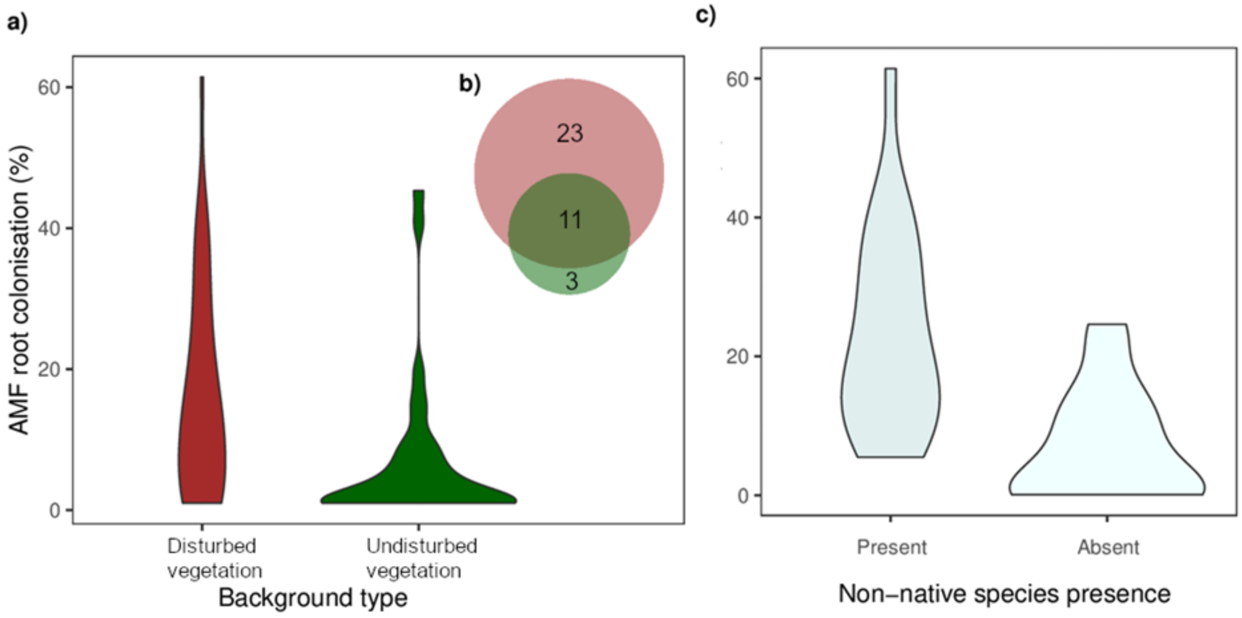

Figure 2.3: Effects of road disturbance on arbuscular mycorrhizal fungi (AMF) distribution along roadsides. a) Violin plots (boxplot-like plots with horizontal width depending on number of samples at that specific percentage) of background AMF colonisation in the disturbed vegetation and in the undisturbed vegetation. **b)** Venn-diagram of AMF operational taxonomic unit (OTU) overlap between disturbed vegetation background (brown) and undisturbed vegetation background (green) communities. **c)** Violin plots of background AMF colonisation in the disturbed vegetation for plots with or without presence of non-native plant species.

The random sampling approach across a thousand replications (Table **S2.5**) also shows little effect of elevation with less than 1 % of replicates resulting in a *P*-value inferior to 0.05. The same approach, when looking at the effect of disturbance, showed around 40 % of all replicates resulting in a *P*-value inferior to 0.05. This does denote a tendency for the AM fungal community composition to differ between the disturbed and undisturbed vegetations as we would expect only 5 % of replicates to have *P*-values inferior to 0.05 if there was no difference between to two environments.

The elevation range of most OTUs extended from the lowest elevations upwards (Fig. **2.4**, dots on the red line); only few, typically infrequent, OTUs were present exclusively at higher elevations (Fig. **2.4**, dots below the red line). Furthermore, the most common OTUs were also those with the largest elevation range and were mostly found all the way up to the highest elevations, with 18 OTUs being found across the whole elevation gradient (Fig. **2.5b**). Additionally, the distribution of many of the infrequent OTUs seemed similar to the distribution of the non-native plant species, with their maximum observed elevation range being mostly similar or slightly lower than that of the non-native plant species (Fig. **2.4**, dots on the red line).

Relationship between non-native plant species presence and AM fungal root colonisation

We found that the rate of AM fungal colonisation in background samples was higher in transects where non-native plant species were present than in transects where no non-native plant species were found meaning that the vegetation was only composed of native plant species (Table **2.1d**). We confirmed that this effect was not due to the former background samples potentially including non-native plant species roots as there was no difference in the rate of AM fungal root colonisation between background samples in proximity of non-native plants (i.e. non-natives occurred in accompanying vegetation survey of sample within plot, which was true for 35 % of all background samples) and the ones that did not (*t*-test, *df*= 18, *t*(18) = 0.42, *p* = 0.68). Our variation partitioning approach showed a higher degree of variance in the rate of AM fungal root colonisation being explained by the presence of non-native plant species ($R^2 = 0.297$) than by soil pH (as a proxy for soil factors, $R^2 = 0.195$), while the two factors' shared variance was $R^2 = 0.093$.

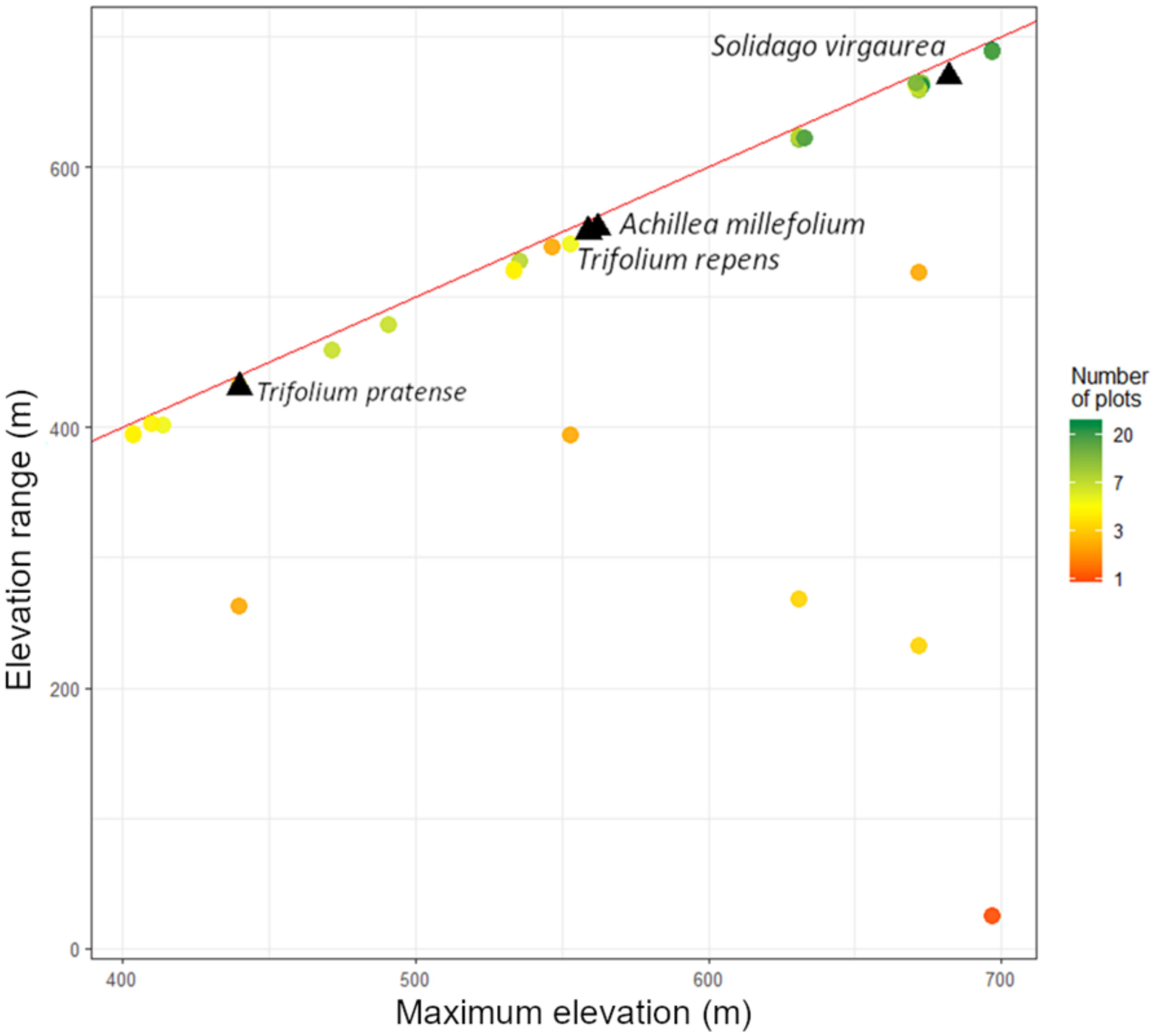

Figure 2.4: Elevation range of arbuscular mycorrhiza OTUs and focal plant species. Relationship between elevation range and maximum elevation for each AM fungi OTU (circles; both background and focal samples combined) and each focal plant species (triangles; *Achillea millefolium* L., *Trifolium repens* L., *Trifolium pratense* L., *Solidago virgaurea* L.). OTUs and plant species close to the red line are found along the whole gradient from the lowest elevation up to their maximum elevation of occurrence. Exceptions are likely caused at least in part by limited sample sizes, as illustrated by the colour gradient. The 18 most common OTUs (green, note some overlap of points) were present across most of the elevation gradient and above the current maximum elevation of non-native plant species.

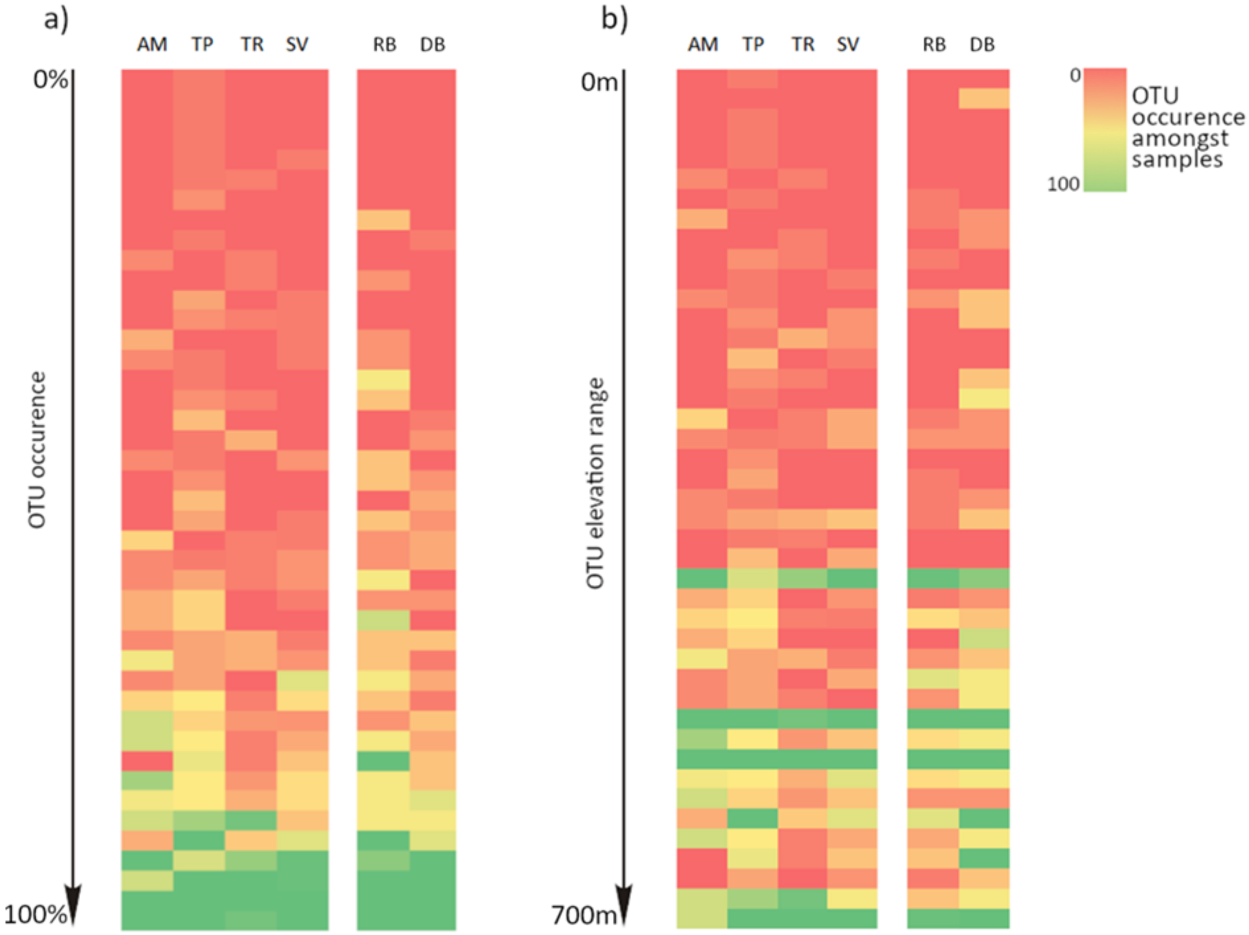

Figure 2.5: Pattern of OTU occurrence by focal plant species and background types. The color scale represents for each focal plant species (columns, named at the top, AM: *Achillea millefolium*, TP: Trifolium pratense, TR: Trifolium repens and SV: Solidago virgaurea) and background type (DB: Disturbed vegetation background and UB: undisturbed vegetation background) the percentage of samples in which each of 43 OTUs was found (ranging from 0 % to 100 %). a) OTUs ordered from low to high total occurrence following the direction of the arrow over the whole dataset. All the focal plant species associated with the most common OTUs (in green) and those OTUs are present in both undisturbed and disturbed vegetation backgrounds. b) OTUs ordered by their elevation range, from OTUs found in only one transect (i.e. a range of 0 m) to a range of 700 m, following the direction of the arrow. The most common OTUs (in green) are found across the largest elevation range.

2.4 Discussion

Mycorrhizal fungi and other soil biota are increasingly recognized as key determinants of plant invasions and subsequent ecosystem transformations (Dickie et al., 2017; Waller et al., 2020). Disturbance along mountain roads is known to facilitate non-native plants, but whether mycorrhizal fungi play a decisive role in this process is hitherto unknown. Our results indicate a strong correlation between mountain road disturbance and AM fungal distribution, with higher AM fungi occurrence, a more diverse AM fungal community (Fig. **2.3b**), and higher root colonisation rate in the disturbed vegetation (Fig. **2.2b**, Fig. **2.3a**) compared to the undisturbed natural vegetation, which is in line with our first hypothesis (H1). This difference most likely results from the striking contrast in biotic and abiotic conditions between the two environments (Müllerová et al., 2011). Arctic forests and heathlands such as found in the Scandes are known for slow nutrient cycling, high organic matter content and low soil pH conditions favorable to ecto- and ericoid-mycorrhizal species, while AM fungi and AM plants tend to be more abundant in environments with faster nutrient cycling and are less tolerant of low soil pH conditions (Soudzilovskaia et al., 2015; Steidinger, Liang, et al., 2019). As expected from the literature (Müllerová et al., 2011), we did find a clear relationship between road disturbance and changes in soil pH which was higher in the disturbed vegetation (Fig. **S2.2**). That difference in soil pH should lead to a more benign environment for AM fungi compared to the undisturbed vegetation (Van Aarle et al., 2002), and help explain our observed pattern of AM fungal distribution. This is reinforced by the results of the models testing our measured abiotic factors against the residuals of our initial models (respectively Table **2.1a** and **2.1c**) which show soil pH to be a strong additional predictor of increased AM fungal root colonisation rate whereas soil N and P play only a marginal role. The effect of disturbance on AM fungi is also illustrated by an increased abundance of native ruderal species known to associate with AM fungi in the disturbed vegetation compared to the undisturbed vegetation, which is dominated by plants typically associated with ecto- and ericoid-mycorrhizal fungi (Lembrechts et al., 2014).

This strong contrast between disturbed and undisturbed conditions, combined with the reliance of the non-native plant species on their AM fungal symbionts, suggest that a lack of, and unsuitable conditions for, AM fungi are likely to be an overlooked barrier to the spread of non-native plant species from roadsides towards the undisturbed vegetation (McDougall et al., 2018). The fact that out of our three non-native focal plant species, only the facultative *A. millefolium* was observed in the undisturbed vegetation as opposed to the two obligatory mycorrhizal *Trifolium* species is another observation supporting this argument. It is however difficult through observational data alone to infer the importance of AM fungal limitation amongst other factors preventing non-native success in the undisturbed vegetation (Lembrechts et al., 2016; McDougall et al., 2018). For example, we observe higher soil temperatures along roadsides in this region (unpublished data in the context of the SoilTemp project (Lembrechts et al., 2020)) which could lead to a faster phenological cycle compared to the undisturbed vegetation and partly explain the distribution patterns of non-native plants. Further factors such as reduced biotic interactions between plant species, or shorter growing seasons in the undisturbed vegetation could also play a role in limiting the success of non-native plant species away from the disturbed roadsides. Disentangling all these possible explanations behind the observed patterns will however require further experimental effort. Nevertheless, the coincidence between the observed current distribution of non-native plants and root colonisation intensity by AM fungi, combined with the strong positive correlation between road disturbance and root colonisation rate by AM fungi, suggests that the impact of disturbance on belowground symbiosis plays an important role in driving the plant invasion patterns that were observed in our system.

Contrary to our expectation (H2), there was little effect of elevation on AM fungal distribution. The rate of AM fungal root colonisation only slightly diminished with elevation, in both disturbed and undisturbed vegetation (Table **2.1a**, Fig. **2.2a**). This shows that AM fungi are already present above the current upper limit of the studied non-native plant species' elevational ranges thanks to their association with native AM plant species, such as for example *S. virgaurea,* which are widely present, but not dominant, in the natural vegetation. Furthermore, there was no effect of elevation on AM fungi community composition amongst both disturbed and undisturbed background samples. Importantly, we also found all our focal plant species to associate with any of the most common AM fungal OTUs which we found to be already present across the whole elevation gradient, including above the current upper elevational range limit of the non-native plant species (Fig. **4, 5b**). These observations indicate that an absence of suitable AM fungi is currently not a limiting factor for the upward spread of non-native plants in the region, as has also been concluded by others, for example by Oehl & Körner (2014) in the Swiss Alps and by Kotilínek *et al.* (2017) in the Himalayas. The spread of non-native plant species to the higher elevation disturbed roadsides is thus more likely to be limited by climatic factors (for example colder temperatures leading to reduced winter survival (Haider et al., 2011)), weaker propagule pressure or even reduced efficiency of AM fungi mycorrhizal symbiosis due to the slow decomposing litter types most found under Arctic climates (Steidinger, Crowther, et al., 2019), rather than by the unavailability of mycorrhizas themselves (Alexander et al., 2016; Lembrechts et al., 2016; Ruotsalainen et al., 2004).

Finally, we observed a pattern of overall higher AM fungal colonisation rates in disturbed vegetation plots when non-native plant species were present (Fig. **2.3c**). This could have multiple cause: high rates of AM fungal colonisation being a driver of non-native plant success, AM fungi being passengers by following changes in non-native plant distribution, or a third factor – for example disturbance – positively affecting both AM fungi colonisation and non-native plant species success in a concomitant manner (Zobel & Opik, 2014). Our observation that non-native presence is a better predictor of AM fungal colonisation rate than soil pH, even though the latter is likely to be a dominant environmental filter in this system, suggests that the presence of non-native plant species is likely to be driving increases in AM fungal colonisation rate. Neither explanations are however mutually exclusive, and both non-native plant species promotion of associated mycorrhizas and the mycorrhizal facilitation of non-native plant species success have been previously observed across different habitats (Reinhart & Callaway, 2006; Richardson et al., 2000; Shah & Reshi, 2009; A. Yang et al., 2018). It is also important to keep in mind that changes in mycorrhizal fungi are not the only possible explanation to the observed patterns but only a likely mechanism given our observations. However, it is most likely that this effect is only part of a general impact of non-native plants on their environment benefiting further non-native success. For example, the known N-fixing capacity of *Trifolium repens* and *Trifolium pratense* could be one such mechanism by shifting the nutrient availability in their surrounding soil which should benefit further success or ruderal plant species (Dornbusch et al., 2018).

We also observed a number of less frequent AM fungi OTUs which happen to have similar ranges as the non-native plants. It is tempting, though speculative, to suggest that the higher AM plant densities brought about by invasion could lead to a richer AMF community by recruiting from co-dispersing AM fungi or from rare locally present AM fungal taxa (Chaudhary et al., 2020), explaining the matching distribution patterns. This would mean that these OTUs matching non-native plant species distribution could be a sign of further changes in mycorrhizal background, potentially also facilitating further non-native plant success (Thakur et al., 2019). Regardless, it is not possible to conclusively determine which of these mechanisms are at play in our system without access to a time series of AM fungal root colonisation rate and AM fungi community composition or experimental data. This should be an important avenue for future research, as a non-native driven positive effect on AM fungi could be self-reinforcing by facilitating the invasion success of other non-native plant species. This could increase our understanding of invasion dynamics and help develop successful intervention methods.

Conclusion

Our results align with a possible facilitating role of mycorrhizal fungi on the establishment success of non-native plants through disturbance along roads, because (1) AM fungal abundance was elevated along the disturbed roadsides, to which non-native plants are largely restricted, along the whole elevation gradient in the northern Scandinavian mountains, and (2) increased AM fungal abundance correlated with high abundance of non-native plants within these roadsides. We conclude however that the movement of non-native plant species to higher elevation is not limited by mycorrhizal fungal presence per se as AM fungi occurred along the whole elevation range, including the AM fungal taxa the non-native plants were found to interact with most. Our results represent a crucial first step in understanding the combined effects of disturbance and mycorrhizal interactions on non-native plant species invasions and offer us new insights into the potential self-reinforcing effect of non-native species through their fungal interactions, which will require further research to be fully disentangled.

CHAPTER III

Roadside disturbance promotes arbuscular mycorrhizal communities in mountain regions worldwide

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Summary

Aim: We aimed to assess the impact at the global level of physical anthropogenic disturbances on the dominant mycorrhizal types in ecosystems and how this mechanism can potentially lead to lasting plant community changes.

Location: Globally distributed study regions

Time Period: 2007-2018

Taxa studied: Plants and mycorrhizal fungi

Methods: We used a database of coordinated plant community surveys following mountain roads from 894 plots in 11 mountain regions across the globe in combination with a database of mycorrhizal-plant associations in order to estimate the relative abundance of mycorrhizal types in natural and disturbed environments.

Results: Our findings show that roadside disturbance promotes the cover of plants associated with arbuscular mycorrhizal (AM) fungi. This effect is especially strong in colder mountain environments and in mountain regions where plant communities are dominated by ectomycorrhizal (EcM) or ericoid-mycorrhizal (ErM) associations. Furthermore, non-native plant species, which we confirmed to be mostly AM plants, are more successful in environments dominated by AM associations.

Main Conclusions: These biogeographical patterns suggest that changes in mycorrhizal types are a crucial factor in the worldwide impact of anthropogenic disturbances on mountain ecosystems by promoting AM-dominated systems and potentially weakening biotic resistance against non-native species invasion. Restoration efforts in mountain ecosystems will have to contend with changes in the fundamental make-up of EcM- and ErM plant communities induced by roadside disturbance.

3.1 Introduction

Mycorrhizal associations are found in the majority of terrestrial plants (Kivlin et al., 2011; Wang & Qiu, 2006) and are increasingly seen as fundamental drivers of plant community composition and ecosystem functioning (Klironomos et al., 2011; Neuenkamp et al., 2018; Cameron Wagg et al., 2014). Different mycorrhizal types have different nutrient provision traits: arbuscular mycorrhizas (AM) are more important in the uptake of inorganic nutrients, thereby improving nutrient and carbon cycling (Phillips et al., 2013), whereas many ectomycorrhizas (EcM) and ericoid mycorrhizas (ErM) can directly free up nutrients locked up in organic matter (David J Read et al., 2004; Sulman et al., 2017). Therefore, different ecosystems are dominated by different mycorrhizal types. It has been shown that the dominance of a certain mycorrhizal strategy is determined both by environmental conditions and by mycorrhiza-mediated feedbacks, implying that even relatively minor disturbances could cause lasting changes in mycorrhizal communities (Averill et al., 2022; Carteron et al., 2022). Existing studies in natural (Clavel et al., 2020; Gerz et al., 2019) and agricultural settings (Schnoor et al., 2011; van der Heyde et al., 2017) show a range of responses of mycorrhizal communities to different types of anthropogenic disturbances. However, these studies remain limited to local scales, and all took place in Europe or North America, and thus generalizing their results remains challenging (Öpik et al., 2011; Soudzilovskaia et al., 2017). Here, we explore the impact of anthropogenic disturbances on the dominant mycorrhizal types of mountain plant communities, using a global database from the Mountain Invasion Research Network (MIREN, www.mountaininvasions.org) (Haider et al., 2022).

The MIREN database consists of 2822 surveys of both native and non-native plant diversity and cover for 100 m² plots, both close to and away from 62 mountain roads covering 11 mountain ranges (Haider et al., 2022). Mountains are highly valuable ecosystems, being home to a disproportionately large share of global biodiversity (Rahbek, Borregaard, Colwell, et al., 2019) and providing crucial ecosystem services (A. Grêt-regamey et al., 2021), that are under increasing pressures from anthropogenic disturbance (Dainese et al., 2017; Kohler et al., 2010). Mountain roads are ideal systems to study the impact of human-induced disturbances as they have a strong but locally limited effect on their surroundings (Müllerová et al., 2011; O'Farrell & Milton, 2006; Pollnac et al., 2012) while covering large elevational, and consequently, large climatic gradients. The plant cover data from MIREN was combined with the FungalRoots database (Soudzilovskaia et al., 2020) to estimate the proportions of plant cover associated with each mycorrhizal type in disturbed roadsides and in the adjacent undisturbed vegetation.

Our expectation was that the disturbance caused by roads would cause a shift in types of mycorrhizal associations, as such an effect was found previously in Norway (Clavel et al., 2020) and road disturbance is known to alter plant community composition (Lembrechts et al., 2016; McDougall et al., 2018). As roads tend to favour ruderal species, commonly AM or non-mycorrhizal (NM) plants, to the detriment of more perennial and woody plants, more likely to be associated with EcM and ErM fungi, we expected road disturbance to correlate with increased AM and NM representation and decreased EcM and ErM. However, we also expected the diversity present within the MIREN database to be reflected in a variety of responses to anthropogenic disturbances along environmental gradients (Barcelo et al., 2019; Gerz et al., 2019; van der Heyde et al., 2017). As non-native plant species are typically AM or NM (Pringle et al., 2009) and non-native species are favored by disturbance, we hypothesized that high non-native plant species cover would correspond with regions and plots with AM- or NM-dominated native communities and, in turn, be higher along roadsides (Lekberg et al., 2013).

More specifically, we tested the following hypotheses:

1) Road disturbance is associated with changes in the distribution of mycorrhizal types in mountain plant communities at the global scale.

2) The strength and direction of the disturbance effect on mycorrhizal type distribution depends on local environmental conditions and on the dominant mycorrhizal type of the region's native community.

3) Non-native plant success is highest in AM- and NM-dominated communities and in plots affected by road disturbance.

3.2 Materials and Methods

MIREN dataset

Vegetation composition along mountain roads has been recorded by the MIREN network since 2007 across 18 mountain regions on 5 continents (Haider et al., 2022). For the current study we focused on eleven mountain regions, as the remaining seven did not offer plant cover data both along the roadside and in the neighboring natural vegetation at the time of analyses. These regions cover environmental gradients with elevations ranging from 0 to 4000 m above sea level (m a.s.l) and mean annual soil temperatures ranging from -5.8 to 27.9 °C. The studied mountain regions were: the Caucasus Mountains in Armenia; the Argentine Andes in Argentina; the Australian Alps in Australia; the Chilean Andes in Chile; the European Alps in Switzerland; the Changbai Mountains in China; Mount Teide in the Canary Islands; the Himalayas in India; the Rocky Mountains in Montana; the Blue Mountains in Oregon; and the Northern Scandes in Norway. In each of these 11 regions, three roads which covered large elevational gradients and were actively used by motorized traffic were selected. The surveyed road edges were typically highly disturbed, with shallow soils and often altered surface material due to the road construction, and reduced vegetation cover (Müllerová et al., 2011), see Figure S2.1 for examples. All surveys were first executed between 2007 and 2018, depending on the region. Surveys are repeated every 5 years, resulting in currently one (two regions), two (five regions) or three (four regions) repeated surveys depending on the region.

Sampling design

Twenty transects were set up along each road at approximately equal elevational steps following the MIREN protocol (Haider et al., 2022). Each of these transect was then divided in three 2 m x 50 m plots organized in a T-shape. The first plot was laid out parallel to the road, encompassing the vegetation directly impacted by the road-associated disturbance, and both the second and third plots were set up perpendicular to the road, respectively from 2 to 52 m and from 52 m to 102 m, covering the less disturbed vegetation communities adjacent to the road.

Henceforth, we will refer to these plots as “roadside plots” and “adjacent plots”. While anthropogenic disturbance was almost always higher in the roadside plot, some of the adjacent plots were also subject to anthropogenic disturbances, especially so at lower elevations. The presence and total cover of all vascular plant species present in each plot were then recorded using the following semi-continuous scale: 1 = <1%; 2 = 1 to 5%; 3 = 5 to 25%; 4 = 25 to 50%; 5 = 50 to 75%; 6 = 75 to 95%; and 7 = 95–100%. Plant species were also categorized as either native or non-native following local and regional floras (Haider et al., 2022). Additionally, average yearly soil temperature was extracted from the SoilTemp global maps of soil temperature at a 1 km resolution (Lembrechts et al., 2022). In total, our dataset included 894 plots spread out across 62 separate mountain roads and 11 regions.

Mycorrhizal type

Using the FungalRoot database (Soudzilovskaia et al., 2020), as many plant species as possible were grouped according to their mycorrhizal types: arbuscular mycorrhizal (AM); ericoid mycorrhizal (ErM); ecto-mycorrhizal (EcM); orchid mycorrhizal (OM); or non-mycorrhizal (NM). Scores of 0, 0.5 or 1 were attributed to each plant species for each mycorrhizal type, depending on whether they were recorded as either associating (1) or not (0) with a certain mycorrhizal type, or associating with two different types (0.5) (e.g., AM and NM; there were no species associated with more than two types). We included plant species which were referred to in the FungalRoot database as “AM, others not addressed” and “EcM, other not addressed” meaning that they were confirmed to associate with the respective mycorrhizal type, but that uncertainty remained regarding their potential to form associations with more than one mycorrhizal type. The potential resulting error is minimal as amongst all the species for which mycorrhizal types were assessed, only 0.3% were found to associate with more than one type of mycorrhizal fungi, therefore these species were attributed a score of 1 for the corresponding mycorrhizal type and of 0 for all other types. We could extract mycorrhizal types at the plant species level for 65.0 % of all observations, ranging from a minimum of 30.2% in Argentina to a maximum of 93.2% in Norway. For the remaining species the dominant mycorrhizal type at the genus level was used instead, resulting in 98.4 % of all observations being assigned a mycorrhizal type, ranging from a minimum of 94.2% in Argentina to a maximum of 99.8% in India. This categorization by genus was done by Soudzilovskaia et al., 2020 by assigning a mycorrhizal type or NM status to a genus if more than 2/3 of the available observations in that genus converged. It is however important to remember that the genus level data comes associated with a higher degree of uncertainty, therefore making results in regions with less known species level mycorrhizal types slightly more unreliable.

We multiplied the cover percentage for each species in a plot by its score for each mycorrhizal type (0, 0.5 or 1). The resulting percentages of total vegetation cover were added up for each mycorrhizal type and then divided by the total vegetation cover of the plot obtained by adding up the cover values of all species present. This resulted in a percentage of total plot vegetation cover associated with each mycorrhizal type (for example: 65% of all vegetation in a plot is associated with AM fungi) which was then converted to proportions between 0 and 1 for further analysis; this value is henceforth referred to as 'mycorrhizal type cover'. It is worth noting that this value is a percentage of a plot total vegetation cover and not of the plot total surface. OM mycorrhizal type cover was excluded from further analysis as it represented less than 0.1% of total cover so it is unlikely to be relevant when looking at changes in mycorrhizal type distribution.

Statistical analysis

We fitted generalized linear mixed-effects models (GLMMs) for each mycorrhizal type to test for the effects of disturbance, temperature and elevation on the respective mycorrhizal type covers (2822 data points from 894 individual plots). We used beta regressions with the glmmTMB package (Brooks et al., 2017) in R (R Core Team, 2021) after transformation of the response variable (i.e. proportion data) to avoid extreme values of 0 and 1: (response variable value * (number of observations – 1) + 0.5) / number of observations (Cribari-Neto & Zeileis, 2010). The explanatory variables used were: (i) disturbance as a three-level factor for each plot (0 to 2 m from the road, 2 to 52 m from the road and 52 to a 102 m from the road); (ii) mean annual soil temperature; (iii) elevation; as well as (iv) the two-way interactions between these two factors. The elevation values used were relative to each region's elevation gradient obtained by scaling elevation individually for each region using the scale function in base R (Becker, 2021) resulting in gradients between -1 and 1 for all regions, with the lowest elevation of each gradient being the valley where the roads start, i.e. the point at which the elevation gradient starts and not sea level. We chose this because we were interested in the elevational distance relative to the bottom of the gradient and not in the absolute elevation of a region. The initial model contained both elevation and temperature, as elevation can serve as a measure of non-climate driven and more local gradients while temperature takes into account the differences between regions as well as large-scale climate-driven trends within a region. However, after testing for multicollinearity using the VIF (Variable Inflation Factor) through the vif function in R (Fox & Weisberg, 2018), elevation and temperature were found to be too strongly correlated (VIF value of 5.812 for elevation). We consequently omitted the effect of elevation from the final model. However, to make sure that temperature and elevation patterns did indeed behave similarly we also ran the model selection strategy in parallel with elevation instead of temperature. The random intercept term of transect nested in road nested in region was added to consider the hierarchical nature of our design, as well as a random intercept term for year of observation to consider repeated surveys, and a random slope term for plots. Candidate models with all possible combinations of fixed effects were then derived from the complete model and compared using AICc (Akaike Information Criterion, corrected for small sample sizes). Only models with a ΔAICc of less than 2 units compared to the best candidate model were retained (Zuur et al., 2009). We then applied a variation partitioning approach to the selected models using the performance package in R to determine the proportion of variation in mycorrhizal type proportions explained by disturbance, mean annual soil temperature and elevation (Lüdecke et al., 2021).

To investigate the variation between regions, we used a partial pooling approach (Harrison et al., 2018). This was done using models derived from the aforementioned beta regressions for each mycorrhizal types with the same explanatory variables that were retained after model selection, i.e. temperature and disturbance, but with additional random slope terms for both of these terms. The addition of these random effect allows for the intercept and slope associated with each region and each variable to deviate while still capturing the overall trends from the larger dataset. We then extracted the coefficients associated with each variable for every region, allowing for comparisons of trends between these regions and the global models.

A similar modeling approach was then used to investigate how the proportion of plants associated with the different mycorrhizal types correlated with the proportion of non-native plant cover. We ran separate tests for the percentage of total non-native plant cover in the roadside plots (0-2 m from the road) and in the neighboring vegetation in the furthest adjacent plots (52-102 m from the road). The 2-52 m plots were left out because we know from previous studies (Clavel et al., 2020; McDougall et al., 2018) that non-native plant species can spread away from the road in the adjacent vegetation and using the 2-52 m adjacent plot could be misleading as it in some cases still included roadside vegetation when the roadside was more than 2 m wide. As the presence of non-native species is linked to changes in the local balance of mycorrhizal association types, we used the percentage cover of vegetation associated to each given mycorrhizal type amongst native species only as a predictor instead of the proportion of total vegetation cover. The models included native cover percentage of a certain mycorrhizal type and mean annual soil temperature as well as their interaction as explanatory variables and either roadside plot or adjacent plot non-native plant cover percentage as a response variable in order to distinguish between non-native species simply benefitting from the road disturbance and more established non-native species present in the surrounding vegetation. As previously, a random intercept of road nested into region was included to account for the survey's hierarchical design. Model selection was then performed by comparing AICc values as described above.

3.3 Results

Global patterns in mycorrhizal types

Over the whole dataset, we observed 456 plant species (from 125 in Armenia to 856 in Switzerland), 97.1% of which could be assigned a mycorrhizal. AM plants accounted for 71.3% of all plant species with an assigned mycorrhizal association, EcM plants accounted for 7.0%, ErM for 4.2%, NM for 15.8% and Orchid Mycorrhizas (OM) for 0.1%. These numbers are similar to the proportions per mycorrhizal type found by Soudzilovskaia et al., 2020 across the entire FungalRoot database (AM: 70%, EcM: 8%, ErM: 2%, OM: 1%, NM: 17%). The distribution of mycorrhizal types amongst native species in our data was likewise broadly similar to that of the FungalRoot database (AM: 79.3%, EcM: 6%, ErM: 3%, OM: 0.6%, NM: 10.3%). We observed 452 non-native species (from one in Armenia to 150 in Australia). As expected, the majority of non-native species were found to associate with AM or to be non-mycorrhizal (AM: 85.6%, EcM: 0.3%, ErM: 0%, OM: 0%, NM: 14.1%). Mycorrhizal type distribution also varied with region (Fig. **3.1**): AM plant species made up more than half of all vegetation in almost all regions except for Norway, where ErM vegetation was dominant. In Argentina, Armenia, Chile and India there was no or almost no EcM and ErM vegetation, with all plants being either AM or NM.

Globally, our models showed that both EcM and ErM vegetation cover decreased with increasing mean annual soil temperatures, while AM and NM vegetation cover increased (Table **3.1**). This effect of temperature on EcM and ErM vegetation remained mostly consistent across regions while there was a high variability of responses for AM and NM vegetation (Table **3.2**).

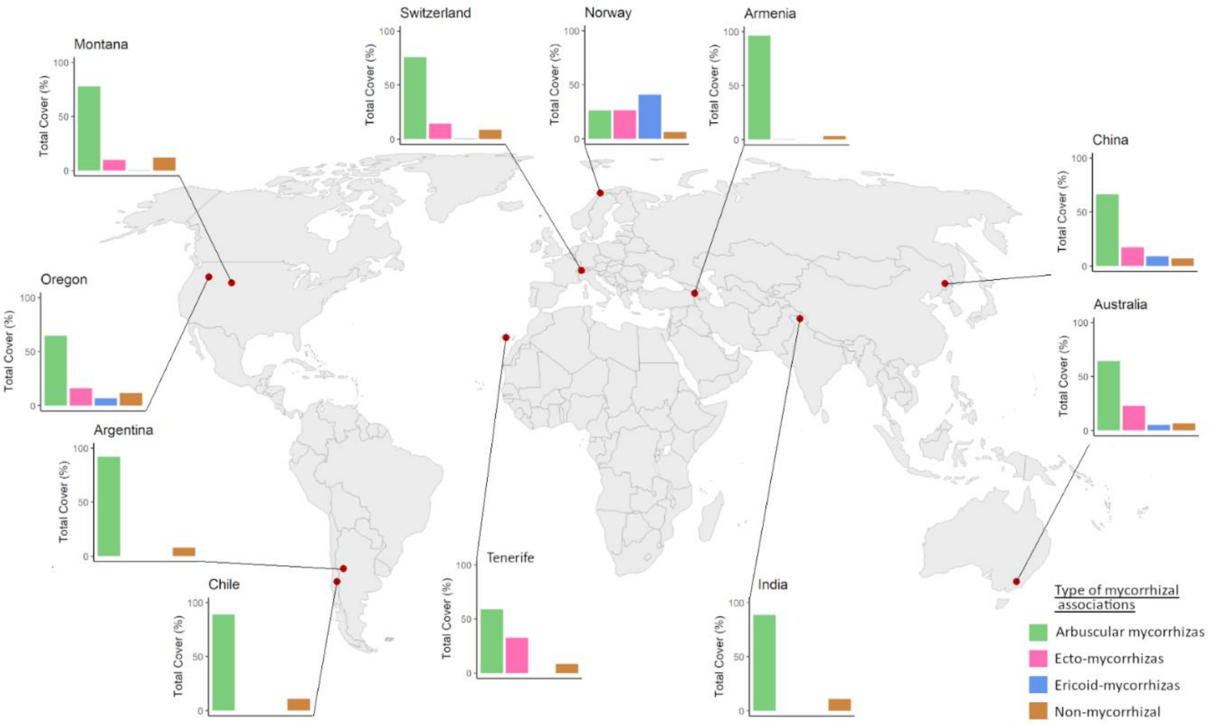

Figure 3.1: Map of mycorrhizal type associations in the 11 studies mountain regions. Percentage of total vegetation cover for plants associated with arbuscular mycorrhizas, ecto-mycorrhizas and ericoid-mycorrhizas, as well as non-mycorrhizal plants. For each region the total cover of plant species was recorded along multiple mountain roads as well as in the adjacent vegetation, plotted here are only the results from this adjacent vegetation independent of the road effect.

Impact of mountain roads on mycorrhizal types

At the global scale, the percentage of vegetation cover associated with AM fungi was higher on average in plots following the roadsides (roadside plots) than in the adjacent plots away from the roadside (adjacent plots) (Table **3.1**, Fig. **3.2**). Conversely, the proportion of EcM and ErM vegetation cover was lower on average in the roadside plots. In general, the proportion of EcM and ErM vegetation cover behaved in an opposite manner to that of AM plant cover where increased levels of AM vegetation cover corresponded to decreased levels of EcM and ErM vegetation cover and vice-versa. NM vegetation cover did not significantly differ between the two environments (Table **3.1**). All the patterns we describe regarding soil temperature remained the same when using elevation instead as the environmental explanatory variable (Table **S3.1**).

Table 3.2: Partial pooling results showing variation of mycorrhizal type cover responses across regions: Region-specific coefficients for each type of mycorrhizal association: Arbuscular Mycorrhizas (AM), Ecto-Mycorrhizas (EcM), Ericoid Mycorrhizas (ErM) and Non-Mycorrhizal (NM) across all regions where a given mycorrhizal type was present. Explanatory variables were mean annual soil temperature (Temp), disturbance (Dist) as the three-level plot with 1: 52 to 102 m from the road, 2: 2 to 52 m from the road, and 3: 0 to 2 m from the road and the interaction between disturbance and temperature. Green and orange fields are respectively positive and negative correlations. Gray fields are factors that were not included in a given model following the results of model selection (see Table S1). Region abbreviations are as follows: ARC for Argentina, ARM for Armenia, AUN for Australia, CHE for Switzerland, CLC for Chile, CNN for China, TEN for Tenerife, IND for India, MTN for Montana, NOR for Norway and ORE for Oregon. Regions are ordered from lowest total proportion of AM vegetation cover (Norway) to highest (Armenia) and points are colored from low temperatures (blue) to high temperatures (red) scaled within region.

AM

Region	Intercept	Temp	Dist	Temp* Dist
ARC	1.78	-0.01	0.00	0.03
ARM	2.28	0.00	0.25	0.01
AUN	2.11	-0.06	0.58	0.01
CHE	1.19	0.04	0.28	0.01
CLC	2.46	-0.19	0.10	0.12
CNN	1.05	0.06	0.52	0.01
TEN	0.09	0.04	1.16	0.04
IND	1.86	-0.03	0.13	0.03
MTN	2.40	-0.03	0.62	0.04
NOR	-0.15	0.06	0.47	-0.06
ORE	-0.54	0.23	0.39	0.04
EcM				
AUN	-3.54	0.16	-0.45	
CHE	-2.25	-0.05	-0.17	
CNN	-1.74	0.06	-0.10	
TEN	0.32	-0.13	-0.62	
MTN	-2.84	-0.03	-0.35	
NOR	-0.94	0.00	-0.05	
ORE	-0.57	-0.17	-0.14	

ErM

Region	Intercept	Temp	Dist	Temp* Dist
AUN	-3.01	-0.03	-0.36	-0.02
CHE	-3.01	-0.03	-0.32	-0.03
CNN	-3.08	-0.05	-0.18	-0.05
NOR	-1.33	-0.10	-0.50	0.06
ORE	-1.89	-0.11	-0.20	-0.01
NM				
ARC	-2.00	0.00	0.16	-0.02
ARM	-2.02	-0.12	0.26	-0.01
AUN	-1.87	-0.12	-0.26	-0.02
CHE	-1.73	-0.06	0.02	-0.01
CLC	-3.89	0.52	-0.78	-0.15
CNN	-2.26	-0.08	0.25	0.00
TEN	-1.24	-0.19	0.39	0.01
IND	-2.07	0.05	-0.14	-0.03
MTN	-2.85	0.02	-0.45	-0.04
NOR	-2.02	-0.06	0.19	-0.02
ORE	-0.90	-0.21	0.07	0.00

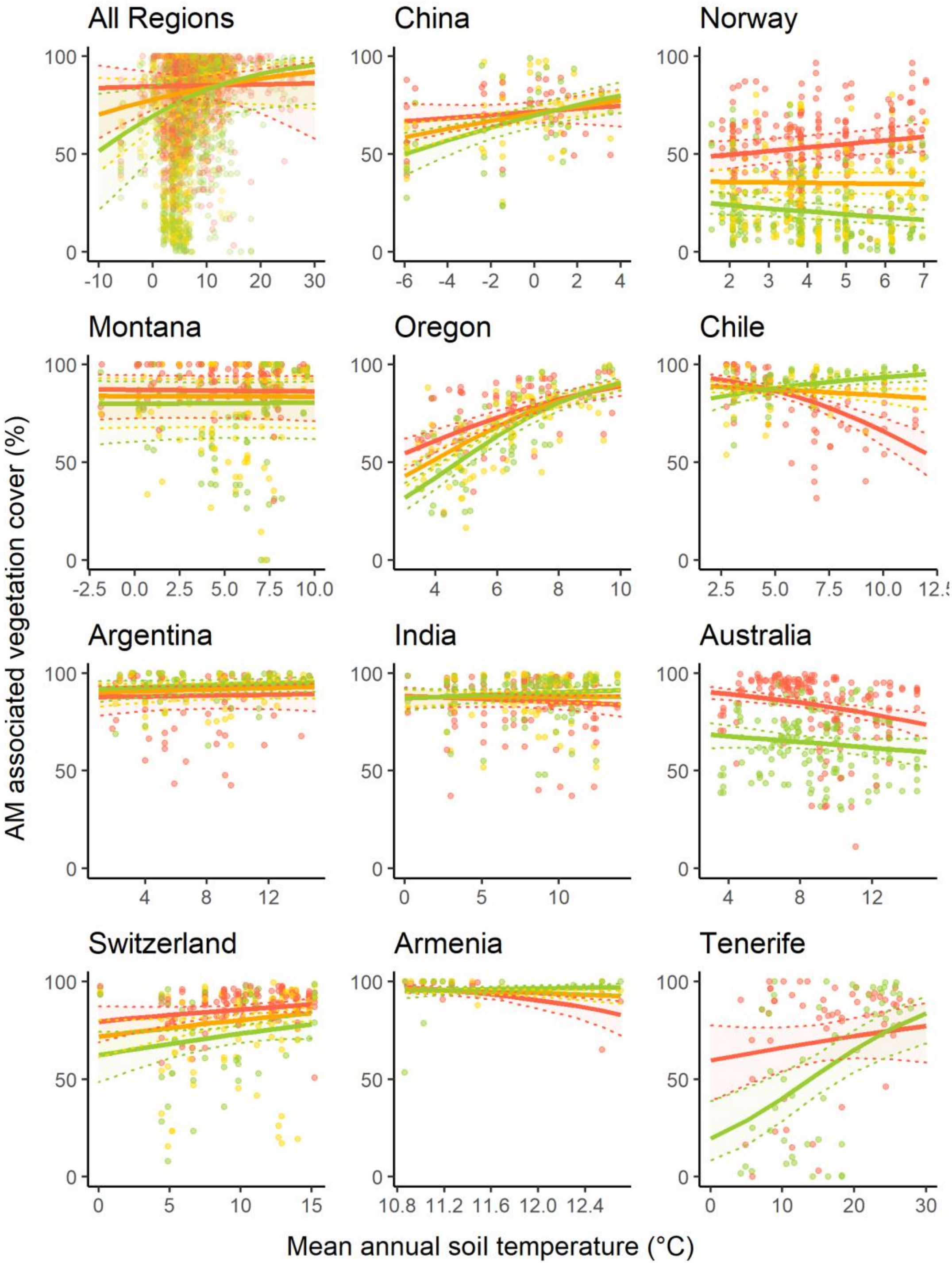

Figure 3.2: Effect of road disturbance on the proportion of total vegetation cover associated with Arbuscular Mycorrhizal (AM) fungi across regions and along temperature gradients. AM vegetation cover was measured along roadsides from 0 to 2 m (red) and in the adjacent vegetation both from 2 to 52 m away from the road (orange) and from 52 to 102 m (green). Mean annual soil temperature was extracted from global maps of soil temperature at a 1 km resolution. The regions are plotted in order from lowest to highest average yearly soil temperature and confidence intervals are represented by the dotted lines. Trendlines come from a partial pooling approach on a linear mixed model (see Methods).

Table 3.1: Selected models explaining percentage of vegetation cover associated with a certain mycorrhizal type: Coefficients and their p-values (between brackets) for each type of mycorrhizal association: Arbuscular Mycorrhizas (AM), Ecto-Mycorrhizas (EcM), Ericoid Mycorrhizas (ErM) and Non-Mycorrhizal (NM). Model selection was performed by selecting all models with a ΔAICc < 2 from the best model (i.e. Model 1). Explanatory variables were soil temperature (Temp), disturbance (Dist) as the three-level plot with 1: 52 to 102 m from the road, 2: 2 to 52 m from the road, and 3: 0 to 2 m from the road, and the interaction between temperature and disturbance. Green and orange fields are respectively positive and negative correlations. Gray fields represent explanatory variables that were not retained in a given model.

	Model	Intercept	Temp	Dist	Dist* Temp
AM	1	1.6177 (p<0.001)	0.0132 (p=0.178)	0.3909 (p=0.004)	-0.0293 (p<0.001)
EcM	1	-3.0698 (p<0.001)	-0.0377 (p<0.001)	-0.2520 (p=0.010)	
ErM	1	-4.1528 (p<0.001)	-0.0165 (p=0.060)	-0.2671 (p=0.003)	0.0111 (p=0.098)
	2	-4.1141 (p<0.001)	-0.0378 (p<0.001)		
NM	1	-2.3977 (p<0.001)	0.0079 (p=0.412)	-0.0022 (p=0.978)	0.0156 (p=0.025)
	2	-2.3341 (p<0.001)		0.1103 (p=0.130)	
	3	-2.2948 (p<0.001)	-0.0051 (p=0.498)	0.1104 (p=0.126)	

Influence of the climate gradient

The aforementioned effect of disturbance on mycorrhizal type distribution was strongest in plots with low mean annual soil temperature as shown in Figure **3.2** and as indicated by a positive interaction between disturbance and temperature (Table **3.1**). While the percentage of AM vegetation cover was on average higher in roadsides, this pattern tended to be reversed at the upper ranges of the regional temperature gradients where the percentage of AM vegetation cover in high temperature plots was lower in the roadside when compared to the adjacent vegetation with NM vegetation instead being higher in these roadside plots. This effect was less clear at the inter-regional level: while the effect on disturbance was mostly higher in cold regions compared to warmer regions, both Australia and especially Tenerife where outliers with comparatively high average temperatures as well as a strong effect of road disturbance on the proportion of mycorrhizal association types observed in the vegetation (Fig. **3.2**, Table **3.3**).

Table 3.3: Selected models explaining percentage of non-native plant species cover amongst roadside (a) and adjacent (b) vegetation for the different mycorrhizal types: Coefficients and their p-values (between brackets) for each type of mycorrhizal associations: Arbuscular Mycorrhizas (AM), Ecto-Mycorrhizas (EcM), Ericoid Mycorrhizas (ErM) and Non-Mycorrhizal (NM). Model selection was performed by selecting all models with a ΔAICc < 2 from the best model (i.e. Model 1). Explanatory variables were mean annual soil temperature ('Temp'), the percentage cover of native vegetation associated to each given mycorrhizal type ('% cover') and the interaction between these two factors. Green and orange fields are respectively positive and negative correlations. Gray fields represent explanatory variables that were not retained in a given model.

a) <u>Roadside non-native plant species</u>

	Model	Intercept	% Cover	Temp	% Cover*Temp
AM	1	-3.29 (p<0.001)	0.60 (p=0.003)	0.06 (p<0.001)	
	2	-3.09 (p<0.001)	0.34 (p=0.279)	0.03 (p=0.212)	0.03 (p=0.305)
EcM	1	-2.82 (p<0.001)	-0.39 (p=0.364)	0.07 (p<0.001)	-0.02 (p=0.609)
	2	-2.81 (p<0.001)	-0.57 (p=0.010)	0.07 (p<0.001)	
ErM	1	-2.87 (p<0.001)	-0.54 (p=0.050)	0.07 (p<0.001)	
	2	-2.90 (p<0.001)	-0.04 (p=0.920)	0.07 (p<0.001)	-0.12 (p=0.139)
NM	1	-2.99 (p<0.001)	0.65 (p=0.098)	0.06 (p<0.001)	
	2	-3.02 (p<0.001)	1.09 (p=0.160)	0.08 (p<0.001)	-0.06 (p=0.517)

b) <u>Adjacent vegetation non-native plant species</u>

	Model	Intercept	% Cover	Temp	% Cover*Temp
AM	1	-3.10 (p<0.001)	0.34 (p=0.279)	0.03 (p=0.212)	0.03 (p=0.305)
	2	-3.29 (p<0.001)	0.60 (p=0.003)	0.06 (p<0.001)	
EcM	1	-2.81 (p<0.001)	-0.58 (p=0.014)	0.06 (p<0.001)	
	2	-2.83 (p<0.001)	-0.39 (p=0.364)	0.07 (p<0.001)	-0.02 (p=0.609)
ErM	1	-2.91 (p<0.001)	-0.04 (p=0.920)	0.07 (p<0.001)	-0.12 (p=0.139)
	2	-2.87 (p<0.001)	-0.54 (p=0.050)	0.07 (p<0.001)	
NM	1	-2.98 (p<0.001)	0.65 (p=0.098)	0.07 (p<0.001)	
	2	-3.03 (p<0.001)	1.08 (p=0.160)	0.08 (p<0.001)	-0.06 (p=0.517)

Overall, disturbance had larger and more consistent effects on the proportion of mycorrhizal association types than elevation or temperature. Using variation partitioning, we found that disturbance explained 9.8% of the total variation in AM vegetation cover and mean annual soil temperature 4.0%. For EcM, these numbers were 2.8% for disturbance and 2.2% for temperature, for ErM these were 21.6% against 7.9% respectively, and 1.0% against 0.6% for NM. Again, we found similar results when replacing mean annual soil temperature with elevation as the environmental explanatory variable: 8.9% for disturbance and 1.2% for elevation in AM vegetation and respectively 2.7% and 1.9% for EcM, 15.3% and 1.3% for ErM, 3.0% and 1.9% for NM. The direction of the temperature effect on the proportion of mycorrhizal association types also greatly varied across regions for all mycorrhizal types except ErM, while the disturbance effect was consistent in its direction across all regions for all four mycorrhizal types (Table **3.2**).

Influence of regionally dominant mycorrhizal type

In spite of the wide variation in ecosystems, all 8 regions where EcM and ErM associations occurred (all except India, Argentina and Chile) showed the pattern of increased AM vegetation cover and decreased EcM and ErM vegetation cover in roadside plots (Table **3.2**). Importantly, however, the difference in proportion of mycorrhizal-type associations between the vegetation of the roadside plots and that of the adjacent plots was larger in plots (and regions) where AM plant species had a lower representation in the native natural vegetation (Fig. **3.3**). In general, the lower the percentage cover of AM-dominated species in the natural vegetation, the higher the increase in AM-dominated species in the roadside plot (Fig. **3.1**, as indicated by a flatter line above the 1:1 in Fig. **3.3**). This effect mirrored the pattern we described for soil temperature, in plots for which the natural vegetation was in the upper range of AM vegetation cover we did observe a reversal of the general pattern: AM vegetation cover was lower in the roadside when compared to the adjacent vegetation with the vegetation being more NM instead (Fig. **3.3**, regression line crossing the 1:1-line).

Non-native species

The proportion of mycorrhizal association types amongst the native vegetation was significantly correlated with the proportion of non-native species in the total vegetation cover, both in the roadside plots (Fig. **3.4**, Table **3.3**) and in the adjacent plots (Fig. **S3.2**, Table **3.3**). Coincident with the AM or NM status of almost all non-native species, we observed that higher proportions of AM and NM native plant cover correlated with higher proportion of non-native cover both in the roadside plots (Fig.

3.4) and in the adjacent plots (Fig. **S3.2**). The opposite pattern was true for EcM and ErM vegetation cover. Elevation was as expected also strongly correlated with non-native plant cover, with higher elevation plots being on average less colonised by non-native plant species.

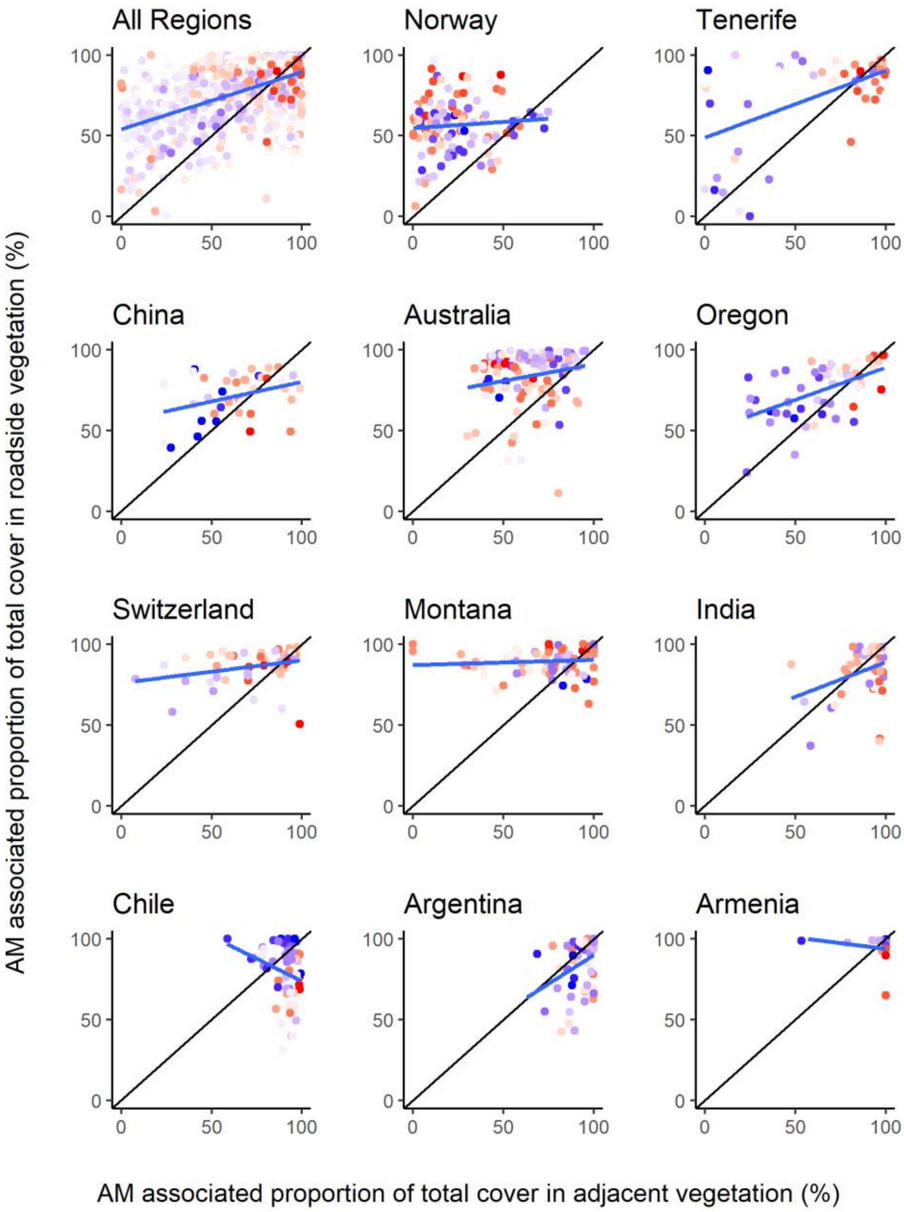

Figure 3: Pairwise comparison of arbuscular mycorrhizal (AM) proportions of total vegetation cover between disturbed (roadside plot) and undisturbed (adjacent plot) vegetation. For each region, every individual transect along the studied mountain roads was plotted as the intersection between the percentage of AM vegetation cover in 2 × 50 m plots parallel to the road (0 m to 2 m from the road) and in 2 × 50 m plots of adjacent vegetation perpendicular to the roadside (52 to 102 m from the road). Points above the identity line correspond to transects in which the AM proportion of the total vegetation cover was higher along the roadside compared to the proportion in the adjacent vegetation. The blue regression line was plotted using the results of linear models, highlighting that the relationships between AM vegetation cover proportion in the disturbed and undisturbed vegetation are different from what would be expected if disturbance had no effect (i.e., relative to the 1/1-line). Regions are ordered from lowest total proportion of AM vegetation cover (Norway) to highest (Armenia) and points are colored from low temperatures (blue) to high temperatures (red) scaled within region.

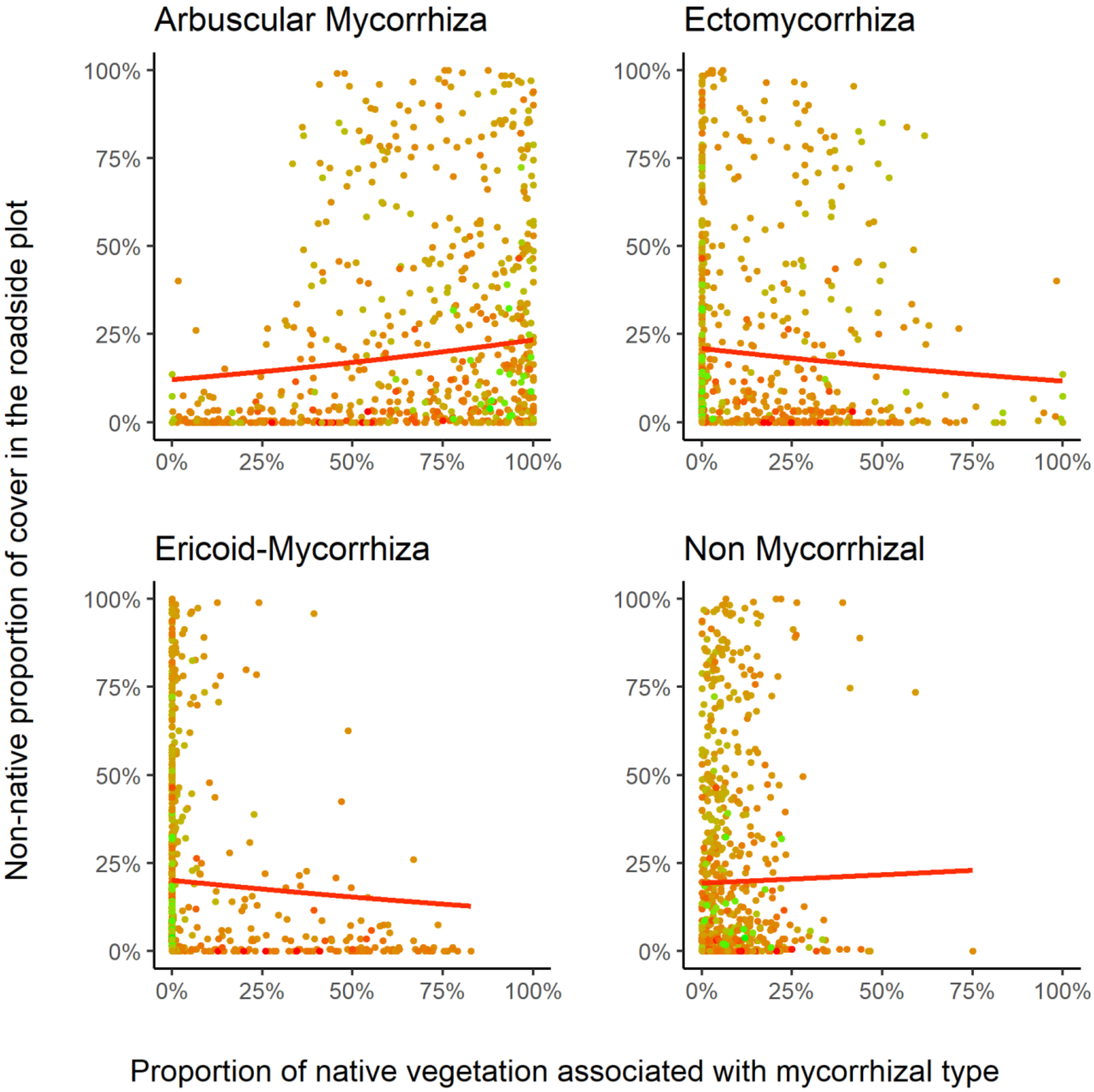

Figure 4: Correlation between native mycorrhizal associations and cover of non-native species. For each mycorrhizal-type association, the proportion of the vegetation cover in roadside plots (0 m to 2 m from the road) which was of non-native origin was plotted against the proportion of the native vegetation in plots perpendicular and away from to the road (52 m to 102 m from the road) which was associated with each mycorrhizal type. Each dot represents an individual pair of plots. Regression lines were plotted using the results of generalized linear models (see methods) and individual dots were colored according to elevation from low elevation (green) to high elevation (red).

3.4 Discussion

On average, roadsides harbored an increased share of AM plant species in the total vegetation cover and a decreased share of EcM and ErM vegetation (Fig. **3.2**). This mostly followed our initial expectations as road conditions and management are known to be detrimental to the more perennial and often tall and/or woody vegetations types that commonly associate with EcM and ErM fungi, while more ruderal and opportunist plant species are most often AM and tend to be more successful in these environments (Müllerová et al., 2011; S. E. Smith & Read, 2010). These differences were especially true in colder environments, i.e. at high elevations and high latitudes, but were less pronounced or even reversed at the upper end of the temperature gradient where roadside vegetation showed lower AM vegetation cover than in the adjacent vegetation.

However, despite these intra-regional fluctuations, we did observe that for almost all regions the effect of disturbance on the regional scale followed the global trend of increased AM vegetation along roadsides coupled with decreased ErM and EcM vegetation (when present; Table **3.2**). Only Argentina was an exception as we found no correlation between road disturbance and changes in mycorrhizal type, which was not the case in other regions where the vegetation was entirely or almost entirely AM-associated or NM. These findings suggests that the impact of mountain road disturbance on mycorrhizal type distribution follows a systematic pattern that can be influenced, but is mostly not overruled by, local factors. As we observed in a recent study in the northern Scandes (Clavel et al., 2020), this change in AM vegetation cover is likely also reflected in the prevalence of the AM fungi themselves, as disturbance in these cold-climate mountains strongly promoted both the colonisation of roots by arbuscular mycorrhizas as well as overall AM fungal diversity.

While largely constant in its direction, the strength of the roadside disturbance effect on mycorrhizal types varied between regions (Table **3.2**, Fig. **3.2**, Fig. **3.3**) as it was lowest, but still present, in regions already dominated by AM and NM plants, and in warmer regions. This was most obvious in the three regions where EcM and ErM vegetation was entirely absent (Argentina, Chile and India) as could be expected since there is less margin for increases in AM vegetation cover when they already dominate the regional vegetation. Conversely, the regions with large proportions of EcM- and ErM vegetation cover, such as Norway or Tenerife, exhibited the largest differences in mycorrhizal associations between the vegetation of the roadside and of the adjacent plots (Fig. **3.3**). In a similar fashion the effect of road disturbance on mycorrhizal type distribution was on average strongest in plots with lower average soil temperature, which is to be expected as EcM and ErM plant species are more common in colder conditions (Brundrett & Tedersoo, 2018). However, this pattern was less clear at a regional level with examples of warmer regions such as Tenerife and colder regions such as China not following the global pattern (Fig. **3.2**). Despite these region-specific patterns, disturbance was overall the strongest predictor of mycorrhizal proportion, above elevation and temperature, and this for all mycorrhizal types. This confirms, at the global level, findings from previous regional studies showing that local anthropogenic disturbance predicts plant species composition better than macro-environmental drivers (Fuentes-lillo et al., 2021; Lembrechts et al., 2016). The distribution patterns of the different mycorrhizal types that we observed across our sites was overall consistent with those previously reported in the scientific literature across climate and elevation gradients (Barcelo et al., 2019; Soudzilovskaia et al., 2017).

The relationship between disturbance and the different mycorrhizal types seems to suggest that ErM and EcM plant species are more sensitive to the effects of road disturbance when compared to AM plant species, thus resulting in increased success for AM plant species along roadsides in environments where they would usually be competing with EcM and ErM plant species (Segre et al., 2016). This would also help explain the discrepancies between our results of increased AM vegetation cover in disturbed roadside conditions and the results of previous studies on disturbance in agricultural settings, where physical disturbances in the form of tillage led to reduced colonisation by AM fungi (Schnoor et al., 2011; van der Heyde et al., 2017). This disparity would then be explained by the effect of altered biotic interactions (through a proportionally higher disruption of EcM and ErM plants) outweighing the negative effects of physical disturbance in a setting that is not already entirely AM-dominated.

Supporting this hypothesis is the fact that the proportion of AM vegetation cover in the roadside plots tended to be comparatively lower when the proportions of AM vegetation cover in the adjacent vegetation was high. This pattern also aligned with the relationship between soil temperature and road disturbance, i.e., the fact that the difference between mycorrhizal proportions in the roadside and in the adjacent plots grew smaller following the regional temperature gradients, and higher following the regional elevational gradients. In both cases, when reaching the upper limits of the temperature gradients and at the highest saturation of AM natural vegetation, the effect of disturbance tended towards reversing, with lower proportions of AM vegetation cover along roadsides than in the adjacent vegetation (Fig. **3.2**, roadside plot line in red crossing over the adjacent vegetation line in green; Fig. **3.3**, points below the 1/1-line, blue line falling below 1/1-line). These congruent patterns are to be expected as EcM and ErM vegetation tends to be most common in cooler conditions and at higher elevations (Barcelo et al., 2019; Bueno, Gerz, et al., 2021; S. E. Smith & Read, 2010). Understanding these interactions and the general patterns that result from them is a crucial first step in disentangling the nexus between disturbance and changes in mycorrhizal type distribution.

While we cannot come to a definitive conclusion as to the mechanisms driving the increase in AM plants to the detriment of EcM and ErM plants due to the strictly observational nature of our study, a possible hypothesis would be that roadside disturbance leads to increased soil nutrient mineralization which AM fungi are more apt at taking advantage of (David J Read et al., 2004; Sulman et al., 2017). This, combined with the fact that AM plant species (as well as NM species) are generally faster growing and more disturbance tolerant due to their ability to take advantage of vegetation gaps (García de León et al., 2016), could begin to explain the patterns we observed: increased nutrient availability, less extreme pH and reduced competition in roadsides all act in favor of AM plant species.

Importantly, we also observed a clear difference in mycorrhizal association type between native and non-native plants species: almost all non-native plant species were NM or AM, with 0.2% of EcM non-native plant species being the exception. This matches the results of previous studies in the U.S. that found non-native plant species to be mainly AM or NM (Pringle et al., 2009). Although as we saw earlier that EcM/ErM dominated vegetation saw a greater increase in AM plant abundance upon disturbance, increases in non-native vegetation were more pronounced in AM-dominated communities. Additionally, non-native species had greater potential for establishing in the native community when that native community was already dominated by AM-associated native plant species (Fig. **3.4**, Fig. **S3.2**). This suggests that plant communities that predominantly associate with the same mycorrhizal fungi as the non-native plant species are also more susceptible to potential invasions by these non-native plant species (Q. Yang et al., 2013). The importance of anthropogenic disturbances in facilitating the success of non-native plant species outside of their natural range is well documented (Jauni et al., 2015; Lembrechts et al., 2016). However, the mechanisms linking these disturbances to increased non-native success remain poorly understood. We suggest that anthropogenic disturbances being favorable to the most common types of mycorrhizal association amongst non-native species could partly help explain the pattern of increased non-native species success in roadside-adjacent environments, especially in communities already dominated by AM or NM-associated species.

However, it remains important to keep the limitations of our study in mind when considering potential mechanisms that drive changes in plant species composition and non-native success. Indeed, our observations remain based on a proxy of mycorrhizal abundance shown previously to have limitations (M. Brundrett & Tedersoo, 2019): aggregated datasets such as FungalRoot are bound to accumulate small errors and imprecisions and should be used with caution when looking at distribution patterns. This is partly why we limited our analyses to broad categories of mycorrhizal associations. Nevertheless, using large collections of datasets remains valuable as long as direct mycorrhizal measurements are not sufficiently available. Our results should thus be seen as a first investigation of global relationships between anthropogenic disturbances, mycorrhizal distribution and their potential impacts on non-native plant invasions, and the resulting observations as potential openings for further studies investigating how human activities can impact mycorrhizal communities and plant-fungal interactions.

Conclusion

Our results show a global pattern of anthropogenic disturbance influencing the distribution of plant cover associated with the different types of mycorrhizal fungi in mountains. AM and NM vegetation cover increased along mountain roads, while the cover of EcM and ErM vegetation decreased. This pattern was consistent across regions but varied in intensity along gradients of environmental factors and depending on the prevailing type of mycorrhiza in the natural vegetation. Indeed, cold-climate regions with higher representation of EcM and ErM vegetation showed greater increases in AM vegetation as a result of road disturbance. Non-native plants were almost exclusively associated with AM fungi or NM, and in turn more successful in environments strongly dominated by AM associations, suggesting that disturbance could be facilitating non-native plant invasion through changes in local mycorrhizal communities. While we hypothesize that this shifting effect of disturbance on the distribution of mycorrhizal types could be caused by changing abiotic factors and in particular by changes in nutrient availabilities, further research with a focus on testing individual drivers associated with disturbance in an experimental setting would be required to truly understand which underlying processes drive the shifts we observed. Regardless, our results represent an important first global study of the role of anthropogenic disturbances in shaping plant communities through the mycorrhizal fungi they associate with. These findings have important implications for vegetation restoration worldwide, as they suggest that roadside disturbance can change the fundamental make-up of EM- and ErM-dominated plant communities, potentially shifting communities between alternative stable states of mycorrhizal dominance that could be very difficult to reverse (Averill et al., 2022; Fukami et al., 2017).

CHAPTER IV

An experimental approach to understanding the impact of anthropogenic disturbance on non-native plant species and fungal communities

Clavel, J., Lembrechts J.J., Verbruggen, E. & Nijs, I.

Summary

Anthropogenic disturbance is known to be a crucial driver in the successful establishment of non-native plant species in high elevation and high latitude cold climates. This disturbance effect manifests itself through a combination of multiple interconnected processes such as reduced competition, increased propagule pressure or increased nutrient availability which have been the targets of previous studies aiming to disentangle their respective role in driving non-native plant success. Here, we add another axis of investigation to this approach in the form of mycorrhizal symbiosis which is increasingly known to be crucial in driving the distribution and success of plants and the resulting plant community composition. To this effect we setup a seed-addition experiment in the subarctic mountains of Sweden testing for the effects of physical disturbance, nutrient addition and mycorrhizal inoculum on non-native plant species establishment success and biomass production and on the local fungi community. Our results confirmed the previously known increase in non-native plant success following disturbance and nutrient addition and showed an accompanying shift in the fungal community composition, especially to the detriment of ectomycorrhizal fungi. Contrary to our expectations we did not observe a significant shift in arbuscular mycorrhizal fungi or increases in the success of non-native plants following the addition of mycorrhizal inoculum.

4.1 Introduction

In the face of mounting pressure from climate change and other anthropogenic activities, invasive non-native species are among the most impactful agents disrupting ecosystems worldwide (Facon et al., 2006). To prevent or at least mitigate their effects on native ecosystems, a great deal of research has focused on understanding the processes leading to their initial invasion success (Kumar Rai & Singh, 2020). Specifically in cold climates, evidence has shown that successful invasion of non-native plant species is closely tied to the disturbances caused by human activity during their introduction in a new ecosystem (Dainese et al., 2017;

Lembrechts et al., 2016). Most non-native plant species are ruderal and opportunistic species and thrive in disturbed systems such as construction sites, roadsides and agricultural fields (Chiuffo et al., 2018; Montesinos, 2022). Therefore, the early success of invasive species in cold environments, such as mountainous and high latitude ecosystems, often happens in areas which have been disturbed following the aforementioned anthropogenic activities. To limit the early spread of non-native plant species to previously undisturbed areas and avoid long-term damage to the ecosystem, it is essential to understand the mechanisms that underlie this apparent facilitation of invasion (Pauchard et al., 2009).

In this context, research has been pursued in disentangling the drivers associated with anthropogenic disturbance such as reduced competition, increased nutrient availability, increased propagule pressure or changes in temperature and how their combined effect can lead to facilitating the success of non-native plant species invasions (Biswas & Mallik, 2010; Blumenthal, 2006; Vilà & Ibáñez, 2011). In this study, we aim to add another axis to our understanding of the effect of anthropogenic disturbance: its impact on the mycorrhizal fungi associated with both native and non-native plants and the possible role it could play in explaining patterns of invasions success. Indeed, mycorrhizal fungi are known to associate with the majority of terrestrial plant species (Kivlin et al., 2011; Wang & Qiu, 2006) and are increasingly being recognized as crucial drivers of plant community composition and ecosystem functioning (Klironomos et al., 2011; Neuenkamp et al., 2018; Cameron Wagg et al., 2014). As could be expected, these interactions also play an important role in regulating invasion success of non-native plant species (Aslani et al., 2019; Dickie et al., 2017).

Previous studies on mycorrhizal communities in natural and agricultural settings have shown that their response to anthropogenic disturbance can be highly dependent on the nature of the mycorrhizal and plant communities, the form of disturbance and regional environmental condition, and therefore difficult to predict (Schnoor et al., 2011; van der Heyde et al., 2017). In the specific context of mountain systems, previous studies seem to suggest that anthropogenic disturbances, such as road construction and presence, may increase the presence of arbuscular mycorrhizal

(AM) fungi and AM-associated plant species (Clavel et al., 2020). However, these patterns were observed through observational studies, and road disturbance is a complex issue that makes it difficult to isolate individual drivers. Roadside disturbance, for example, does not only result in physical disruption but also causes increases in nutrient availability, reduced competition, pH changes, increased propagule pressure and, we argue, changes in belowground fungal communities (Müllerová *et al.*, 2011; Pollnac *et al.*, 2012, Clavel et al. 2020). In this study, our aim is to use an experimental approach to isolate and assess the individual contributions of these drivers to non-native plant success and to evaluate the importance of changes in fungal communities in this process.

To answer these questions, a multifactorial seed-addition experiment was set up in the mountains of northern Sweden, testing for establishment rate and biomass production of four non-native species across four elevation levels and under different combinations of three treatments: nutrient addition, anthropogenic disturbance through the physical removal of vegetation cover, and inoculation with a soil suspension from heavily disturbed lowland sites with or without AM fungi (Fig. **4.1**). Our expectations were that 1) disturbance would be the strongest driver of non-native establishment success and biomass production as previously observed (Lembrechts et al., 2016), 2) nutrient addition would lead to increased biomass production, 3) the addition of soil microbiota from invaded sites would further facilitate non-native success and growth in the disturbed treatment, and 4) all three treatments would lead to shifts in fungal community composition in the soil, generally favoring AM fungi to the detriment of EcM and ErM fungi.

4.2 Methods

Location

The experiment for this study was conducted in the Scandes mountains of northern Sweden, near the Abisko Scientific Research Station (68°21’N, 18°49’E). We chose this location to maintain continuity with a previous survey that showed a correlation between disturbance and increased levels of AM fungal root colonisation and diversity in the same environment (Clavel et al., 2020). The vegetation in the region transitions from birch and willow-dominated forest with a diverse understory including ferns, grasses and dwarf shrubs at lower elevation to alpine shrublands dominated by a few species of ericaceous dwarf shrubs such as *Vaccinium* species, *Empetrum hermaphroditum* or *Betula nana.* We know from previous studies that the vegetation in the region is predominantly associated with Ericoid Mycorrhizas (ErM), which make up 40.9% of the total plant cover. Ecto-mycorrhiza (EcM) and AM-associated plant species are however also strongly represented, comprising 26.1% and 26.4% of the total vegetation cover, respectively. The remaining 6.3% of plant species do not form associations with mycorrhizal fungi.

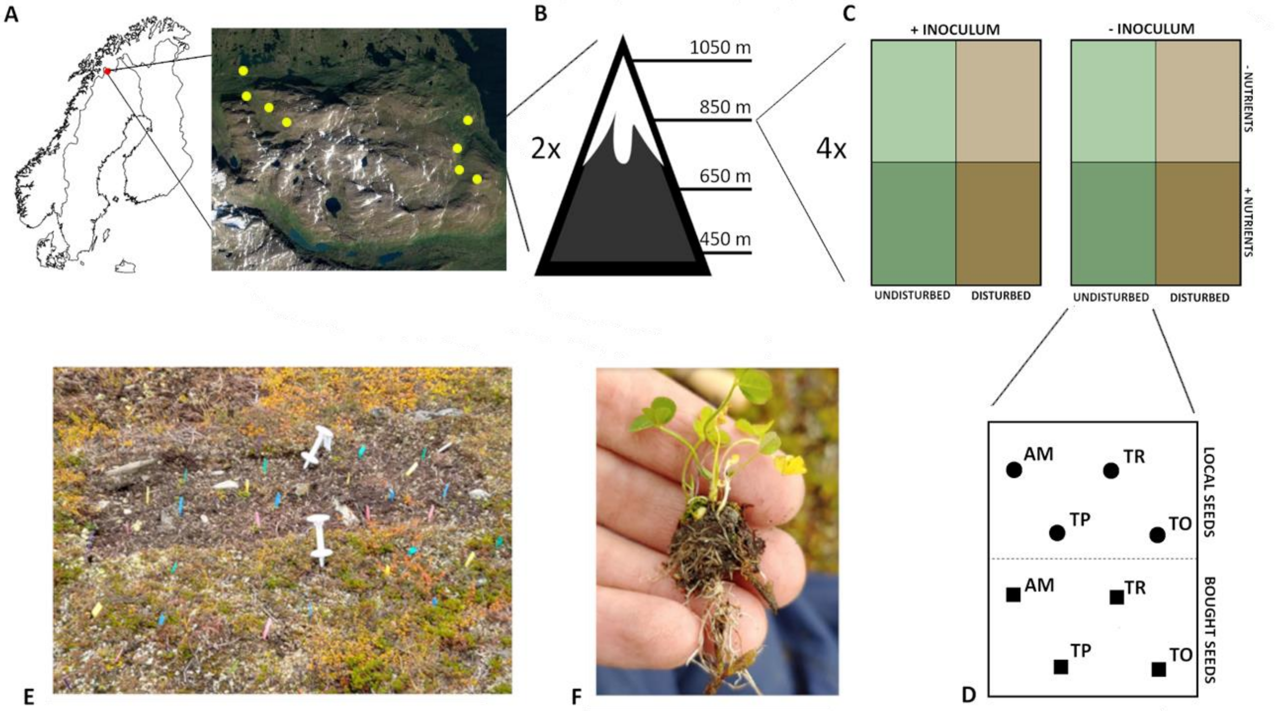

Figure 4.1: Experimental Design (A) Location of the experimental plots in the north of Sweden. (B) Four sites were set up along each of two elevation gradients, with 200 m elevational intervals. (C) Each site was composed of eight 160 × 120 cm plots. Half of the plots received a microbial inoculum originating from highly disturbed lowland sites, each plot was divided vertically between disturbed and undisturbed conditions and horizontally between added nutrients and no added nutrients. (D) For each treatment combination, four non-native plant species (AM: *Achillea millefolium,* TR: *Trifolium repens,* TP: *Trifolium pratense* TO: *Taraxacum officinale*) were sowed in clusters of ten seeds, with two replicates for each species, one with locally harvested seeds and one with bought seeds. (E) Picture of one plot with TMS probes measuring temperature in both disturbed and undisturbed parts of the plot. Note that the rocky soil did not allow for accurate soil moisture measurements. (F) Example of one of the more successful harvested clusters of *Trifolium pratense* seedlings one year after the experiment was setup.

Eight experimental sites were chosen, with four sites along each of two elevational gradients, the first ranging from the settlement of Björkliden along the northern facing side of mount Nuolja, and the second ranging from the Låktatjåkka train station towards the Låktatjåkka Mountain Lodge, following the northeast facing side of the valley (left and right cluster of points in Fig. 1A, respectively). In both cases, sites were set up at regular elevation steps (450 m, 650 m, 850 m and 1050 m a.s.l.), far enough away from any trail or construction to avoid confounding disturbance effects, and in as flat an area as possible.

Seeds

Four non-native species commonly found in the Scandes mountains were planted at each site: *Achillea millefolium, Trifolium repens, Trifolium pratense* and *Taraxacum officinale*. These species were selected based on the data on non-native species from the Mountain Invasion Research Network's long-term vegetation survey protocol, as used in previous studies in the region (Haider et al., 2022; Lembrechts et al., 2017). All four of these species are AM-associated species, as are the vast majority of invasive species globally (Pringle et al., 2009c). Both *Trifolium* species are obligatory mycorrhizal species, while *A. millefolium* and *T. officinale* are facultative mycorrhizal plants (Wang & Qiu, 2006).

Two types of seeds were used: half of the seeds were store bought in Belgium (purchased from https://www.cruydthoeck.nl/) and half were harvested directly within the study system. Local seeds were harvested during the second half of August 2019 across three low elevation and highly disturbed sites: Abisko's research station, train station and tourist station. These sites see a large amount and of both foot and vehicle transit and are surrounded by numerous ruderal species, both local and non-native. Flower heads were harvested and dried at room temperature before extracting the seeds through sieving. The store-bought seeds were initially bought to compare local and foreign seeds as well as a backup solution in case locally harvested seeds would not germinate successfully, ultimately both store bought Belgian seeds and locally harvested seeds were sown but did not show significant differences in performance (see results).

To minimize the influence of microbial contaminants on our results, all seeds were surface sterilized prior to the experiment. This treatment involved immersing the seeds for 5 minutes in a 5% Sodium hypochlorite solution, followed by a thorough rinse with distilled water (Oyebanji et al., 2011). We then conducted germination tests for all species on moist filter paper in Petri dishes. All species showed germination rates above 50%. Following the germination tests, the seeds were stored in dry, sterile conditions until they were sowed two weeks later.

Microbial inoculum

To investigate the role of belowground microbial changes following disturbance, we used a microbial inoculum approach in which microbial communities from low elevation and highly disturbed communities were transposed to the experimental sites. Soil, including root pieces, was collected and pooled from the three locations where local seeds were collected and in close proximity to thriving communities of the four sown species. A microbial wash was then obtained by mixing this soil with sterile water (Koide & Li, 1989). Some of this microbial wash was then filtered using "Whatman Grade 1 Qualitative Filter Papers" with a particle retention of 11 µm (Schnitzer et al., 2011). The original, unfiltered soil was then sterilized using an autoclave and half of it was inoculated with the non-filtered microbial wash while the other half was inoculated with the filtered microbial wash. This was done to focus on the effect of AM as they would be present in the non-filtered half of the microbial wash (henceforth referred to as 'AM soil') but should have been filtered out in the other half which was then used as a control (henceforth referred to as 'control soil'). These inoculated soils were then stored in a fridge at 3°C. About 5 g of either control or AM soil was then added to each seed cluster (see below).

Experimental design

At each of the eight previously described sites, we installed eight 160x120 cm plots (Fig. **4.1**). Half (160 × 60 cm) of each of these plots was then disturbed by removing all vegetation and about 3 cm of the top soil layer to simulate the substantial amount of physical disturbance and disruption of biotic interactions that would be caused by anthropogenic disturbance such as construction works or roadside conditions

(Lembrechts et al. 2016). In each of these 160 × 120 cm plots, we then sowed 32 seed clusters at regular distance intervals. Each seed cluster was composed of ten seeds of one of the four focal species, for a total of eight seed cluster per species, per plot. Half of these seed clusters were sourced from the locally harvested seeds, while the other half was sourced from the store-bought seeds. Seeds clusters in half of the eight plots were inoculated with AM soil, while the other half of the plots received the control soil. These plots were installed several meters apart to avoid as much cross-contamination as possible. Nutrient addition was done using slow-release fertilizer (Osmocote® Plus 15% N - 9% P - 12% K - 8-9 Months), five beads of the fertilizer were added in proximity to half of all seed clusters. All experimental plots were oriented in the same direction against the slope such as to have the nutrient treatment downslope from the nutrient control and avoid the potential confounding effect of nutrient runoff. Furthermore, temperature sensors were placed in both the disturbed and undisturbed parts of each plot, in each site TMS sensors (Wild et al., 2019) were used for two of the plots, while HOBO sensors (Onset Computer Corporation, U.S.A.) were used for the six other plots. The TMS sensors were used to inform about inter-site variation while the HOBO sensors were used to measure intra-site variation. Temperature over the year and across plots ranged from a low of -18.18 °C to a high of 34.69 °C with the lowest mean annual temperature of -0.79 °C being recorded at the 1050 m elevation site of Låktatjåkka and the highest of 3.27 °C in the 450 m elevation site of Nuolja. As expected, elevation was significantly correlated with temperature: there was a negative correlation between elevation and mean annual temperature ($r(2046) = -0.79, p < 0.001$) as well as minimum annual temperature ($r(1598) = -0.44$, $p < 0.001$), and a positive correlation between elevation and maximum annual temperature ($r(1598) = 0.25, p < 0.001$) as higher elevation plots are more exposed to direct sunlight.

The experiment was installed in late August of 2019 and plant performance was measured at the start of September in 2020. The success of the focal species was measured as the percentage of seeds that germinated and survived over this one-year period for each cluster of 10 seeds. Aboveground biomass for all focal species was harvested for each seed cluster separately before being dried and weighed. We initially aimed to measure the abundance of AM fungi in the roots of the harvested species using microscopy, however the belowground biomass proved to be too low for most clusters to be usable for further measurements. Biomass was measured by whole clusters instead of dividing cluster biomass by establishment success as intraspecific competition in more successful seed clusters could otherwise lead to underestimated biomass values. Soil was also collected at each site by taking one soil sample per treatment combination in each of the eight subplot present at each experimental site and pooling these samples by treatments, for example pooling the four soil samples from undisturbed plots with nutrient addition and no AM fungal inoculum, resulting in eight pooled soil samples per experimental site.

Molecular analysis

DNA was extracted from 250 mg of pooled soil samples using the DNeasy PowerSoil Kit from QIAGEN (Venlo, the Netherlands). We targeted the fungal ITS1 region using the primer pair ITS1f/ITS2 (Gardes & Bruns, 1993; White et al., 1990). The mix for the first step polymerase chain reactions (PCR) was composed of 16.3 µL of water, 5 µL of buffer, 0.5 µL of dNTPs, 0.5 µL of both primers, 0,2 µL of Phusion DNA polymerase and 1 µL of template for a total of 25 µL per reaction. The PCR conditions were: initial denaturation at 98°C for 1 min, 35 cycles of 98°C for 30 s, 55°C for 30 s, 72°C for 30 s and finishing with 72°C for 10 min. After a dilution of 1/50 the products from the first PCR were used to run a second PCR using dual barcoded primer with Illumina adapters with a concentration of 0.1 µM. The conditions for this second PC were: initial denaturation at 98°C for 1 min, 35 cycles of 98°C for 30 s, 63°C for 30 s, 72°C for 30 s and finishing with 72°C for 5 min. The PCR products were then purified and pooled into one library using the NucleoSpin kit (Macherey-Nagel, Germany) for gel extraction (1.5% agarose). Real time PCR was then used to quantify the library (Kapa Library Quantification Kits, Kapa Biosystems, Wilmington, USA). The concentration of the library was then changed to 4 nM in a volume of 20 µL and sequenced using the Illumina platform (Illumina Inc., USA) with 2 x 300 cycles paired-end sequencing.

Statistical analysis of plant data

All statistical and bioinformatic analysis were conducted in R (R Core Team, 2021). Generalized Linear Mixed Models (GLMMs) were run to test for variations in both the percentage of seeds that successfully germinated and survived for each ten-seed cluster (N=2048), henceforth referred to as ‘establishment success’, and dry weight of total above ground biomass for each of the clusters, henceforth referred to as biomass. We ran separate GLMMs with success and biomass as response variables and the four following explanatory variables: disturbance as a two-level factor (disturbed vs undisturbed), nutrient as a two-level factor (with or without added fertilizer), microbial inoculum as a two-level factor (AM soil vs control soil) and mean annual temperature as a continuous factor. The four separate sites on each mountain (450, 650, 850 and 1050 m elevation sites) were included as random factors nested in mountain (Nuolja and Låktatjåkka), and species (*A. millefolium, T. repens, T. pratense* and *T.officinale*) was included as a separate random factor. We used the function glmmTMB (Brooks et al., 2017) to run these models using a Beta distribution for establishment success and a Gamma distribution for biomass, the latter after transformation to avoid values of 0 and 1: (response variable value * (number of observations – 1) + 0.5) / number of observations (Cribari-Neto & Zeileis, 2010). We used the ziformula argument in glmmTMB to correct for zero inflation when using Gamma distributions. The two-way interactions between the three treatment factors (disturbance, nutrients and inoculum) were included for both models to investigate potential combined effects of the treatments. We further used a partial pooling approach (Harrison et al., 2018) to investigate the differences in treatment effects between the four species, and a Student t-test to test establishment success and biomass between bought and wild seeds.

Bioinformatic and statistical analyses of sequencing data

Amplicon Sequence Variants (ASV) were generated from the raw sequences using the package DADA2 in R (Callahan et al., 2016). Primers were removed with cutadapt version 2.1 (Martin, 2011), a quality filtering step was then applied to the resulting sequences, allowing for 2 errors maximum for both the forward and reverse reads. Sequences were then dereplicated and merged, chimeras were removed and all samples were rarefied to 20,000 reads for further analysis. This process resulted in a total of 3,770 separate ASVs which were then aligned to the fungal sequences of the UNITE database (Kõljalg et al., 2001) by using the assignTaxonomy function from the

DADA2 package. We then used the FungalTraits database to assign lifestyles to the ASVs which were identified to the genus level. Additionally, all glomeromycota were assigned an arbuscular mycorrhizal lifestyle and all instances of *Pezoloma ericae* were assigned an ErM lifestyle (S. E. Smith & Read, 2010), which all in all resulted in 1,712 ASVs being assigned a primary lifestyle.

The adonis function from the R package vegan (Oksanen et al., 2019) was used to run PERMANOVAs in order to test for the effects of disturbance, nutrient addition and inoculum on the ASV community composition. Furthermore, diversity and relative abundance for AM, ErM mycorrhizal and EcM fungi as well as potential plant pathogens were calculated for each soil sample and their response to disturbance, nutrient addition and AM fungi inoculum were tested for by using GLMMs with Poisson and Beta distributions, the latter correcting for zero inflation.

4.3 Results

General patterns in plant establishment and biomass production

Overall, 36.4% of the 2048 seed clusters resulted in successful establishment of non-native plants one year after being planted. Amongst these successful seed clusters, 2.54 seeds out of the 10 planted established on average, with an average aboveground dried biomass of 17 mg per seed cluster. We found no difference between seeds obtained commercially in Belgium and local seeds harvested in the region in the rate of establishment success (t(2043.1)=1.19, P=0.232) or in biomass production (t(1989.7)=0.89, P=0.374). For this reason, we treated both seed origins as equivalent for all subsequent analysis. Conversely, there were differences in establishment success and biomass production between the four species that were planted. *A. millefolium* was the most successful of the four species with an average successful establishment rate of 51.5% compared to 40.2% for *T. officinale,* 34.4% for *T. repens,* and 19.5% for *T. pratense*, and an average biomass production of 23.9 mg, compared to 18.7 mg for *T. officinale,* 11.7 mg for *T. repens,* and 11.0 mg for *T. pratense.* Both establishment success and biomass were correlated positively with higher mean annual temperature, with the only exception being *A. millefolium*'s establishment success which seemed to be independent of the effect of temperature (Fig. **S4.1**, Table **4.1**).

Table 4.1: Model results for non-native plant establishment success and biomass production: Coefficients and their p-values for a) establishment success of non-native plant species as a percentage of the sown seeds that successfully established after one year, and b) biomass production of these plants measured in grams. Explanatory variables were mean annual temperature (meantemp) and three separate two-level treatments: 1) physical disturbance through topsoil removal, 2) nutrient addition with slow-release fertilizer, and 3) microbial inoculum with or without AM fungi. A partial pooling method was then used to unravel the difference in response between the four tested non-native plant species. Two-way interactions were included in the models but were never significant. Green and orange fields are respectively positive and negative correlations.

a) Success models

	Intercept	Disturbance	Nutrients	AMF Inoculum	Meantemp
Full Model	-1.127 p<0.001 ***	0.438 p<0.001 ***	0.017 p=0.763	0.099 p=0.186	0.108 p=0.008**
A. millefolium	-0.761	0.777	0.009	0.264	0.011
T. pratense	-1.364	0.105	-0.022	-0.032	-0.158
T. Repens	-1.317	0.158	-0.014	-0.009	-0.144
T. officinale	-1.136	0.359	0.023	0.080	-0.941

b) Biomass models

	Intercept	Disturbance	Nutrient	AMF	Meantemp
Full Model	-5.031 p<0.001 ***	1.377 p<0.001 ***	0.511 p<0.001 ***	-0.039 p= 0.841	0.489 p=0.032 *
A. millefolium	-4.236	1.650	0.808	0.055	-0.270
T. Pratense	-5.608	1.295	0.848	-0.623	-0.506
T. Repens	-4.449	1.337	0.169	-0.268	-0.186
T. officinale	-5.396	1.486	0.551	0.184	-0.474

Treatment effects on establishment rate and biomass production

We found no clear effect of two-ways interactions between treatments on either establishment success or biomass production across all models (Table **S4.1**). Disturbance treatment led to both increased establishment success rate and increased biomass production across all species (Table **4.1**, Fig. **4.2**). The nutrient addition treatment did not produce any changes in establishment success rate but led to increased biomass production across all species. Finally, we found no general effect of the AMF inoculum on either establishment success rate or biomass production. However, there was a notable degree of variation between species in the impact of the AMF inoculum treatment, with both *A. millefolium* and *T. officinale* showing slightly increased values for both establishment success and biomass production in plots with AMF inoculum whereas both *T. repens* and *T. pratense* showed the inverse pattern of reduced establishment success and biomass production (Table **4.1**).

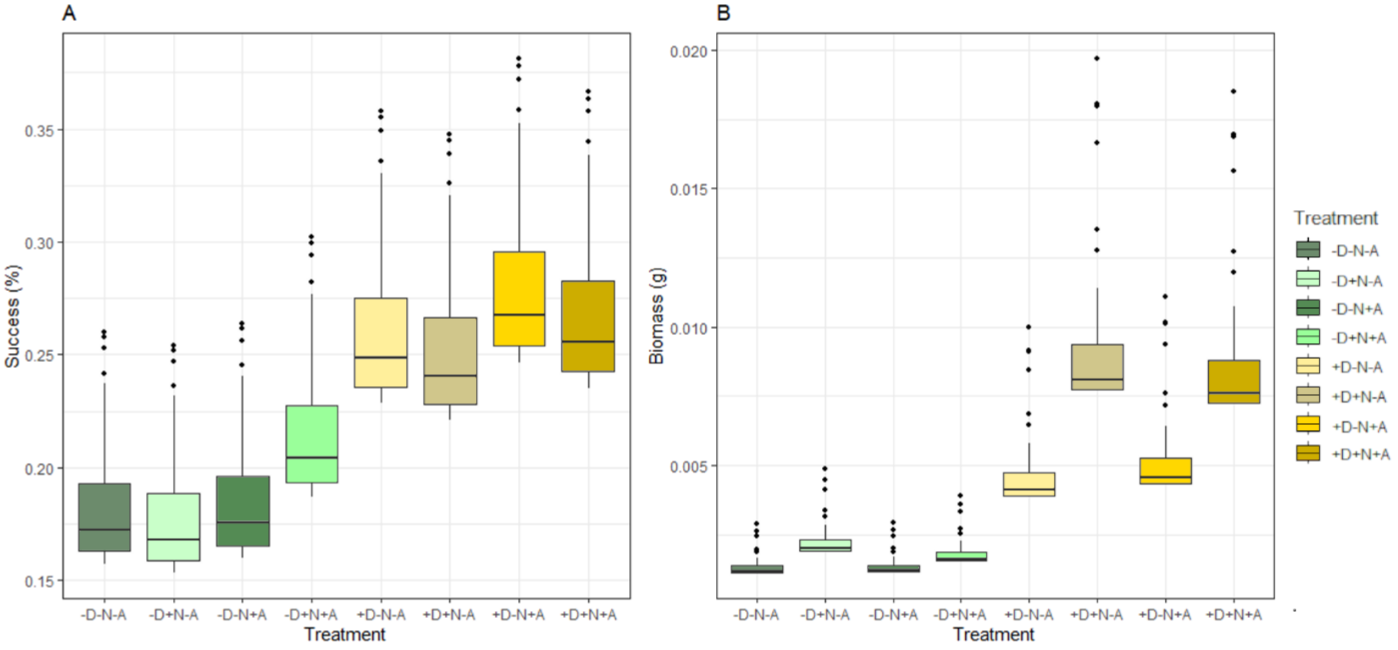

Figure 4.2: Effect of treatments on non-native plants' success and growth. Three two-level treatments were applied to seeds of non-native plant species with a) establishment success of non-native plant species as a percentage of sown seed that successfully that germinated, and b) biomass production of these plants measured in grams being measured after a year. The treatments were disturbance through topsoil removal (disturbed vs undisturbed: +D and -D), nutrient addition with slow-release fertilizer (added nutrients vs no added nutrients: +N and -N) and microbial inoculum from low elevation invaded sites (including Arbuscular Mycorrhizal Fungi or not: +A and -A).

Fungal community results

Across all analyzed soil samples, 76.1 % of reads belonged to ASVs which could be associated to a primary lifestyle using the FungalTrait database. Soil saprotrophs were by far the most common lifestyle as their relative abundance across all samples was of 54.4 % (Fig. **4.3**). Arbuscular mycorrhizas were overall very scarce as they were found only in 14 of the 64 analyzed soil samples and represented only 0.03 % of the total relative fungal abundance. Comparatively, ErM and EcM fungi were respectively present in 60 and 62 of the 64 soil samples and represented 3.41 % and 4.92 % of the total fungal abundance.

Fungal community composition changed significantly in disturbed plots (PERMANOVA, $R^2 = 0.026$, $F_{1,57} = 1.52$, P=0.006) and in plots with added nutrient (PERMANOVA, R^2 0.020, $F_{1,57} = 1.17$, $P=0.043$) (Fig. **4.4**). However, we did not observe a change in fungal community composition when comparing plots with AMF inoculum instead of filtered inoculum (PERMANOVA, R^2 0.017, $F_{1,57} = 1.05$, $P=0.123$) or across the temperature gradient (PERMANOVA, R^2 0.043, $F_{1,57} = 2.55$, $P=0.308$). None of the two-way interactions between treatments turned out to have statistically significant effects ($P>0.05$).

Relative abundance of EcM fungi was negatively correlated with both disturbance, nutrient addition treatments and mean annual temperature (Table **4.2a**). There was also a small but non-significant increase in relative abundance of plant pathogens in disturbed plots compared to undisturbed. Furthermore, the main patterns emerging from the fungal diversity analysis were a significant decrease in EcM fungal diversity and increase in plant pathogen diversity in disturbed plots compared to undisturbed plots (Table **4.2b**).

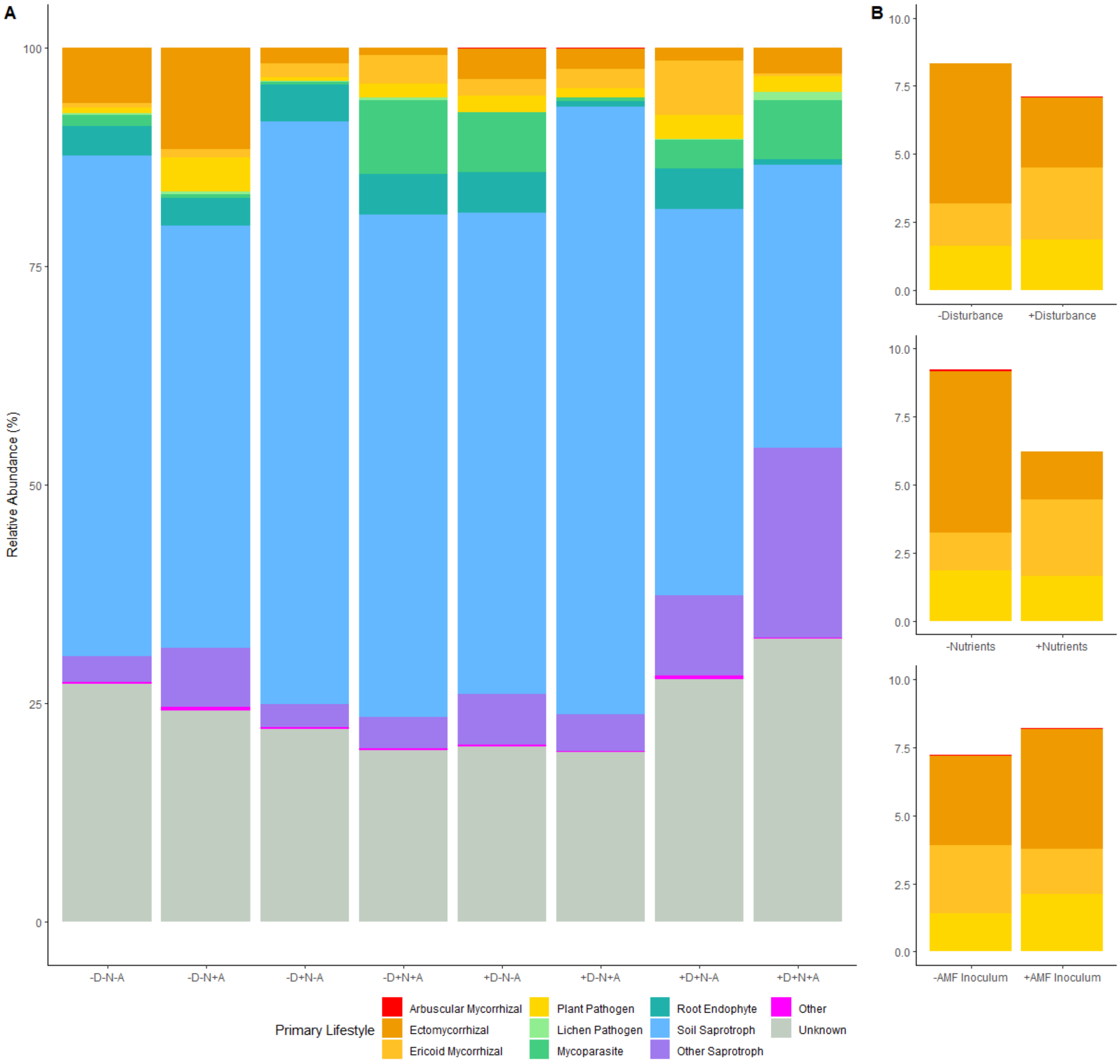

Figure 4.3: Relative abundance of fungi primary lifestyles across treatment combinations. Primary lifestyles were associated to all fungi ASVs obtained after sequencing, using the FungalTrait database. The unknown category includes ASVs that could not be associated with a lifestyle and the Other category pools together lifestyles with under 1% of relative abundance. A) All lifestyles across all possible combinations of the three following two-level treatments 1) disturbance through topsoil removal (disturbed vs undisturbed: +D and -D), 2) nutrient addition with slow-release fertilizer (added nutrients vs no added nutrients: +N and -N), and 3) microbial inoculum from low elevation invaded sites (including Arbuscular Mycorrhizal Fungi or not: +A and -A). 2) Single factor level comparison for mycorrhizal fungi and plant pathogenic fungi. Note that arbuscular mycorrhiza were virtually absent across all treatments.

Table 4.2: Model results for relative fungal abundance and fungal diversity: Coefficients and their p-values for a) relative abundance of the three main mycorrhizal fungi categories and plant pathogenic fungi across all soil samples as a percentage of the total reads for each sample, and b) fungal diversity across these same soil samples, expressed as the number of individual ASVs belonging to the same fungi categories. Explanatory variables were mean annual temperature and three separate two-level treatments: 1) physical disturbance through topsoil removal, 2) nutrient addition with slow-release fertilizer, and 3) microbial inoculum with or without AM fungi. Two-way interactions were included in the models but were never significant. Green and orange fields are respectively positive and negative correlations, with a darker color indicating significance.

a) Relative abundance models

	Intercept	Disturbance	Nutrients	AMF Inoculum	Meantemp
Arbuscular Mycorrhizal	-3.163 p<0.001 ***	0.795 p=0.222	-0.877 p=0.124	-0.538 p=0.392	-0.091 p=0.670
Ericoid Mycorrhizal	-3.536 p<0.001 ***	0.214 p=0.383	0.300 p=0.224	-0.167 p=0.507	-0.003 p=0.980
Ecto-Mycorrhizal	-3.163 p<0.001 ***	-0.423 p=0.047*	-0.568 p=0.036 *	-0.328 p=0.763	-0.466 p= 0.001**
Plant Pathogen	-3.890 p<0.001 ***	0.449 p=0.061 .	0.297 p=0.217	0.240 p=0.311	0.233 p=0.095.

b) Diversity models

	Intercept	Disturbance	Nutrients	AMF Inoculum	Meantemp
Arbuscular Mycorrhizal	-0.957 p=0.534	0.845 p=0.063.	-0.649 p=0.156	-0.320 p=0.463	0.280 p=0.570
Ericoid Mycorrhizal	1.368 p=0.004**	0.155 p=0.305	-0.037 p=0.805	-0.118 p=0.380	-0.068 p=0.653
Ecto-Mycorrhizal	3.159 p<0.001 ***	-0.391 p=0.018 *	-0.183 p=0.264	-0.167 p=0.312	-0.225 p=0.005**
Plant Pathogen	1.332 p=0.008 **	0.512 p=0.003**	0.045 p=0.794	0.061 p=0.725	0.008 p=0.931

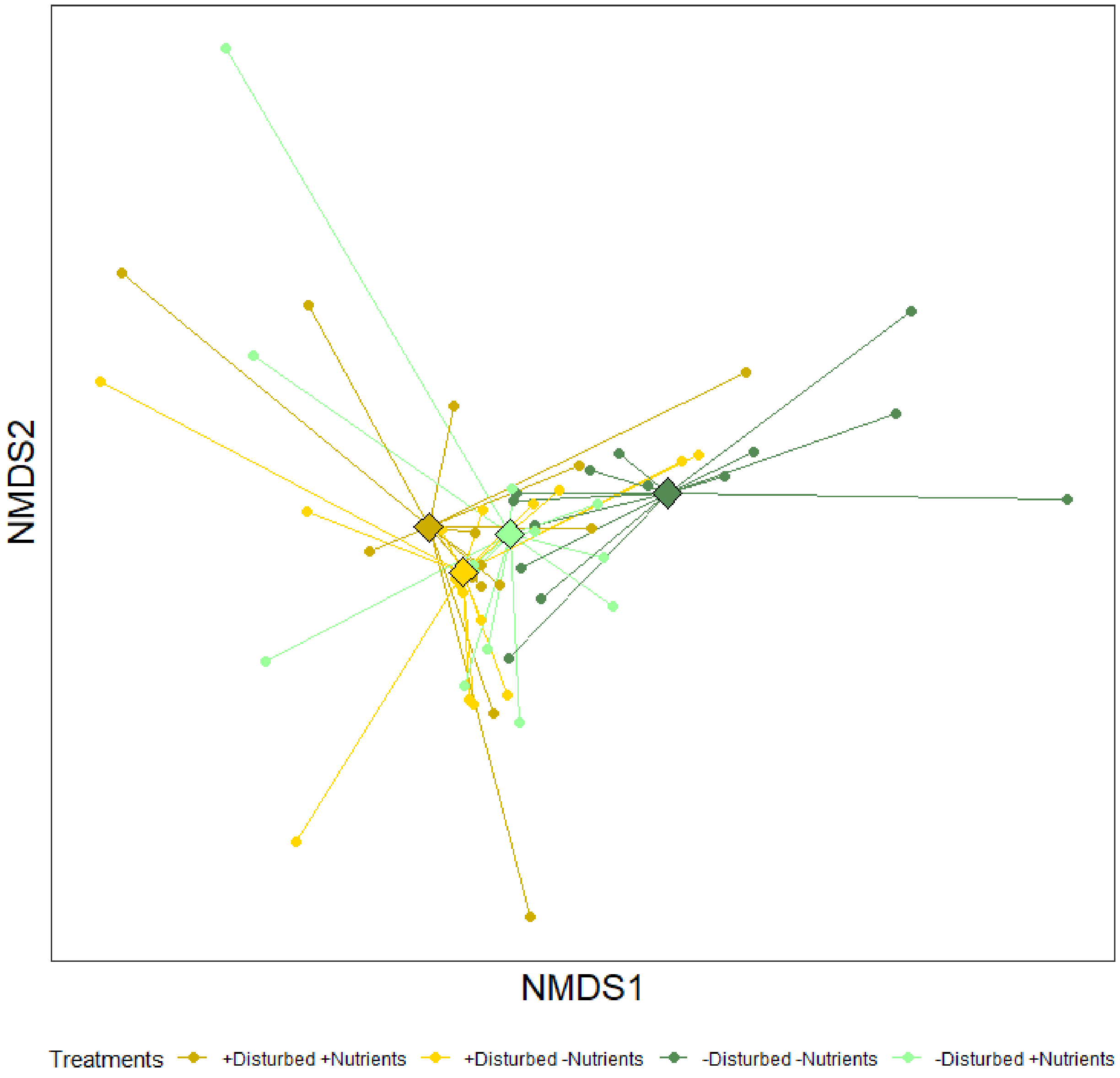

Figure 4.4: Non-metric multidimensional scaling ordinations (NMDS) of fungal community composition. Using Bray-Curtis variation to visualize community dissimilarity between the four different treatment combinations of two-level factors 1) Disturbance through topsoil removal 2) Nutrient addition with slow release fertilizer. Individual points represent soil samples and are each attached to the centroid corresponding to their combination of treatments.

4.4 Discussion

Overall, our results show that physical disturbance was the strongest driver of non-native species establishment success and biomass production, over both nutrient addition and temperature (Table **4.1**, Fig. **4.2**). Nutrient addition was also a strong driver of increased biomass production but not increased establishment success. Both these results are in line with previous experimental work in similar conditions (Lembrechts et al., 2016). Notably these effects were unrelated to any changes in AM-fungi relative abundance or diversity, as AM-fungi presence remained consistently very low across all treatments. These low values of abundance and diversity were not expected as we had previously observed a much higher abundance and diversity of AM fungi compared to this experiment in very similar conditions in the Norwegian side of the Scandes where all samples showed some degree of AM fungal presence contrary to our present results (Clavel et al., 2020). In this previous study we had used the AM specific primer pair AMV4.5NF/AMDG which focuses AM-fungi 18S rDNA (Sato et al., 2005; Maarten Van Geel et al., 2014) and we found the a substantial presence of AM fungi in roots of the natural vegetation, mainly of fungi belonging to the Glomeromycota division. Here we chose to use the primer pair ITS1f/ITS2 targeting the fungal ITS1 region to include all fungi with the goal of extending our analysis to other mycorrhizal types which are bound to play an important role in fungi-plant interactions of the regions. This was done with the knowledge that ITS1f/ITS2 can be less precise when looking specifically at AM fungi when compared to more targeted primers (Gardes & Bruns, 1993; White et al., 1990) but are expected to perform well in identifying glomeral fungi specifically which we expected to be most common in this system (Kohout et al., 2014; Stockinger et al., 2010). Furthermore, in the present study we had a very high sequencing depth (over 20 000 reads) meaning that if AM fungi, were present in our samples they would most likely have been found. This general lack of AM fungi and the absence of any shift in AM fungi relative abundance or diversity following the treatments also clearly shows that the observed changes in non-native plants success and biomass production were entirely separate from changes in AM fungi availability. As previous studies showed a correlation between anthropogenic disturbance and simultaneous increases in AM fungi presence and in non-native plant presence (Clavel et al., 2020), the question arose whether the increased non-native species success was a consequence of disturbance leading to increased AM fungi presence in the environment or conversely, if the increased AM fungi presence was a consequence of the increased presence of non-native species. Our results seem to suggest that in such an AM fungi poor environment the latter explanation of AM fungi being driven indirectly by disturbance through non-native species is the most likely to be true, even if ultimately it is still possible that once both AM fungi and non-native AM-associated plant are present in the system, they could then reinforce each other. It is also possible that the reason we did not observe AM fungi increases following disturbances in this experiment whereas such patterns were observed along disturbed roadsides is a lack of available sources of AM fungi. These could exist in the form of the transport activities related to roads, possibly alongside seeds which could be carrying AM fungi spores (Correia et al., 2018), contrary to our study in which seed surfaces were sterilized. It is also worth noting that we did not expect, and did not observe, the non-native plants in this experiment to be able to affect their surrounding soil enough to have an impact on AM fungi community composition and abundance measurements, as they had only been present in the system for one year and represented only a very small portion of the plant biomass and cover across the experimental plots.

As one could expect for such an AM fungi poor environment, the two AM-facultative species *A. millefolium* and *T. officinale* were overall more successful compared to the AM-obligatory *T. repens* and *T. pratense*. While all four species' establishment success and biomass production were positively impacted by disturbance, the size of that effect was higher for *A. millefolium* and *T. officinale* than for *T. repens* and *T. pratense*, suggesting that the two former species might be more suited to taking advantage of the conditions brought on by the disturbance treatment. Whether this is the consequence of differences in mycorrhizal association status (obligatory vs facultative) is however difficult to assess, as it could reasonably be suggested that other differences in the species' strategies might be at play as well to explain the observed effect.

Contrary to our expectations, the AM fungi treatment showed next to no effect on any of the response variables we analyzed (Table **4.1&4.2**, Fig. **4.3b**). Stemming from the absence of difference in AM fungi relative abundance or diversity between plots treated with inoculum filtered to exclude AM fungi and the plots treated with unfiltered inoculum, the most likely explanation is that the treatment failed to significantly alter the fungal community. Whether the treatment was entirely ineffectual or only too weak to be measurable one year after administration is difficult to answer with only one time step for measurements. The only pattern suggesting a potential effect of AM fungi inoculum is the difference in establishment success between species (Table **4.2**) which seemed to favor *A. millefolium* and to a lesser extent *T. officinale,* which could suggest that there was an initial effect of AM fungi inoculum in the early stages of the experiment following sowing. However, even if this is true, it is quite evident that the chosen inoculum approach was too conservative for the in-situ experiment in question. Answering whether increased AM fungi presence is a driver of non-native species and whether it interacts with the effects of anthropogenic disturbance might require a more controlled in-vitro approach or a much more forceful method of AM fungi inoculation.

We however did observe a significant shift in fungal community composition following disturbance and nutrient addition treatments (Fig. **4.4**). The nature of these shifts can be difficult to pinpoint as we have to rely on relative abundance and diversity to quantify the shifts, and functional traits could be assigned to only 45% of the ASVs in our dataset (Schoch et al., 2012). Despite these limitations, we did observe decreases in EcM fungi relative abundance following both nutrient addition and disturbance treatment and an increase in plant pathogen fungi after the disturbance treatment, which can help explain how fungal communities in ErM and EcM dominated environment are impacted by anthropogenic disturbance. The observed pattern of decrease in EcM fungi following nutrient addition is in line with previous research, as nitrogen deposition is known to alter EcM fungal community composition and to reduce their abundance (Kjøller et al., 2012). Contrary to our initial expectations, we did not see a decrease in ErM fungi following nutrient addition. This does not seem to be an isolated case, however, as similar results have been observed before in comparable cold climate shrub dominated systems (Kiheri et al., 2020).

Reduction in EcM fungi following physical disturbance is likely due to EcM plants in our systems, i.e. mainly tree species (Soudzilovskaia et al., 2020), being more sensitive to this type of treatment than other mostly ErM associated plant species and having recovered more slowly only one year after the initial disturbance. The changes in plant pathogen fungi following nutrient addition are also in line with previous studies in grassland which found nutrient addition to favor pathogenic fungi over mutualistic ones (Lekberg et al., 2021). While less of a clear pattern, the increase in plant pathogenic fungi following physical disturbance is an interesting result that to our knowledge has not been described previously and might justify further study, as it could help better understand plant and fungal community changes resulting from anthropogenic disturbance. The shifts in fungal community composition following disturbance and nutrient addition treatments did line-up with the patterns we observed in non-native plant establishment success and biomass production, as reduced EcM fungi abundance could lead to the improved success of non-native species through reduced competition from EcM plants and the increase in plant pathogens is more likely to harm native plant species than non-native ones (Callaway & Lucero, 2020). However, these results can only point to broad patterns of fungi abundance as they are only derived from relative abundance based on sequencing data which are not a direct measurement of abundance due to different primer affinity between fungi. It is also important to remember that these coinciding patterns in fungal community composition and non-native plant success can only be established as correlations from the results of this study, testing whether there is actual causation would require further research in a more controlled environment to disentangle the exact effects of fungal community shifts.

Conclusion

Our results align with the idea that anthropogenic disturbance leads to shifts in mycorrhizal fungi communities. However, we did not find evidence that these changes are favorable to AM fungi, and therefore to AM-associated non-native plants. This suggests that previously observed increases in abundance and diversity of AM fungi following anthropogenic disturbance in cold mountainous systems were not directly caused by the abiotic effects of disturbance. Instead, the increased presence of non-native AM-associated plant species in the environment would have in turn led to increases in AM fungi in their surroundings. However, the immediate decrease in EcM fungi and the increase in pathogenic fungi we observed following the disturbance treatments suggest that other fungal taxa might play a role in the initial increased success of non-native plant species following anthropogenic disturbance.

CHAPTER V

Non-native plants and arbuscular mycorrhizal fungi presence are increasing over time along arctic mountains roads

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Summary

Roads in cold climate mountains are known to be important vectors in the introduction and spread of non-native plant species. In the same context mycorrhizal communities are also altered in disturbed roadsides with known positive effect on arbuscular mycorrhizal (AM) fungi diversity and abundance. However, to which degree these two effects of roads are intertwined and how they are evolving over time is not well understood. In this study we conducted surveys of non-native plants and AM fungi with three timesteps each, respectively from 2012 to 2022 and from 2017 to 2021, in the Norwegian mountains of the northern Scandes to investigate temporal changes and interactions between disturbance, mycorrhizal fungi and non-native plants. We found that upward spread of non-native plants and lateral spread away from roadside into the natural vegetation are currently extremely limited, however diversity and total cover of non-native plant species did see an increase over the ten years period, especially at lower elevations. Likewise, we saw an increase in AM fungal abundance over four years along the roadsides at lower elevations. Furthermore, our results suggest that increases in non-native species is unlikely to be the driving cause of the observed increase in AM fungal abundance.

5.1 Introduction

Non-native plant invasions are an important component of how anthropogenic activity is currently increasingly impacting mountain ecosystems (Alexander et al., 2016). With increased human activity in previously remote areas (Price 2006) and climate change pushing the viable range of plant species upward (Petitpierre 2015), it is expected that the rate of non-native plant invasions will further increase across mountains ecosystems and, without proper management, will lead to losses in biodiversity and ecosystem services (Körner 2004). A better understanding of the patterns of introduction and consequent spread of invasive species in mountains is therefore crucial for proper management aiming to limit the negative impacts of future plant invasions. The role of anthropogenic disturbance in facilitating non-native plant success is particularly important in this context (Alexander et al., 2016; McDougall et al., 2018). Previous studies have shown that the early success of these plant species is most often associated with cases of physical disturbance associated with activities such as construction, logging, or agriculture (Lembrechts 2016, Dainese 2017). Most non-native plants are ruderal and opportunistic species that thrive in such conditions, which allows them to establish their presence in the system before potentially spreading into the surrounding natural vegetation (Chiuffo 2016, Montesinos 2022). The way non-native species benefit from these disturbances to establish themselves and then spread over time along and away from these disturbed sites is therefore a crucial step in the process of invasions at which management could be at its most effective in stopping potential spread of non-native species.

One axis through which disturbance could impact local vegetation and potentially facilitate non-native plant success is the impact of mycorrhizal fungi and their symbiosis with both native and non-native plant species. Mycorrhizal associations are known to be almost ubiquitous among terrestrial plants and to be crucial drivers of plant community composition (Wang & Qiu, 2006; Kivlin et al., 2011; Wagg et al., 2014; Neuenkamp et al., 2018) and, in turn, are an important factor in regulating plant invasions (Dickie et al., 2017; Aslani et al., 2019). However, the effects of anthropogenic disturbance on mycorrhizal associations, and the potential implications for non-native plant success, are still poorly understood. Previous studies in agricultural settings have shown that tillage-related disturbance leads to highly context dependent shifts in mycorrhizal associations (Schnoor et al., 2011; Van Der Heijden et al., 1998). A previous study using a large dataset of plant surveys along mountain roads across the globe has shown that anthropogenic disturbance leads towards an increased representation of Arbuscular Mycorrhizal (AM)-associated plant species, to the detriment of Ectomycorrhizal (EcM) and Ericoid Mycorrhizal (ErM)-associated plant species (chapter 3). Direct observations of mycorrhizal abundance and diversity in the northern part of the Scandes mountains in Norway has also shown similar patterns of increased AM fungi diversity and abundance in disturbed mountain roadsides (Clavel et al., 2020). The latter correlated strongly with higher presence of non-native plant species. As the vast majority of non-native plant species that form mycorrhizal associations are AM-associated (Pringle 2009), we hypothesized that the increased representation of AM fungi caused by disturbance would lead to facilitation of non-native plant success. In this study we aim to further investigate the dynamics of non-native plant species spread and the hypothesized accompanying increases in AM fungi, using a repeated survey of plant species distributions over 10 years and of AM fungi abundance over 4 years along mountain roads leading up into the northern Scandes mountains.

The system of the Northern Scandes is an EcM and ErM-dominated environment with low disturbance rates, where non-native species currently seem to be limited to lowlands and disturbed sites only (Clavel et al., 2021; Lembrechts et al., 2014). This makes it an ideal system to study the early stages of the impact of anthropogenic disturbance on non-native species spread and on mycorrhizal association shifts. Our initial vegetation survey in 2012 seemed to indicate that non-native plant species were increasing their range upwards along disturbed roadsides but did not yet expand into the surrounding natural vegetation due to the high resistance of the native EcM and ErM-dominated vegetation (Lembrechts et al., 2014). Likewise, increased AM fungi presence seemed to be consistent across but limited to disturbed sites (Clavel et al., 2020). We therefore set out to test the three following hypothesis:

1) The pattern of higher AM fungi abundance and higher non-native plant cover in disturbed sites is true across all three repeated surveys.
2) Non-native plant species elevational range limits and roadside colonisation increased over time, yet not their spread away from the roadsides.
3) AM fungi abundance along the disturbed roadsides increased over time, in the roots of non-native species but also in roots of the entire plant community.

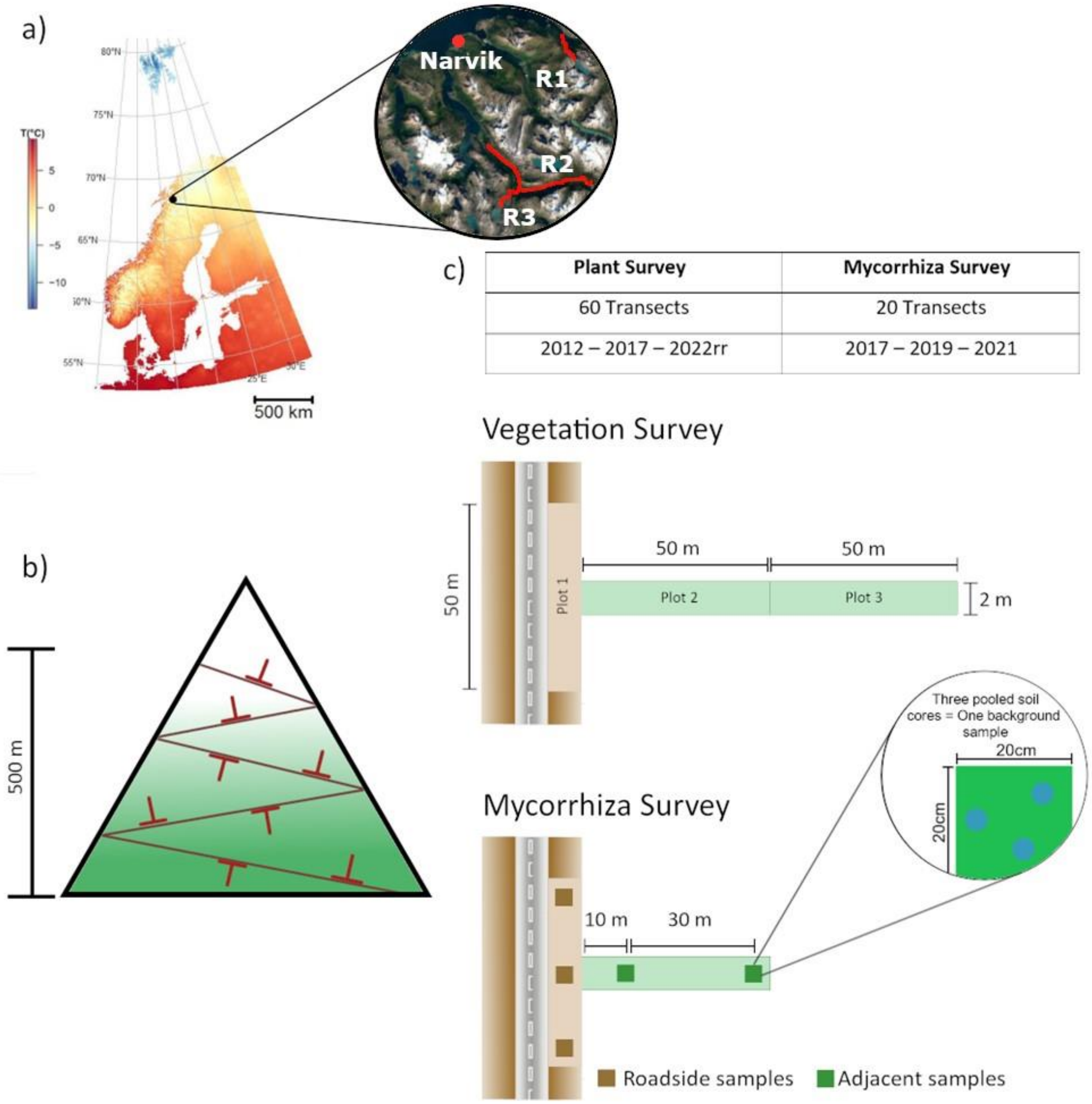
5.2 Material and Methods

The region in which the study was conducted is the subarctic Northern Scandes mountain range in the proximity of the city of Narvik, Norway (68°26’N, 17°25’E). The vegetation in these mountains includes *Betula* dominated forests at lower elevation with occasional *Salix* and *Pinus* trees and an understory of ericaceous shrubs and ferns, which gradually transition towards alpine shrublands dominated by ericaceous shrubs such as *Empetrum hermaphroditum, Betula nana* and various *Vaccinium* species. These vegetation types predominantly consist of ErM plants which represent 40.9% of the total vegetation cover, with EcM and AM plants respectively being 26.1% and 26.4% of that cover and the remaining 6.3% being non-mycorrhizal species.

Three mountain roads were chosen around Narvik along which we conducted vegetation surveys and AM abundance measurements in three timesteps, respectively over 10 and 4 years, both along the disturbed roadside and in the adjacent undisturbed vegetation for comparison. These roads, henceforth called R1, R2 and R3 (Fig. **5.1**) cover elevation gradients from sea level up to 610, 634 and 690 m a.s.l, are respectively 7.1, 26.4 and 20 km long, are regularly maintained through yearly mowing and occasional gravel addition and are actively used in summer for tourism and access to high elevation power plants. The monitoring of vegetation along these roads has been ongoing since 2012 as part of the Mountain Invasion Research Network’s (MIREN, www.mountaininvasions.org) effort to study patterns of plant invasions across mountain ecosystems globally (Haider et al., 2022). For this study we conducted two types of measurements, 1) vegetation surveys of abundance and cover for all plant species present along the roadside and in the adjacent natural vegetation and 2) measuring the abundance of AM fungi in the roots found in representative soil samples, as well as in the roots of specific non-native focal species. The vegetation surveys were conducted in 2012, 2017 and 2022, while the mycorrhizal surveys were conducted in 2017, 2019 and 2021. Furthermore, temperature was measured over the years using sensors placed in both the roadside and the adjacent vegetation, one TMS sensor (Wild et al., 2019) was placed at 40 m from the road in the adjacent vegetation plot and one HOBO sensor (Onset Computer Corporation, U.S.A.) was placed in the middle of the roadside plot.

The vegetation surveys were conducted following the MIREN road survey protocol (Haider et al., 2022), which is designed to allow for comparison between the vegetation composition of the disturbed roadsides and the adjacent undisturbed vegetation as well between lower and higher elevations. Additionally, it allows to assess the spread of non-native species, both upwards along the roads and into the natural vegetation away from the roads. 20 transects were set up for each of the three roads at consistent elevational intervals. These transects consisted of three 50 m x 2 m subplots organized in a T-shape with the first plot being parallel to the road from 0 m to 2m away from it, the second plot being perpendicular to the road from 2 m to 52 m away and the third plot following along the second plot from 52 m to 102 m (Fig. **5.1**). The first plot will henceforth be referred to as the “roadside plot”, while the second and third plots will be referred to as the “adjacent plots”. For each of these transects and their subplots, all plant species present were identified and assigned an abundance and a total cover value. All three surveys were conducted between the months of July and August, with uneven and even transects being surveyed respectively at the start and at the end of the survey period to reduce potential sampling bias caused by changes in the phenology of the observed plants during the sampling season. Species were considered as non-native following the list established by the Norwegian generic ecological impact assessment of alien species (GEIAA) (Sandvik et al., 2019) which defines non-native species as originating from outside of Norway, being established after the year 1500 and being capable to reproduce in the wild, resulting in a total of 1892 known non-natives plant species across Norway.

The mycorrhiza surveys were conducted in a selection of transects along which the vegetation surveys were done, resulting in mycorrhizal samples being taken in 19 of the total 60 transects, 7 along R2 and R3 and 5 along the shorter R1. Soil samples (henceforth called background samples) were taken for AM fungal measurement in roots both in the roadside plot and the first adjacent plot. A background sample consisted of the roots collected in three topsoil cores of 5 cm diameter and depth taken inside a 20 x 20 cm square, as to best represent the local AM fungal colonisation across the roots of multiple species without biases towards species more visible above ground. The soil was then sifted to isolate the roots before being frozen until analysis. During the first year of survey in 2017, 5 background samples were taken in each roadside plot but ultimately only two could be analyzed due to resource constraints. Henceforth, in 2019 and 2021, three background samples were taken and analyzed in each roadside plot. In the adjacent plots, two samples were taken each year, one 10 m away from the roadside plot and one 40 m away from the roadside plot, to test for a potential effect related to distance from the road. Contrary to the first root survey in 2017, we did not repeat nutrient measurements, as they had proven to have only little influence in our previous analyses. This low accuracy might have been due to the technical difficulties related to the coarse gravel sizes in the roadside vegetation and the poor soils with high levels of organic material in the adjacent vegetation, possibly making measurements unreliable.



Plant Survey	Mycorrhiza Survey
60 Transects	20 Transects
2012 – 2017 – 2022rr	2017 – 2019 – 2021

Figure 5.1: Sampling setup along three mountain roads in the Northern Scandes. a) Geographical location of the three studied mountain roads (R1, R2 and R3) in the Northern Scandes near Narvik, Norway. b) 20 transects were spread out with even elevation distances along each of the three mountain roads. c) Each transect was subdivided in three 50 x 2m plots, one following the disturbed roadsides and the two others oriented perpendicularly away from the road into the adjacent natural vegetation. Vegetation surveys following the Mountain Invasions Research Network (MIREN) road survey protocol were effectuated along these plots in 2012, 2017 and 2022 while surveys of root colonisation by arbuscular mycorrhizal fungi were effectuated in 2017, 2019 and 2022.

Furthermore, we selected three focal non-native species: *Achillea millefolium* L., *Trifolium repens* L., and *Trifolium pratense* L. These species are the most common non-native species found along our studied roads as established in the vegetation survey of 2012 (Lembrechts et al., 2014). All three of these species are known to associate with AM fungi with *T.repens* and *T.pratense* being obligate mycorrhizal while *A.millefolium's* AM fungi association is only facultative (Wang & Qiu, 2006). Whenever these species were present in a plot, up to four samples of their roots were harvested, with one sample corresponding to the roots of one individual plant.

All root samples were cleaned of soil material using demineralized water and a 2 mm mesh sieve before being cut into 1 cm long pieces. Most samples yielded at least 20 such pieces which were then used for analysis, cases were material was limited were only kept if they yielded at least 10 standardized root pieces and were discarded otherwise. AM fungal root colonisation was measured for every background and focal species root sample. This was done using the gridline and intersection method in which mycorrhizal structures are stained using black ink and counted under the microscope (McGonigle et al., 1990). The root samples were first cleared using a 5% KOH solution then stained using a mix of 10% Schaeffer black ink and 10% acetic acid (Vierheilig et al., 2005). This method produces a count of mycorrhizal structures, including hyphae, arbuscules and vesicles, and in a turn a percentage of root length colonised by AM fungi.

Generalized linear mixed-effects models (GLMMs) were fitted to analyze the patterns of non-native plant distribution and of AM colonisation over the years, along the elevation gradients, and between road and adjacent plots. All models were made using the glmmTMB package (Brooks et al., 2017) in R (R Core Team, 2021). Three different response variables were tested for: 1) total non-native vegetation cover in a given plot as a percentage of the 2 x 50 m plot area (*n*=540), 2) non-native species' richness in a given plot (n=540) and 3) AM fungal root colonisation as a percentage of root samples where AM mycorrhizal structures were observed. In the case of AM fungal root colonisation, models were separated in two categories, one including only background samples from the road and from the adjacent plots (n=294) and another including only focal species' roots (*n*=215). Both 1) and 3) were modelled using beta regressions after transforming the response variable to avoid extremes of 0 and 1 (response variable value * (number of observations – 1) + 0.5) / number of observations (Cribari-Neto & Zeileis, 2010) and 2) was modelled using a Poisson distribution. The response variables tested for were as follows: 1) year as a three-level factor, either 2012, 2017 and 2022 for the vegetation surveys or 2017, 2019 and 2021 for the mycorrhizal surveys. 2) Plot as a three-level factor corresponding to the three plots of the survey design, for which plot 1 is the plot furthest from the road from 52 to 102 m away and therefore least affected by disturbance, 2 is the closest adjacent plot mostly unaffected by road disturbance ranging from 2 to 52 m from the road and 3 is the roadside plot parallel to the road, i.e. the most disturbed part of the transect ranging from 0 to 2 m from the road. 3) Elevation, which was scaled using the scale function in R to avoid issues of different scales amongst predictor variables, with the lowest elevation point being the valley where the first transect on each road is situated. Both elevation and mean annual temperature were initially included in the models but were found to be too correlated after testing for multicollinearity using the VIF function in R (Fox & Weisberg, 2018) which returned values above 5 (5.45 for elevation and 5.33 for temperature when both included) resulting in only elevation being retained. These three response variables were included in all aforementioned models expect for the one pertaining to AM fungi colonisation in the focal species samples for which the plot effect was omitted as the vast majority of non-native individuals were found and sampled in the roadside plot. Subsets of the focal species model were also made for each individual species.

A random intercept of road was included in all models and an additional random intercept for species identity was included in the grouped focal species model. The relevance of all two-ways interactions were tested for using a model selection approach in which models with all possible combinations of interactions were made and compared and models with the lowest AICc (Akaike Information Criterion, corrected for small sample sizes) were retained (Zuur et al., 2009). In all cases this corresponded to models including all possible two-way interactions.

Finally, we investigated whether the rate of change in non-native species cover, species richness and in AM fungal abundance over the surveyed time period was different across the elevation and disturbance gradients, and if it was higher in plots were non-native plant species were already most present at the start of the survey. For this, a two-step approach was used. First, GLMMs were run for each response variable at the plot level with only year as an explanatory variable to obtain the slope of change across years. These slopes were then used as the response variable in linear models with elevation and distance to the road as explanatory variables. Additionally, in the case of AM fungi root colonisation changes, the total cover of non-native species present in a given plot in 2017, the first year of mycorrhizal surveys, was included as a supplementary explanatory variable.

5.3 Results

Vegetation surveys

Non-native plant species cover and richness were found to both be consistently higher in the road plots than in the adjacent vegetation (Fig. **5.2**, Table **5.1a**). The road effect on non-native success was confirmed by the fact that non-native species were found in 94% of all roadside plots against 27% in adjacent plots 2 to 52 m from the road and only 15% in the plots 52 to 102 m from the road. Non-native species cover was also found to decline with increasing elevation, yet such a decline was less clear for non-native species richness. Furthermore, the difference in non-native species total cover and richness between roadside plot and adjacent plots was strongest at lower elevation as illustrated by the interaction effect between plot and elevation in Table **5.1a**.

Over the ten-year period of vegetation surveys, we observed no significant changes in non-native species richness at the plot level (Table **5.1**). We did however observe an increase in the total number of non-native species observed across our study system which went from 17 non-native species observed in 2012 to 19 in 2017 and 23 in 2022 (Table **S5.1**). This change is not reflected in the outcome of our models at the plot level as these new species, such as for example *Polygonatum vulgare* or *Poa trivialis,* do not currently significantly affect overall plot-level species richness since they remain rare and are only found in very few low elevation plots (with the notable exception of *Poa trivialis* which was found up to 447 m).

Table 5.1: Model results for non-native plant cover and species richness and arbuscular mycorrhizal root colonisation: Coefficients and their p-values for a) the total cover percentage of non-native plants and the total species richness of non-native plants and b) the percentage of roots colonised by arbuscular mycorrhizal fungi in pooled samples of randomly selected roots (background samples) and for the roots of a selection of focal non-native species. Explanatory variables were 1) plot, i.e., the three plots described in Fig. 1, for which a higher value means a higher proximity to the road, 2) elevation, as a gradient from the valley to the highest point of the surveyed roads and 3) year, as the timesteps between repeated surveys: 2012, 2017, 2022 for a) and 2017, 2019 and 2021 for b).

a) Non-Native Plants

	Intercept	Plot	Elevation	Year	Plot* Elevation	Plot* Year	Elevation* Year
Non-Native Plant Cover	-3.08 P<0.001***	0.624 P<0.001***	-0.54 P<0.001*	0.273 P=0.019***	-0.325 P<0.001***	0.122 P=0.009***	-0.105 P=0.082*
Non-Native Species Richness	2.731 P<0.001***	1.506 P<0.001***	-0.36. P=0.062.	-0.026 P=0.801	-0.229 P=0.017*	-0.005 P=0.95	-0.097 P=0.15

b) Mycorrhiza colonisation

	Intercept	Plot	Elevation	Year	Plot* Elevation	Plot* Year	Elevation* Year
Background Samples	-2.322 P<0.001***	1.319 P=0.002**	-0.003 P=0.947	-0.142 P=0.528	-0.003 P<0.001***	0.571 P=0.001**	0.001 P=0.862
All Focal Species	-0.521 P=0.358		-0.001 P=0.734	0.153 P=0.268			-0.001 P=0.733
Trifolium repens	0.762 P=0.142		-0.001 P=0.512	-0.006 P=0.979			-0.001 P=0.901
Trifolium pratense	0.414 P=0.399		-0.002 P=0.531	0.027 P=0.891			-0.001 P=0.807
Achillea millefolium	-1.998 P<0.001***		0.002 P=0.288	0.568 P=0.045*			-0.001 P=0.168

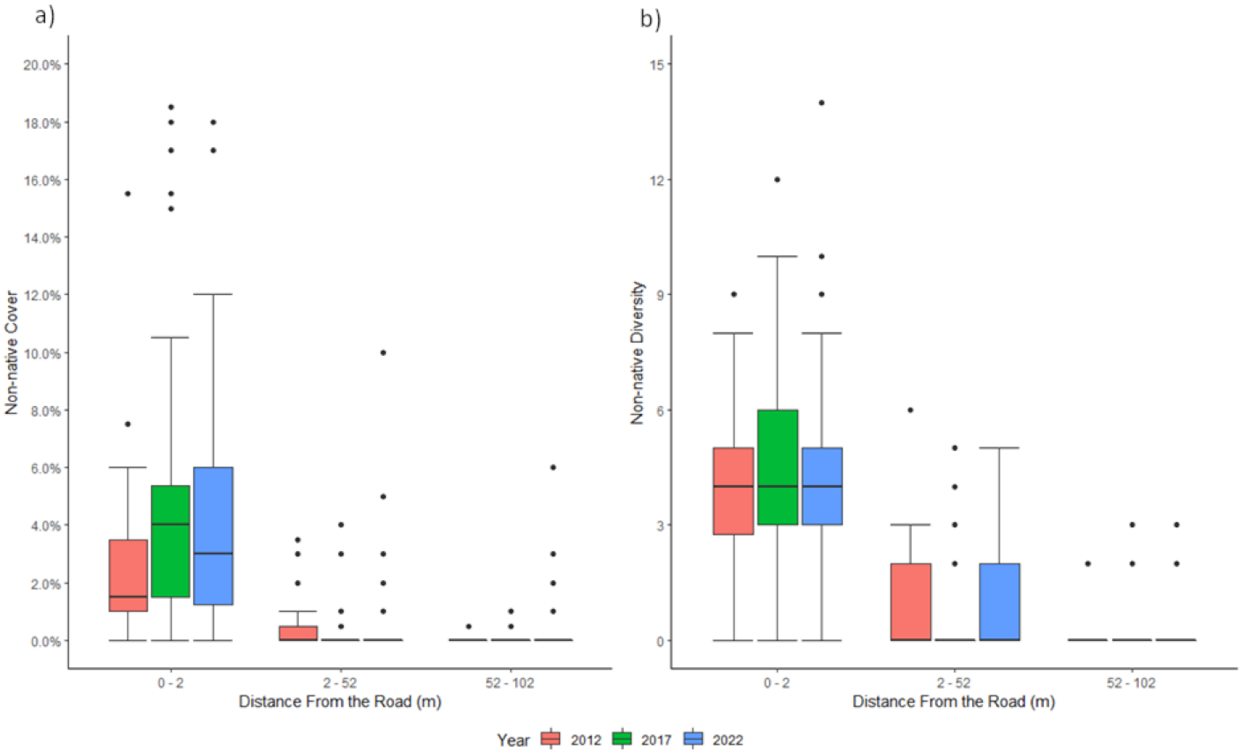


Figure 5.2: Changes over time in non-native plant cover and species richness along mountain roads: The percentage of total non-native plant species cover (a) and non-native plant species richness (b) were measured along mountain roads in three plots at increasing distance from said road.

When looking at shifts in maximum elevational range only two of the 23 observed non-native species, *Ranunculus repens* and *Plantago major,* showed a clear increase (Table **S5.1**). None of our focal species showed a clear increase in their maximum elevational range, as both *T.pratense* and *T.repens* were observed at the same highest elevation (428 and 556 m a.s.l., respectively) in all three surveys, and *A.millefolium* reached 426 m a.s.l in both 2012 and 2022, yet was found at 556 m a.s.l. in 2017. Conversely, we did observe an increase in total cover of non-native species over the same time frame (Table **5.1a**, Fig. **5.3**). As shown by the interactions effect in Table 1, this increase was restricted to low elevation roadside plots, an observation confirmed by the plot-level slope-based models (Table **S5.2**).

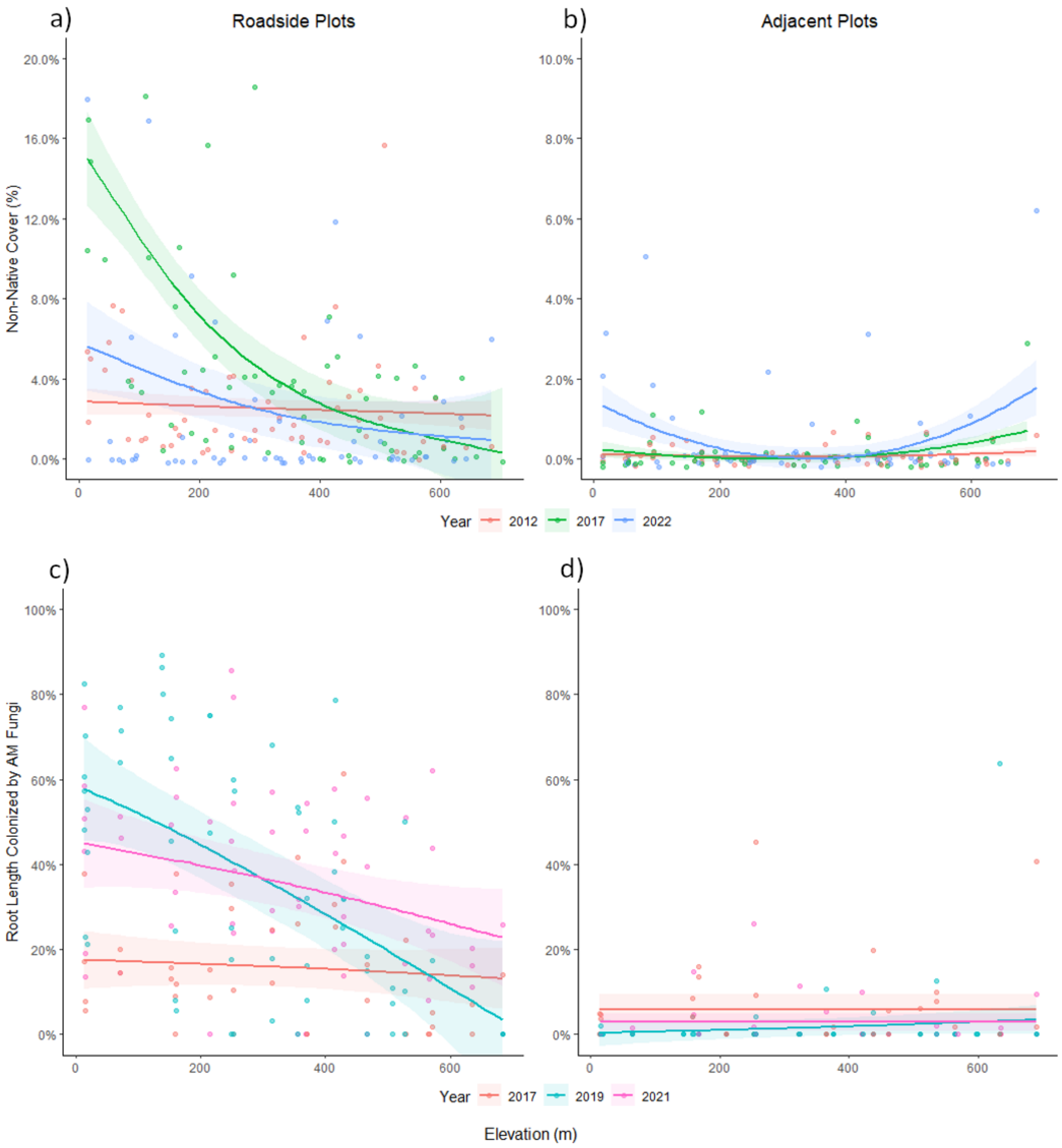


Figure 5.3: Elevation effect on the non-native plant cover and on arbuscular mycorrhizal root colonisation: Total percentage cover of non-native plant species (a & b) and the percentage of roots colonised by arbuscular mycorrhizal fungi structures (c & d) were measures along mountain roads across a 700 m elevation gradient. These measurements were taken both directly in the disturbed roadside (a & c) and in the adjacent vegetation less impacted by the effects of the road (b & d).

Mycorrhizal colonisation

Across all background samples, the degree of root colonisation by AM fungi was higher in roadside plots than in the adjacent plots (Fig. **5.4**, Table **5.1b**) and there were only 19% of roadside background samples where no AM mycorrhizal structures were observed, against 77% of the adjacent background samples. While there was no effect of elevation on AM root colonisation in adjacent background samples, roadside background samples were on average more colonised by AM fungi at lower elevations (Table **5.1b**, Fig. **5.3**). Over the four-year period of mycorrhizal surveys, we did observe an increase in AM root colonisation in the roadside plots and no changes in the adjacent background samples (Table **5.1b**, Fig. **5.4**). There was no clear effect of elevation on the rate of increase of AM root colonisation across roadside plots (Table **S5.2**). Furthermore, the rate of increase in AM root colonisation in background samples was not higher in plots which had higher non-native plant species cover in the 2017 survey.

When looking at the degree of AM root colonisation across an aggregate of our focal species there were no effects of either elevation or year (Table **5.1b**). At the species level this remained true for both *T.repens* and *T.pratense* whereas we did observe an increase in AM fungi colonisation over years in the roots of *A.millefolium* .

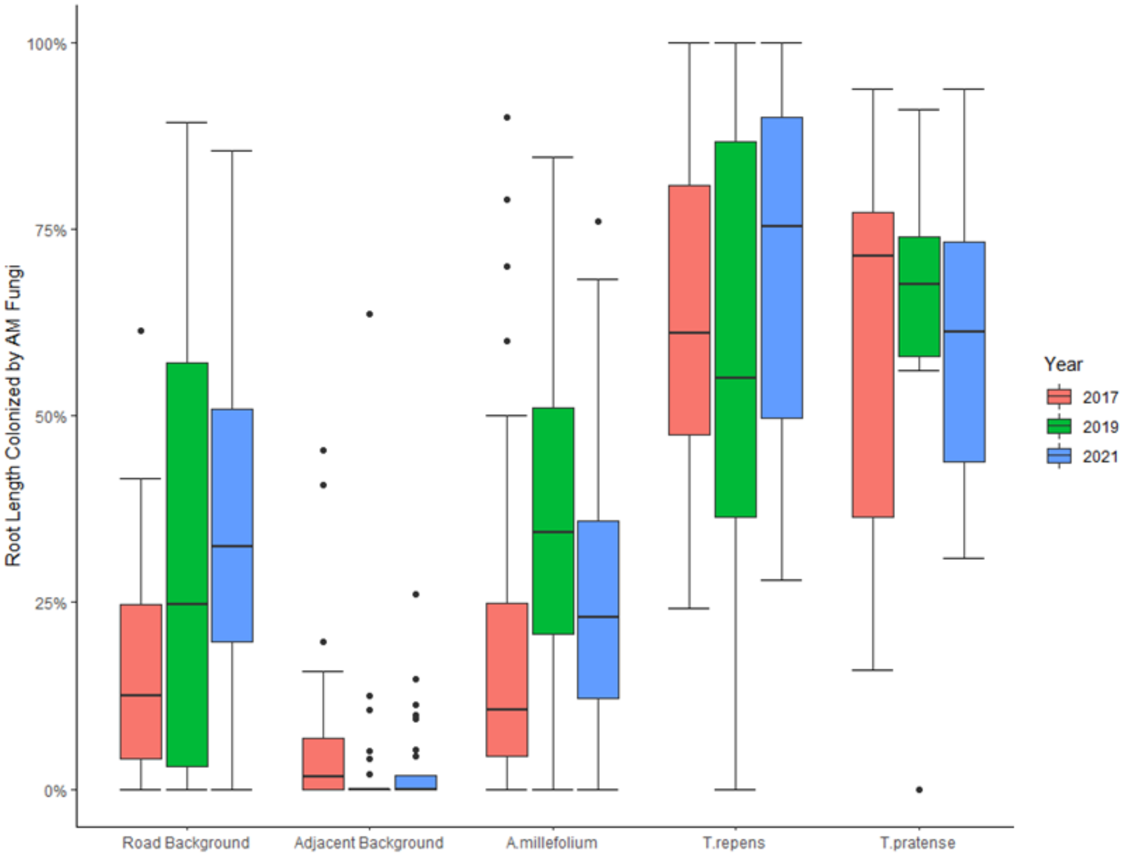


Figure 5.4: Changes over time of arbuscular mycorrhizal root colonisation along mountain roads: The percentage of roots colonised by arbuscular mycorrhizal fungi in each sample. Background samples were composed of roots extracted from randomly selected soil samples taken in the disturbed roadsides or in the undisturbed adjacent vegetation. The roots of three focal non-native species (*Achillea millefolium*, *Trifolium repens* and *Trifolium pratense*) were also sampled whenever present in a plot.

5.4 Discussion

The way anthropogenic disturbance facilitates the success of non-native species is by now a well-known phenomenon (Fuentes-lillo et al., 2021; Lembrechts et al., 2016) and the results of our plant survey align with these expectations. The higher species richness and overall vegetation cover of non-native species in the disturbed roadside plots we observed matches previous observations, in this system and across other mountain systems globally (Iseli et al., 2023; Lembrechts et al., 2017; S.E. Smith, 2008). Those studies show mountain roads to be hotspots for non-native species and generally potent starting points for potential invasions. However, here we did not observe non-native species to be invading the natural vegetation from the roadside as had been observed in these studies, instead non-native species remained highly restricted to the roadside and were still seldom found in the adjacent natural vegetation, without any noticeable changes in the ten last years. This shows that while primary invasion in disturbed sites is possible in cold-climate mountainous systems, it does not seem to be followed by secondary invasion as has been observed in other mountain regions (McDougall et al., 2018). Likewise, the observed pattern of higher AM fungi colonisation in the roadsides plots (Table **5.1b**, Fig. **5.4**) is in line with results from previous studies. Indeed, we have observed previously in this system that roadside disturbance leads to a drastic increase in AM fungi abundance and diversity (Clavel et al., 2020) and at a more global scale that vegetation along roadsides tended to include more AM-associated plant species to the detriment of EcM or ErM plants species (chapter 3). Both the higher non-native success and increased AM colonisation make sense in the high latitude and acidic-soil ecosystems that are most common in the northern Scandes. Indeed, road disturbances are known to substantially alter the physical and chemical properties of mountain soils in favor of non-native and AM-associated plant species, with for example increases in pH and higher nutrient availability (Müllerová et al., 2011). Both AM-associated non-native species and AM fungi themselves thus highly benefit from roadside disturbance but cannot overcome the barrier posed by the natural ecosystem’s conditions and therefore remain for now limited to disturbed sites.

Contrary to our expectations, we did not observe an upward shift in the non-native species’ upper elevational limits. Studies in other regions have often found that non-native plant species after being introduced in the lowlands tend to extend their elevation range over time, either filling in their potential range or being actively helped by climatic shifts (Iseli et al., 2023; Pauchard et al., 2009; Petitpierre et al., 2015). This does not seem to be the case in our system, at least not within the decadal time frame of observations, as almost all non-native species we observed fluctuated around their original observed maximum elevation from 2017. While we did observe some fluctuations for specific species those were less common ones for which these patterns are better explained by stochastic changes than by an intrinsic change in range. Furthermore, our three focal species, which are the most abundant non-native species in the system, saw no increase in their maximum elevation across years.

However, we know from previous studies in the region (Lembrechts et al., 2016, Chapter 3) that these species are capable of successful germination and growth when introduced at higher elevations than those observed along these roads. A possible explanation to this discrepancy would be that while short term success is possible for these non-native species above their current range, fluctuating climatic conditions would prevent self-sustaining populations, meaning that the current upper range of non-native species we observed corresponds to the limit where these species can establish but are not able to then disperse further upwards (Barni et al., 2012). This could be caused by a number of factors such as for example growing seasons that are too short to produce viable seeds, lack of specific biotic interactions or deaths of seedlings due to cold events (Louthan et al., 2015; Rumpf et al., 2018). We also cannot discard the possibility that a ten-year period is too short to see meaningful changes in elevational ranges given the highly variable nature of disturbed roadsides, although such time period was sufficient to observe significant shifts in non-native plant species elevational range limits in other mountain regions worldwide (Iseli et al., 2023). Regardless, we can rather safely assume that there currently is no rapid elevational increase in the distribution of non-native plant species along mountain roads in this Arctic mountainous system.

While upwards movement of non-native species across our study period was limited, across the range where they were already established their share of the total vegetation has been increasing and total non-native richness also increased as six species that were absent during the first step of the study were present by the end of it. All these new non-native species, except for *Poa trivialis*, were present only at lower elevations and in small number suggesting that they were indeed new introduction to the systems and not simply already established rare species that might have been missed during the first survey as they would otherwise likely be found at higher elevation. The fact that no species were only found during the first two surveys but not in the latest one also reinforces this conclusion. These results would suggest that while non-native species that are already present in the system might have reached their current upper elevational limit, they are still in the process of filling in their niches across the range in which they are already present by supplanting native species along roadsides over time. The introduction of new non-native species in the lowlands also suggest that these plots are not in a state of equilibrium and became more suitable to non-native species across the ten-year period of our survey. Notably, while there was on average an increase in total non-native cover over the 10-year period of our survey, it was not a linear increase as we did observe an increase between 2012 and 2017 followed by a small decrease between 2017 and 2022 which did however remain higher than the original levels of 2012 (Fig. **5.2**). This speaks to the variable nature of roadsides and the limitations of a three time-step survey which can be strongly influenced by random events. More specifically in our case, heavier road maintenance than usual was performed in 2021 and to a lesser degree in 2022 before our survey seasons through the addition of gravel and mowing, which is likely to partially explain the fluctuations we observed in non-native cover as total vegetation cover was overall decreased from these heightened disturbed conditions. We also know from a previous study on historical data from the nearby area surrounding Abisko, Sweden, that many of the non-native species we observed have been present in the region for long periods of time, with recorded evidence of their presence going back to the early 20*th* century (Wiegmans et al., 2023). It is possible that these species have already reached an equilibrium and that they fluctuate following the intensity of disturbance, nevertheless the fact that we observed both increases in the total non-native species richness and AM fungal root colonisation in addition to the increased cover of non-native species over the survey period suggest that there is more to the observed patterns than simple stochastic variation. These considerations are the reason why repeated measurements and pooling observation from local surveys such as ours, as is the goal of the MIREN network, is so crucial in detecting and quantifying larger-scale systemic patterns (Haider et al., 2022; Iseli et al., 2023). In short, while the observed increase in non-native cover in the roadsides could be part of a management-related cyclical pattern, it did follow our expectations.

AM fungal colonisation in the roots of background samples also increased over time along the roadside. This effect was consistent across all three years of the survey from 2017 to 2021 which interestingly did not align with the changes in non-native species cover which saw a slight decline between 2017 and 2022. While the end of the surveys being desynchronized by one year due to practical limitations makes this comparison less clear, both 2021 and 2022 saw a higher degree of road maintenance and disturbance compared to previous years which seemingly had different consequences for non-native plant cover and AM fungal root colonisation. This is noteworthy as an important question that arose in our 2017 study was that of the relationship between increased non-native success in the roadside and increased AM fungi presence and whether AM fungi are driving increased non-native success or increased non-native success is driving higher AM fungi abundance (Clavel et al., 2020). While answering this question through observation alone remains difficult our results give us a few interesting patterns that could help further our understanding of this relationship. Indeed, we found no correlation between higher degrees of non-native presence in 2017, at the start of the AM fungi survey, and the speed at which AM fungal root colonisation increased, with elevation being the overall main driver. We also observed no overall increase in the degree of root colonisation amongst our three focal species and, when taken separately, only the AM facultative species *A. millefolium* showed an increase in colonisation over the years. These two results, alongside the aforementioned mismatch of changes for AM fungi colonisation and non-native success from 2017 to 2021 and 2022 respectively, seem to suggest that the increase in AM fungi colonisation we observed in road backgrounds is unlikely to be primarily driven by the increase in non-native species. The reverse is more likely, in that roadside conditions are generally more favorable to AM-associated plant species and to AM fungi through drastic abiotic and biotic changes such as increased pH, higher nutrient availability, or reduced competition (Hansen & Clevenger, 2005; Müllerová et al., 2011). Non-native species would then benefit from this increased AM fungi availability in roadsides. While speculative, this explanation does match previously observed patterns (chapter 4) and could justify further research as a potentially important mechanism for the effect of anthropogenic disturbance on non-native plant success, especially in heavily EcM and ErM-dominated cold-climate systems where AM fungi availability might be a real limitation. This increase in AM fungi presence could potentially also help explain the increase in non-native species richness we observed between 2012 and 2022 (from 17 to 23 non-native species) as these were all AM-associated species.

Overall, our results show that while they currently remain limited to roadsides and lower elevations, non-native plant species in the region are slowly increasing their share of the total vegetation. Likewise, increasing proportions of AM fungi colonisation seem restricted to roadsides and low elevations. We can therefore assume that the Northern Scandes mountains are currently not under immediate threat from the spread of non-native species, unlike many other mountain regions of the globe with substantially higher levels of invasion and significant increases in upper limits and richness (Iseli et al., 2023). However, we know from other cold-climate systems that with increasing effects from climate change and other anthropogenic pressure there is always a risk of reaching a breaking point beyond which vegetation shifts become substantial (Pearson et al., 2013; Vowles & Björk, 2019). Therefore, while currently under little threat from non-native invasions, it is important to keep consistently monitoring these systems to be able to predict and prevent potential future threats.

CHAPTER VI

General discussion

6.1 Overview

In the context of increasing impact of non-native species invasions on ecosystem functioning, services and biodiversity at a global scale it is crucial for ecologist to better understand how these species can establish and naturalize in order to inform adequate management practices (Roy et al., 2023). The role of below-ground interactions in this process, and more specifically that of mycorrhizal symbiosis, has been garnering an increasing amount of attention and research over recent years has made clear that these associations can play pivotal roles in the success of invasive plant species (Aslani et al., 2019). These invasions are also known to be strongly associated with anthropogenic disturbance in as non-native plants can benefit from disturbance events to establish and spread (Fuentes-lillo et al., 2021; Lembrechts et al., 2016). While these two drivers are well understood to be key predictors of non-native plant invasion success when taken individually, knowledge is lacking on what implications their interaction could have for the trajectories of non-native plant invasions.

Therefore, one of the central topics of this thesis is to investigate the relationship and possible interaction between anthropogenic disturbance and mycorrhizal symbiosis and assess the ensuing consequences for the vegetation, both native and non-native, with a focus on mountain and cold climate ecosystem as these environments are expected to see a strong increase in non-native pressure in the near future (Alexander et al., 2016). To achieve this goal, we approached this topic through four different angles: 1) A regional survey of plants and their associated AM fungi along roads in the subarctic mountains of the northern Scandes, 2) A global survey of vegetation along mountain roads using mycorrhizal type associations to investigate broad patterns of road disturbance impact on mycorrhizal interactions across regions, 3) An in situ experimental approach in cold climate mountains by sowing non-native plant species under different combinations of disturbance associated factors to assess the impact of these individual factors on fungal communities and on the success rate of non-native plant establishment, and 4) Repeated mycorrhizal and plant surveys along the roads of the northern Scandes, over 6 and 10 years respectively, to assess their evolution over time and the implications for future potential plant invasions in cold-climate mountain systems. Our results across all four approaches show a consistent effect of disturbance on the mycorrhizal associations as it promoted AM fungi across the board and conversely had a detrimental effect on EcM and ErM fungi. In turn this shift in the mycorrhizal community has the potential to facilitate the success of non-native plant species, however this pattern is less clear and more context dependent.

6.2 Impact of disturbance on mycorrhizal associations

Literature on the effect of disturbance on mycorrhizal fungi in natural habits is quite rare (Brundrett & Ashwath, 2013; García de León et al., 2018; Trejo et al., 2016) as most studies are focused on the role of disturbance in agricultural contexts (Goss & De Varennes, 2002; Jansa et al., 2006; Kabir, 2005; Schnoor et al., 2011). These studies in agricultural settings widely report reduced diversity and abundance of AM fungi following physical disturbance related to tilling (Jansa et al., 2006; Schnoor et al., 2011), albeit in systems that are typically species poor, often monocultures, and nutrient rich making extrapolation to natural habitats tenuous. Nevertheless, those studies that do address the effect of disturbance on mycorrhizal fungi in natural habits tend to observe negative consequences for AM fungi abundance and diversity (Brundrett & Ashwath, 2013; Sharmah & Jha, 2014) more often than positive (Picone, 2000) or neutral consequences (Lekberg et al., 2012). This diversity in observed patterns is likely due to different disturbance types not being equally disruptive for AM fungi and different AM fungi families having differential disturbance tolerance as observed by van der Heyde et al., 2017In this context, our results could come as somewhat surprising as we observed general trends of increased AM fungi success following anthropogenic disturbance. From chapters 2 and 5 we observed a net increase in both AM fungi abundance, through root length colonised (Fig. **2.2** & **5.4**), and AM diversity in the roots of disturbed vegetation along mountain roads of the northern Scandes (Fig. **2.3**). In chapter 3 we also saw a net average increase in AM-associated vegetation in disturbed environments along mountain roads across multiple regions (Table **3.2**). However, what these results reinforce is the context-dependent nature of the effect of disturbance on mycorrhizal associations. Indeed, our studies mainly focus on one type of disturbance vector, road associated disturbance, on mountain environments specifically and, aside from chapter 3, on a subset of mountain environments within sub-arctic mountains.

Roads are a complex source of disturbance as they combine multiple axes of impact on the environments surrounding the road (Müllerová et al., 2011). Physical disturbance is the most obvious of these axes as road construction and maintenance leads to vegetation removal and stirring of the soil which is known to favor ruderal plant species (Catford et al., 2012; Lembrechts et al., 2014) and on its own could be expected to negatively impact AM fungi (van der Heyde et al., 2017). We did observe such a pattern in chapter 3 (Fig. **3.2**) specifically in lower elevation and higher temperature plots where the proportion of total vegetation cover associated with AM fungi was observed to be lower in disturbed sites compared to the adjacent vegetation. However, this pattern was only seen in this sub-selection of plots as with higher elevation and colder temperatures it quickly reversed, with AM-associated vegetation being higher in the disturbed roadside. This, combined with the results of chapter 2 and 5 regarding increased AM fungi abundance and diversity along roadsides, points to the presence of other factors than physical disturbance linking road disturbance and mycorrhizal changes. Indeed, multiple other factors are associated with road disturbance such as changes in nutrient availability or pH linked to construction material (Müllerová et al., 2011), as illustrated in chapter 2 (Fig. **S2.2**), which could be expected to have inverse effects to that of physical disturbance as AM fungi thrive better in more neutral pH conditions and are best at extracting less recalcitrant nutrients compared to other mycorrhizal types (Soudzilovskaia et al., 2015; Steidinger, Liang, et al., 2019).

Our studies taking place in mountain ecosystems is also an important distinction from other research on the effect of disturbance on mycorrhizal fungi as said research has, to the best of our knowledge, always been conducted in lowland conditions, and generally in warm to temperate climates (Brundrett & Ashwath, 2013; García de León et al., 2018; Trejo et al., 2016). As such it stands to reason that the discrepancy between the most often negative effect of disturbance on AM fungi seen in literature and the overall positive effect we observed across our studies is, at least in part, caused by the stark differences in biotic and abiotic factors brought about by mountainous conditions. Indeed, both plants and fungi species in mountains are more adapted to extreme climatic conditions with low temperatures and shorter growing seasons and to lower resource abundance, especially when comparing the higher parts of the elevation gradient with lowlands (Kumar & Vats, 2017). Therefore, the disturbance effect or roads is bound to affect mountain systems differently than lowland ones; soil pH for example is known to be generally lower in mountains compared to global averages (Badía et al., 2016; J. Smith et al., 2002) which in turn makes the higher pH in roadsides due to construction materials a starker effect than it would otherwise be in less acidic conditions, and in turn would be a positive driver for AM fungi and AM plants which prefer more these neutral pH conditions (Liu et al., 2018). This was clearly illustrated in chapter 2 as we measured pH to be much higher in the roadsides than in the adjacent natural vegetation (Fig. **S2.2**) and to be a strong predictor of increased AM fungal root colonisation rate (Table **2.1**). Roads in mountains are also known to alter the temperature in their surroundings, which could also play an effect in facilitating the success of AM plants an AM fungi (Duarte & Maherali, 2022). This contrast between mountain and lowland systems is again shown in the fact that the positive effect of disturbance on AM-associated vegetation observed in chapter 3 is strongest at high elevation and not present at the bottom of the elevation range.

It is also important to realize that most of the studies that looked into the effect of disturbance on mycorrhizal fungi, including ours for both chapter 2 and 5, do so by focusing on AM fungi. As AM fungi are known to associate with more than 70% of all terrestrial plant species (Wang & Qiu, 2006), and are therefore dominant in a majority of ecosystems, they are bound to be the most significant form of mycorrhizal associations when aiming to predict vegetation distribution and community composition and in turn are the most sensible mycorrhizal type to focus studies on. In our case AM fungi changes are also the most relevant as the vast majority of non-native plant species are AM-associated of NM (Menzel et al., 2017). However, this bias leaves us with limited knowledge on the behavior of EcM or ErM symbiosis following anthropogenic disturbance which is problematic in cold-climates and in some high-elevation ranges which are dominated by EcM and/or ErM plants (Barcelo et al., 2019) as competitive release of AM fungi and AM-associated species following declines in EcM/ErM fungi and ErM/EcM plants could be an important factor in explaining the impact of anthropogenic disturbance in these environments. This is why we decided to shift away from focusing on AM fungi exclusively after chapter 2 and towards including all mycorrhizal types in chapter 3 and the whole fungal community in chapter 4. We did find in chapter 4 that both physical disturbance and increased nutrient availability led to reduced EcM relative abundance and that disturbance also led to reduced EcM fungal diversity. Furthermore, we observed in chapter 3 (Table **3.2**) that EcM and ErM-associated vegetation was on average less abundant in disturbed roadsides than in the natural vegetation. This was not the case for AM-associated vegetation which did increase in disturbed roadside and did so especially strongly in regions with higher representation of EcM or ErM-associated vegetation. These results combined seemingly point towards anthropogenic disturbance having a greater impact on EcM and, to a lesser degree, ErM fungi and associated plants, therefore reinforcing the idea that competitive release is likely to be part of the drivers behind increased AM fungi and AM-associated vegetation success following road disturbance. It is however difficult to conclude from our results whether this would be caused by release from EcM fungi directly in favor of AM fungi or if what we have observed is a competitive release of AM plant species from more locally adapted EcM plant species.

Given the numerous factors involved in road disturbance we did not expect the uniform results of chapter 3 in which vegetation mycorrhizal type changes followed similar patterns across all 11 regions with road disturbance correlating with both increases in the percentage of total vegetation associated with AM fungi and decreases of EcM- and ErM-associated total vegetation when present (Table **3.2**). While it is important to not assume that changes in vegetation types correlate one to one with changes in the mycorrhizal fungi themselves (see 6.5), this clear pattern points to a seemingly universal component to the effect of road disturbance in mountain systems. As described previously, a possible explanation to this phenomenon would be that AM fungi and AM plants are the least sensitive amongst mycorrhizal types to the disturbance associated with roads, i.e. physical disturbance favoring ruderal species, changes in soil properties towards higher pH and nutrient availability and increases in temperature (Müllerová et al., 2011). This idea would also explain the reversed pattern we observed in the low-elevation plots of certain regions (Argentina, Central Chile and India) where the native vegetation is almost entirely AM-associated and where total percentage of AM-associated plant cover was lower in disturbed roadside plots. Under this mechanism, the AM plants in these plots would not benefit from reduced competition from plants associated to other mycorrhizal types and the only remaining effect would be the negative consequence of disturbance that has been observed in other studies in lowland environments (Brundrett & Ashwath, 2013; Sharmah & Jha, 2014). However, it is important to keep in mind that these global patterns are gleaned from using mycorrhizal types as a proxy for mycorrhizal fungi which is an imperfect approach, meaning that outside of large-scale patterns our interpretations of these results regarding potential mechanisms remain only informed guesses that would require to be confirmed by further studies. Regardless of these limitations in our understanding of the underlying mechanisms, it remains clear from our results both in the Northern Scandes and using the MIREN dataset that mountain roads have a significant effect on mycorrhizal symbiosis that has the potential to shape vegetation distribution (Dickie et al., 2017).

6.3 Case study of the northern Scandes

Most of our research was carried out in the sub-arctic mountain system of the northern Scandes. A review of historical plant surveys from 1903, 1914 and 1983 along the "Rallarvagen", a trail established alongside the construction of train tracks in Abisko, Sweden and later along the E10 highway in the 1980s, was conducted in 2021 alongside an additional resurvey of this trail and showed that non-native plant species have been present in the region for more than a century following anthropogenic activity (Wiegmans et al., 2023). The mountain roads we investigated in Norway as part of the MIREN protocol are more recent as they were built in the

1980s (around the same time frame as the E10 construction) and we do not have vegetation data of the early stages of disturbance (Lembrechts et al., 2014). However, the vegetation along these roads has been monitored since 2012 at which time multiple non-native plant species were found in the disturbed roadsides (Lembrechts et al., 2014) and as the climate and vegetation types in both the regions of Abisko and Narvik are highly similar it stands to reason that the pattern of non-native plant species establishing soon after construction observed in Abisko was similar along the MIREN roads. Amongst the 23 non-native species matching this definition that we observed to be present along the mountain roads in Norway, three species in particular stood out as being most consistently the most widespread and most abundant across all repeated surveys: *Trifolium repens, Trifolium pratense* and *Achillea millefolium*. Other non-native plant species present in the system were either largely restricted to the bottom of the elevation range or represented by only small proportions of total vegetation along disturbed roadsides whereas the three aforementioned species, henceforth referred to as focal species, often represented a high percentage of the total roadside vegetation. Notably, two of these most common non-native species are also known to have N-fixing capabilities which could help explain their success in this generally nutrient poor environment.

Non-native plant species along mountain roads have been seen to be in the process of expanding upwards across multiple regions (Barros et al., 2023; McDougall et al., 2018), both by following climate change but also often simply as recent introductions filling their potential range (Iseli et al., 2023). However, contrary to our expectations we did not observe an upward trend in the distribution of most non-native plant species along the mountain roads of the northern Scandes over the years with the notable exceptions of *Ranunculus repens* L. and *Plantago major* L. (Table **S5.1**). Therefore, it would seem that most non-native plant species in the region have already reached their maximum elevational range for the current climatic conditions as their introduction to the area is relatively old with most non-native species, including our focal species, having been present in the region for at minimum a century (Wiegmans et al., 2023). Nevertheless, while we did not observe any clear changes in the maximum elevation of non-native species in the region our results still point towards the system not being at an equilibrium as both non-native species diversity and overall abundance have seen an increase over the 10-year frame of our survey. More specifically, the percentage of the total roadside vegetation composed of non-native species has been increasing over time to the detriment of native roadside species.

We did also observe changes in AM fungi abundance over a shorter 6-year period with the AM fungal colonisation in the roots of background samples increasing over time and especially at lower elevations (Fig. **5.3**). It is also notable that non-native plant species are unlikely to be limited in their elevational range in roadsides by a lack of adequate mycorrhizal fungi availability as AM fungi were found to be present across the whole elevation gradient with only a small effect of elevation on both abundance and community composition (Fig. **2.4**). The three AM-associated focal species were all found to be able to form associations with the most common AM fungal OTUs which were already present in the native vegetation and across the whole elevation range (Fig. **2.5**). Furthermore, these non-native focal species were seen in chapter 4 to be able to establish successfully when planted above their current maximum elevational range and in a very AM-poor environment (Fig. **4.2**). However, the fact that these non-native plant species could establish and survive in the context of a one-year experiment does not necessarily mean that they would be able to establish long lasting populations across the whole elevational gradient. Both the AM fungi and the plants seem to be in the process of continuing to fill their current range, and further warming may be required to extend that range beyond its current limits.

Alongside increases in AM fungi abundance and non-native plant cover in lower elevation roadside plots we also observed an increase in non-native species richness with six more non-native species having been found by the end of the survey in 2022 compared to 2012, and all of the non-native species present in 2012 were still found in 2017 and 2022 suggesting that we did observe general enrichment and not merely species turnover (Table **S5.1**). These species (possibly with the exception of *Poa trivialis* L.) all appear to be new introductions as they were only observed at low elevation and in small numbers. Therefore, while the situation of non-native plant spread in the northern Scandes is seemingly stable with their overall distribution not evolving much over the last 10 years, be it elevation wise or spreading into the natural vegetation, we can see that non-native presence is on the rise in plots subject to anthropogenic disturbance with an increase from 17 non-native species in 2012 to 23 species in 2022. Yet, it still seems unlikely that the region could be under threat from non-native plant invasions in the short term as the harsh environmental conditions form a strong barrier to their spread (Petitpierre et al., 2015) and the incremental changes along roadsides we observed were slow and still limited to these highly disturbed environments. In a broader scope our results do support the idea that road disturbance is a strong diver of non-native plant success as even in such an otherwise inhospitable environment non-native plants were still highly common in disturbed roadsides (Fuentes-lillo et al., 2021; Fuentes-Lillo et al., 2021). It stands to reason, and has been observed in other regions, that in environments less resistant to invasions and with increased rise in temperature and anthropogenic activities, these types of disturbance will be the starting points for potential new plant invasions (McDougall et al., 2018b). Roads and other similar sources of disturbance should therefore be focal points for monitoring and managing non-native plant establishment if we want to be able to address plant invasions before they become unmanageable (Iseli et al., 2023).

6.4 Mycorrhiza disturbance and non-native plants

One of the central questions of this thesis is whether the impact of disturbance on mycorrhizal fungi plays a role in facilitating the success of non-native species. From previous research and from our own results it seems clear that disturbance does have an effect on both non-native plants and mycorrhizal fungi separately (Meyer et al., 2021; van der Heyde et al., 2017). It is well established that disturbance is a crucial driver of non-native plant invasion and especially so in mountains (Fuentes-lillo et al., 2021; Lembrechts et al., 2016) which we have also observed across our results. We have also established that disturbance in mountains and especially road disturbance generally favors AM fungi and AM plants to the detriment of EcM and, to a lesser degree, of ErM fungi and associated plants. Furthermore, we have observed that AM fungi abundance was higher in plots where non-native plant species were present than in ones with only native vegetation. However, disentangling how these two factors interact and potentially influence each other is less straightforward.

From our results in chapter 4 we saw that both obligatory (*Trifolium pratense* and *Trifolium repens*) and facultative (*Taraxacum officinale* and *Achillea millefolium*) AM-associated non-native species (Wang & Qiu, 2006) were capable of successfully establishing themselves across all treatments despite the environment the experience took place in being very AM poor (Fig. **4.3**). We also know from chapter 2, as well as from literature (Davison et al., 2015), that AM-associated species, especially non-native species, and AM fungi are rarely specific in their associations. Indeed, our three focal non-native species were found to be able to associate with OTUs of AM fungi which were present across the elevation gradient and in both disturbed and adjacent vegetation (Fig. **2.5**). From this, we see that AM-associated non-native plant species in this system are seemingly not limited in their establishment potential by a lack of AM-fungal abundance or of specific AM fungi. Theoretically, a total absence of AM fungi would be a limiting factor for obligate AM species, nevertheless in practice AM fungi are present in almost all environments even if in low abundance (Smith & Read, 2010) including in the highly EcM and ErM dominated northern Scandes where we have observed a number of native AM plants and root colonisation in the natural background samples. However, while high AM fungi abundance is not a necessity for establishment success it does not mean that AM-associated non-native plants do not benefit from increased AM fungi abundance or diversity (Aslani et al., 2019; Greipsson & DiTommaso, 2006; Menzel et al., 2017). As we saw in chapter 4, non-native species are most successful in regions heavily dominated by AM-associated native plants. We also know from literature that AM symbiosis is generally an advantage and not a barrier for non-native plant success as AM-associated non-native species are generally more successful than non-mycorrhizal ones (Menzel et al., 2017). From these patterns it seems logical that the impact of anthropogenic disturbance on mycorrhizal fungi, and more specifically in favor of AM fungi, could play a role towards increased success of non-native plant species following said disturbances.

From our observations across chapter 2, 4 and 5 we have seen that AM fungi abundance and diversity as well as the proportion of AM plants were higher in disturbed roadsides regardless of the presence of non-native plants, making it clear that this disturbance effect is beneficial for all AM plants and not only non-native ones. Furthermore, we saw in chapter 5 that increases over time in AM fungi abundance were not correlated with non-native plant species presence as the speed of abundance increase over the 6 years of the study was not faster in plots more heavily colonised by non-native plants at the start of that period. The degree of colonisation by AM fungi in the root of non-native plants also did not increase over this same period while that in roadside background roots did. In general, our results seem to point towards non-native plants in our system not playing much of a role in driving increases in AM fungi abundance on their surroundings once established. This is the case despite a large proportion of the non-native population along these roads being *Trifolium* species which one could expect to influence their surroundings through their N-fixing capabilities towards a more fitting environment for AM fungi and AM-associated plants. One exception to that pattern are the rarer AM fungi OTUs observed in chapter 2 which seem to be only associated with the focal non-native species and were not found in the background samples, which could point to non-native species having an effect on AM-fungal diversity and potentially to co-introduction of non-native AM fungi alongside non-native plant species (Moeller et al., 2015). However, as we have seen previously these are not required for the success of these non-native species. Overall, it appears that road disturbance drives increases in AM fungi separately from a specific effect of non-native species. Rather, road disturbance is beneficial for all AM-associated plant species in general leading to higher proportions of the vegetation being AM-associated an in turn increasing abundance of AM fungi in the disturbed environment which then would have beneficial effects for non-native species, especially in EcM and ErM dominated systems. It is however important to keep in mind that AM associated non-native plants, i.e. most non-native plants (Menzel et al., 2017), will also participate in this effect as their presence will most often mean an increase in the proportion of AM-associated vegetation. There is therefore a possible self-reinforcing effect of plant invasions for systems which are not already dominated by AM-associated vegetation, as disturbance facilitates AM fungi success, AM-associated non-natives can establish in otherwise unhospitable environments and in turn shift the mycorrhizal community towards being more AM dominated and ultimately shifting conditions to being more favorable for further non-native plants success (Shah & Reshi, 2009). This is possibly what we are in the process of observing in the Northern Scandes as we have seen that AM fungi abundance and non-native species abundance have been rising over time in lower elevation disturbed roadsides which have also seen new non-native introductions over the last 10 years. This does point to the possibility that these plots are becoming increasingly welcoming for non-native species which could previously not establish in the earlier stages of disturbance. It does remain unlikely in the Scandes that this shift along roadsides could translate to non-native species moving towards the natural vegetation as we have seen that the barrier for non-natives to spread without active disturbance is very high. However, this process could potentially participate in facilitating plant invasions in a less extreme or more disturbed ecosystem .

6.5 Limitations and future prospects

A limitation of this thesis is that our measurements of mycorrhizal fungi were restricted to the one region of the northern Scandes. While access to the MIREN dataset of plant distribution along mountain roads did allow us to confront our local findings to patterns in other regions it is important to acknowledge the intrinsic limitation in using mycorrhizal type amongst plants as a proxy for mycorrhizal fungi. Indeed, aggregated datasets are by their nature subject to accumulating smaller errors resulting in imprecisions and concerns about the methodology involved in FungalRoot in particular have been raised since the publication of the database (Bueno, Davison, et al., 2021; Soudzilovskaia et al., 2022). Therefore, we limited our use of this approach to only very broad categories and only retained conclusions that were strongly supported (such as the uniform effect of mountain roads on AM plants). Consequently, as useful as this method is to investigate broad patterns it is not applicable for smaller scale effects and to understand the mechanisms linking disturbance, mycorrhizal fungi and non-native species. It is also important to keep in mind that the northern Scandes are on the extreme end of an ErM and EcM dominated ecosystem as we saw in chapter three that it is the only region in the MIREN dataset where AM plants do not represent de majority of the total vegetation. This characteristic made the region a good testing ground for the impact of disturbance on a very inhospitable system for non-native plants, showing that disturbance can facilitate non-native establishment even in such extreme conditions. However, it also means that we have to be careful when extrapolating our results to other regions. We did see in chapter three that the broad effect of roads we observed in the northern Scandes was similar across regions, but we cannot make such assumptions about other specific patterns we observed there. For example, the fact that non-native species are strictly restricted to disturbed sites and show no sign of spreading towards the natural vegetation despite a high and increasing presence in the roadsides might not be true in another mountain system. If we want to extrapolate our regional findings to a more global pattern, the only clear approach would be to conduct actual mycorrhizal sampling in other regions with different environmental conditions and mycorrhizal type makeup. Again, the MIREN network would be an ideal framework to pursue this approach as an addon to the core protocol since we could then use the resulting mycorrhizal data in the context of corresponding long term vegetation surveys (Haider et al., 2022).

Another limitation of our approach, and of a large part of the literature on mycorrhizal interaction and disturbance, is the lack of focus on mycorrhizal types other than AM (van der Heyde et al., 2017). As AM-symbiosis is by far the most common and widespread type of mycorrhizal symbiosis (S.E. Smith, 2008) it is also the most logical one to focus one when trying to understand large trends in mycorrhizal symbiosis, and even more so when focusing on non-native plant species as we have seen that they are almost exclusively AM-associated (Menzel et al., 2017). However, we argue that it could be equally important in environments were EcM or ErM plants are a significant part of the ecosystem to understand how these relationships are altered. Indeed, changes in EcM or ErM fungi are bound to also be linked to changes in AM-associations, possibly through reduced competition from diminished EcM and ErM fungi and associated plants. Generally, we believe addressing all present mycorrhizal types together instead of only focusing on AM fungi should be the way forward when trying to assess impact of disturbance, or more broadly of external factors, on mycorrhizal fungi in a natural environment. Furthermore, and depending on the specific question asked, it could also be beneficial to extend this approach to include all fungi known to interact with plants as was done in chapter 4 since mutualistic relationships are only one side of the coin. For example, negative effects of pathogenic fungi may be equally important as the beneficial effects of mycorrhiza and could be especially important for a better understanding of non-native plant invasions (Dickie et al., 2017). Also worth noting is the fact that we have largely not included orchids and orchid mycorrhizas in our analysis, which was a deliberate choice either because the occurrence and abundance of orchids was too low to be relevant for the questions we were asking (chapters 2, 3 and 5), or in the case of chapter 4 because orchids were entirely absent from the experimental sites and their surroundings.

In chapter 4 we encountered an issue with our AM fungi inoculum approach. The aim was to extract AM from disturbed lower elevation plots with high densities of AM-associated non-native species and transplant them in association with non-native plant species' seeds at different elevations to test whether higher AM abundance and diversity was a driver in the success of non-native plant species in disturbed environments. To this effect the soil from these low elevation plots was mixed with sterile water to obtain a microbial wash which was then separated in two batches, one left untouched and the other filtered out to eliminate AM fungi and their spores. These two batches where then separately applied to sterilized soil from the same origin as the microbial wash and the resulting soil was applied as treatment to the seeds planted in the experiment (Koide & Li, 1989; Schnitzer et al., 2011). The goal was to isolate AM fungi presence as the only difference between the two levels of the treatment while otherwise maintaining the microbial community and the abiotic characteristic of the added soil identical. However, we ultimately failed to see any effect of this treatment on non-native plant establishment success or biomass production. This in itself would not have been a concern as a negative result was not entirely unexpected, however we also observed no clear difference in fungal community composition between the treatments with and without AM fungi which is particularly noticeable as the environment was originally very poor in AM fungi. In retrospect, the two inoculums should have been tested separately for AM abundance and community composition before their addition to the experimental setup. As it stands, it seems that our approach to test the effect of increased AM-abundance did not alter the said abundance meaningfully enough to draw definitive conclusions from our results. In the future, such an experiment should either be conducted in a more controlled environment and/or with a stronger source of AM-inoculum.

We also have to address that comparing and interpreting repeated measurements of disturbed systems such as roads can be difficult due to the fluctuations inherent to the stochastic nature of disturbance events, which make disturbed environments often unstable (Fraterrigo et al., 2020; Turner, 2010). As we mentioned in chapter 5, the roads we were studying in the northern Scandes were subject to heavier maintenance through intense mowing and gravel addition in 2021 than in previous years, most likely due a lack of maintenance the year prior during the COVID pandemic. As a result, we observed stagnation or even in some plots a reduction in non-native plants species abundance that is unlikely to be part of a long term downward shift. This issue could be mitigated through more regular surveys so that one outlier year could more easily be identified as such. Similarly, we observed fluctuations in the maximum elevation of some less common non-native species which in all likelihood is again a consequence of stochastic events and of the discrete nature of our sampling, as plots can only cover limited areas and are bound to arbitrarily miss rarer species (Jeliazkov et al., 2022). These limitations are inherent to any observational approach and can take any form of random variation, be it climatic, biotic or anthropogenic and can only be accounted for through regular repeated and diversified measurements, which is why long-term surveys such as conducted throughout the MIREN network are so essential in improving our understanding of natural processes (Haider et al., 2022).

6.6 Conclusion

This thesis brings new insights to the field of linked plant-fungi invasions through a focus on mountain ecosystems and on the consequences of anthropogenic disturbances at both local and global scales. Through observational and experimental approaches, we have shown that anthropogenic disturbance in mountain ecosystems disrupts mycorrhizal associations by promoting AM fungi to the detriment of EcM and ErM fungi. This effect is especially strong in colder climates and at higher elevations, where AM-abundance is typically the lowest, but less noticeable or even detrimental to AM-associated vegetation at lower elevations and in warmer environments most dominated by AM-associated plant species. Our case study in the Northern Scandes reinforces this conclusion as we observed strong increases in AM fungi abundance and diversity following disturbance caused by mountain roads in an EcM and ErM dominated system as well as a decrease in EcM relative abundance following both physical disturbance and nutrient addition. Our research also reinforces the knowledge that anthropogenic disturbance is a key driver of non-native plant invasions, especially so in cold climate, by adding the mycorrhizal dimension to previous known drivers of non-native plant facilitation through disturbance. We have seen that through promoting AM plants and their fungi, anthropogenic disturbance participates in facilitating the establishment and continued success of non-native plants which most commonly form symbiotic relationships with AM fungi themselves.

Overall, our research reinforces the importance of monitoring anthropogenic disturbance in mountains and cold-climate environment as a source of disturbance for both above and belowground native species leading to potential future non-native plant invasions. We also highlighted the context dependent nature of the effect of disturbance on fungi-plant interactions which calls for further studies in a larger range of diverse ecosystems if want to ultimately be able to predict consequences of novel anthropogenic disturbances on plant-fungal communities and plant invasion trajectories.

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Supplementary material

Table S2.1: List of all analyzed root samples. Includes both background root samples and focal plant species samples. AM fungal root colonisation was obtained through microscope counting of arbuscular mycorrhizal structures. Plots are ordered by elevation for each road (R1, R2 and R3), codes summarize sample type and sample number or distance from the road.

Transect	Code	*Sample Type*	Distance from Road (m)	AM fungal root colon.	Elevation (m a.s.l.)
R1-1	DB 1	*Disturbed vegetation background*	0	20%	53
	DB 2	*Disturbed vegetation background pooled*	0	15%	53
	UB 5	*Undisturbed vegetation background*	5	0%	53
	RTR 1	*Focal Species: Trifolium repens*	0	85%	53
	RTR 2	*Focal Species: Trifolium repens*	0	99%	53
	RTR 3	*Focal Species: Trifolium repens*	0	69%	53
	RTP 1	*Focal Species: Trifolium pratense*	0	75%	53
	RTP 2	*Focal Species: Trifolium pratense*	0	53%	53
	RTP 3	*Focal Species: Trifolium pratense*	0	81%	53
R1-2	DB 1	*Disturbed vegetation background*	0	9%	211
	DB 2	*Disturbed vegetation background pooled*	0	15%	211
	RTP 1	*Focal Species: Trifolium pratense*	0	39%	211
	RTP 2	*Focal Species: Trifolium pratense*	0	26%	211
	UB 40	*Undisturbed vegetation background*	40	0%	187
	UB 5	*Undisturbed vegetation background*	5	0%	211
R1-3	UB 40	*Undisturbed vegetation background*	40	0%	310
	UB 5	*Undisturbed vegetation background*	5	0%	304
	DB 1	*Disturbed vegetation background*	0	12%	304
	DB 2	*Disturbed vegetation background pooled*	0	25%	304
	RSOL 1	*Focal Species: Solidago virgaurea*	0	0%	304
	RSOL 2	*Focal Species: Solidago virgaurea*	0	0%	304
R1-3’	RTR 1	*Focal Species: Trifolium repens*	0	26%	360
	RTR 2	*Focal Species: Trifolium repens*	0	59%	360
	RTR 3	*Focal Species: Trifolium repens*	0	80%	360
	RTR 4	*Focal Species: Trifolium repens*	0	43%	360
R1-4	UB 5	*Undisturbed vegetation background*	5	0%	404
	DB 1	*Disturbed vegetation background*	0	25%	404
	DB 2	*Disturbed vegetation background pooled*	0	30%	404

	RTR 1	*Focal Species: Trifolium repens*	0	47%	404
	RTR 2	*Focal Species: Trifolium repens*	0	57%	404
	RTR 3	*Focal Species: Trifolium repens*	0	27%	404
	RTR 4	*Focal Species: Trifolium repens*	0	84%	404
	NSOL 30	*Focal Species: Solidago virgaurea*	30	0%	407
	NSOL 50	*Focal Species: Solidago virgaurea*	50	8%	410
	RSOL 1	*Focal Species: Solidago virgaurea*	0	0%	404
	RSOL 2	*Focal Species: Solidago virgaurea*	0	19%	404
	RSOL 3	*Focal Species: Solidago virgaurea*	0	22%	404
	RSOL 4	*Focal Species: Solidago virgaurea*	0	7%	404
R1-5	UB 40	*Undisturbed vegetation background*	40	0%	502
	UB 5	*Undisturbed vegetation background*	5	6%	491
	NSOL 10	*Focal Species: Solidago virgaurea*	10	0%	500
	NSOL 60	*Focal Species: Solidago virgaurea*	60	8%	490
	DB 1	*Disturbed vegetation background*	0	0%	550
	DB 2	*Disturbed vegetation background pooled*	0	0%	550
R1-5’	RSOL 1	*Focal Species: Solidago virgaurea*	0	0%	550
	RSOL 2	*Focal Species: Solidago virgaurea*	0	20%	550
	NSOL 38	*Focal Species: Solidago virgaurea*	38	20%	589
	NSOL 65	*Focal Species: Solidago virgaurea*	65	0%	593
R2-1	RAM 1	*Focal Species: Achillea millefolium*	0	70%	9
	RAM 2	*Focal Species: Achillea millefolium*	0	20%	9
	RAM 3	*Focal Species: Achillea millefolium*	0	6%	9
	RAM 4	*Focal Species: Achillea millefolium*	0	11%	9
	RTP 1	*Focal Species: Trifolium pratense*	0	71%	9
	RTP 2	*Focal Species: Trifolium pratense*	0	29%	9
	RTP 3	*Focal Species: Trifolium pratense*	0	34%	9
	RTR 1	*Focal Species: Trifolium repens*	0	63%	9
	RTR 2	*Focal Species: Trifolium repens*	0	100%	9
	RTR 3	*Focal Species: Trifolium repens*	0	82%	9
	DB 1	*Disturbed vegetation background*	0	17%	9
	DB 2	*Disturbed vegetation background pooled*	0	38%	9
	UB 50	*Undisturbed vegetation background*	40	0%	14
	UB 5	*Undisturbed vegetation background*	5	5%	9
R2-2	RAM 1	*Focal Species: Achillea millefolium*	0	11%	153
	RAM 2	*Focal Species: Achillea millefolium*	0	13%	153
	RAM 3	*Focal Species: Achillea millefolium*	0	0%	153
	RTR 1	*Focal Species: Trifolium repens*	0	48%	153

	RTR 2	*Focal Species: Trifolium repens*	0	76%	153
	RTR 3	*Focal Species: Trifolium repens*	0	24%	153
	RTR 4	*Focal Species: Trifolium repens*	0	53%	153
	DB 1	*Disturbed vegetation background*	0	38%	153
	DB 2	*Disturbed vegetation background*	0	0%	153
	DB 3	*Disturbed vegetation background*	0	9%	153
	DB 4	*Disturbed vegetation background pooled*	0	12%	153
	UB 40	*Undisturbed vegetation background*	40	14%	160
	UB 5	*Undisturbed vegetation background*	5	16%	153
R2-2’	NAM 20	*Focal Species: Achillea millefolium*	20	0%	168
	NAM 3	*Focal Species: Achillea millefolium*	3	26%	173
R2-3	UB 40	*Undisturbed vegetation background*	40	0%	232
	UB 5	*Undisturbed vegetation background*	5	0%	235
	DB 1	*Disturbed vegetation background*	0	0%	235
	DB 2	*Disturbed vegetation background pooled*	0	10%	235
	RSOL 1	*Focal Species: Solidago virgaurea*	0	27%	235
	RSOL 2	*Focal Species: Solidago virgaurea*	0	12%	235
	RSOL 3	*Focal Species: Solidago virgaurea*	0	28%	235
	RSOL 4	*Focal Species: Solidago virgaurea*	0	14%	235
R2-4	UB 40	*Undisturbed vegetation background*	40	2%	371
	UB 5	*Undisturbed vegetation background*	5	0%	373
	DB 1	*Disturbed vegetation background*	0	0%	373
	DB 2	*Disturbed vegetation background pooled*	0	0%	373
	RSOL 1	*Focal Species: Solidago virgaurea*	0	0%	373
	RSOL 2	*Focal Species: Solidago virgaurea*	0	9%	373
	RSOL 3	*Focal Species: Solidago virgaurea*	0	0%	373
R2-4’	NAM 5	*Focal Species: Achillea millefolium*	5	21%	414
R2-5	UB 40	*Undisturbed vegetation background*	40	0%	410
	UB 5	*Undisturbed vegetation background*	5	5%	414
	RTR 1	*Focal Species: Trifolium repens*	0	54%	414
	RTR 2	*Focal Species: Trifolium repens*	0	70%	414
	RTR 3	*Focal Species: Trifolium repens*	0	44%	414
	RTR 4	*Focal Species: Trifolium repens*	0	66%	414
	DB 1	*Disturbed vegetation background*	0	16%	414
	DB 2	*Disturbed vegetation background pooled*	0	8%	414
R2-6	UB 5	*Undisturbed vegetation background*	5	2%	553

	DB 1	*Disturbed vegetation background*	0	5%	553
	DB 2	*Disturbed vegetation background pooled*	0	2%	553
R2-7	UB 40	*Undisturbed vegetation background*	40	41%	697
	UB 5	*Undisturbed vegetation background*	5	2%	672
	DB 1	*Disturbed vegetation background*	0	0%	672
	DB 2	*Disturbed vegetation background pooled*	0	14%	672
	RSOL 1	*Focal Species: Solidago virgaurea*	0	8%	672
	RSOL 2	*Focal Species: Solidago virgaurea*	0	8%	672
	RSOL 3	*Focal Species: Solidago virgaurea*	0	10%	672
	RSOL 4	*Focal Species: Solidago virgaurea*	0	4%	672
R3-1	RAM 1	*Focal Species: Achillea millefolium*	0	0%	13
	RAM 2	*Focal Species: Achillea millefolium*	0	0%	13
	RAM 3	*Focal Species: Achillea millefolium*	0	0%	13
	RAM 4	*Focal Species: Achillea millefolium*	0	90%	13
	UB 40	*Undisturbed vegetation background*	40	4%	10
	UB 5	*Undisturbed vegetation background*	5	5%	13
	RTP 1	*Focal Species: Trifolium pratense*	0	57%	13
	RTP 2	*Focal Species: Trifolium pratense*	0	93%	13
	RTP 3	*Focal Species: Trifolium pratense*	0	77%	13
	RTR 1	*Focal Species: Trifolium repens*	0	71%	13
	RTR 2	*Focal Species: Trifolium repens*	0	81%	13
	RTR 3	*Focal Species: Trifolium repens*	0	28%	13
	DB 1	*Disturbed vegetation background*	0	5%	13
	DB 2	*Disturbed vegetation background pooled*	0	8%	13
R3-1’	RTP 1	*Focal Species: Trifolium pratense*	0	94%	120
	RAM 1	*Focal Species: Achillea millefolium*	0	6%	159
	RAM 2	*Focal Species: Achillea millefolium*	0	15%	159
	RAM 3	*Focal Species: Achillea millefolium*	0	50%	159
	RAM 4	*Focal Species: Achillea millefolium*	0	19%	159
R3-2	UB 40	*Undisturbed vegetation background*	40	4%	178
	UB 5	*Undisturbed vegetation background*	5	8%	159
	RTR 1	*Focal Species: Trifolium repens*	0	91%	159
	RTR 2	*Focal Species: Trifolium repens*	0	50%	159
	RTR 3	*Focal Species: Trifolium repens*	0	100%	159
	DB 1	*Disturbed vegetation background*	0	16%	159
	DB 2	*Disturbed vegetation background pooled*	0	13%	159
R3-3	RAM 1	*Focal Species: Achillea millefolium*	0	14%	259
	RAM 2	*Focal Species: Achillea millefolium*	0	8%	259
	RAM 3	*Focal Species: Achillea millefolium*	0	10%	259

	RAM 4	*Focal Species: Achillea millefolium*	0	5%	259
	UB 40	*Undisturbed vegetation background*	40	9%	277
	UB 5	*Undisturbed vegetation background*	5	45%	259
	DB 1	*Disturbed vegetation background*	0	30%	259
	DB 2	*Disturbed vegetation background pooled*	0	35%	259
R3-4	RAM 1	*Focal Species: Achillea millefolium*	0	79%	363
	RAM 2	*Focal Species: Achillea millefolium*	0	30%	363
	RAM 3	*Focal Species: Achillea millefolium*	0	40%	363
	RAM 4	*Focal Species: Achillea millefolium*	0	0%	363
	UB 40	*Undisturbed vegetation background*	40	0%	375
	UB 5	*Undisturbed vegetation background*	5	0%	363
	DB 1	*Disturbed vegetation background*	0	26%	363
	DB 2	*Disturbed vegetation background pooled*	0	42%	363
R3-4’	NAM 5-1	*Focal Species: Achillea millefolium*	5	1%	383
	NAM 5-2	*Focal Species: Achillea millefolium*	5	8%	383
	NAM 5-3	*Focal Species: Achillea millefolium*	5	16%	383
	NAM 5-4	*Focal Species: Achillea millefolium*	5	0%	383
	RAM 1	*Focal Species: Achillea millefolium*	0	4%	383
	RAM 2	*Focal Species: Achillea millefolium*	0	5%	383
	RTR 1	*Focal Species: Trifolium repens*	0	76%	383
	RTR 2	*Focal Species: Trifolium repens*	0	30%	383
	NAM 20	*Focal Species: Achillea millefolium*	20	8%	411
R3-5	RAM 1	*Focal Species: Achillea millefolium*	0	41%	440
	RAM 2	*Focal Species: Achillea millefolium*	0	16%	440
	RAM 3	*Focal Species: Achillea millefolium*	0	0%	440
	RAM 1	*Focal Species: Achillea millefolium*	0	48%	559
	RAM 2	*Focal Species: Achillea millefolium*	0	60%	559
	UB 40	*Undisturbed vegetation background*	40	0%	456
	UB 5	*Undisturbed vegetation background*	5	20%	440
	RTP 1	*Focal Species: Trifolium pratense*	0	16%	440
	RTP 2	*Focal Species: Trifolium pratense*	0	77%	440
	RTP 3	*Focal Species: Trifolium pratense*	0	77%	440
	DB 1	*Disturbed vegetation background*	0	61%	440
	DB 2	*Disturbed vegetation background pooled*	0	41%	440
R3-5’	RTR 1	*Focal Species: Trifolium repens*	0	81%	472
	RTR 2	*Focal Species: Trifolium repens*	0	59%	472

	RTR 3	*Focal Species: Trifolium repens*	0	59%	472
	RTR 4	*Focal Species: Trifolium repens*	0	46%	472
R3-6	UB 40	*Undisturbed vegetation background*	40	10%	547
	UB 5	*Undisturbed vegetation background*	5	8%	534
	DB 1	*Disturbed vegetation background*	0	22%	534
	DB 2	*Disturbed vegetation background pooled*	0	0%	534
	NSOL 5	*Focal Species: Solidago virgaurea*	5	0%	534
	RSOL 1	*Focal Species: Solidago virgaurea*	0	0%	534
	RSOL 2	*Focal Species: Solidago virgaurea*	0	37%	534
	RSOL 3	*Focal Species: Solidago virgaurea*	0	6%	534
	RSOL 4	*Focal Species: Solidago virgaurea*	0	24%	534
R3-7	UB 40	*Undisturbed vegetation background*	40	0%	626
	UB 5	*Undisturbed vegetation background*	5	0%	631
	DB 1	*Disturbed vegetation background*	0	0%	631
	DB 2	*Disturbed vegetation background pooled*	0	7%	631
	RSOL 1	*Focal Species: Solidago virgaurea*	0	15%	631
	RSOL 2	*Focal Species: Solidago virgaurea*	0	5%	631

Table S2.2: Soil pH, available P and mineral N (ammonium and nitrate) measurements for each of the plots ordered by elevation.

Transect ID	Plot Type	pH	P	N	Elevation (m a.s.l.)
NO01	Disturbed	4.70	8.73	15.40	9
SO01	Undisturbed	2.80	120.39	10.00	10
SO01	Disturbed	4.10	7.09	2.22	13
NO01	Undisturbed	3.04	17.05	3.66	14
RO03	Disturbed	3.33	9.15	4.47	53
RO03	Undisturbed	5.93	11.10	2.82	53
NO05	Undisturbed	3.35	20.62	13.72	153
NO05	Disturbed	4.65	1.20	0.43	153
SO05	Undisturbed	5.29	17.24	2.87	159
SO05	Undisturbed	3.68	8.02	24.52	178
RO08	Disturbed	2.95	4.26	3.38	187
RO08	Undisturbed	6.57	0.50	0.34	211
NO08	Undisturbed	3.37	9.24	3.08	232
NO08	Disturbed	4.53	0.42	0.03	235
SO08	Undisturbed	4.67	0.90	0.18	259
SO08	Disturbed	3.28	2.50	2.18	277
RO11	Disturbed	5.05	2.32	0.89	304
RO11	Undisturbed	3.52	1.98	1.33	310
SO11	Undisturbed	5.11	1.62	0.58	363
NO11	Undisturbed	2.79	131.32	6.09	371
NO11	Disturbed	3.89	3.89	4.06	373
SO11	Disturbed	3.09	10.81	6.95	375
RO14	Disturbed	4.70	0.87	0.10	404
RO14	Undisturbed	3.34	2.91	2.85	407
NO14	Undisturbed	3.48	7.45	4.59	410
NO14	Undisturbed	4.23	1.72	1.09	414
SO14	Undisturbed	5.04	0.91	0.26	440
SO14	Disturbed	3.77	1.62	5.91	456
RO17	Undisturbed	NA	NA	NA	491
RO17	Disturbed	3.03	3.76	1.89	502
SO17	Undisturbed	4.41	5.06	0.87	534
NO17	Disturbed	3.30	115.35	8.04	536
SO17	Disturbed	3.30	27.74	36.40	547
NO17	Undisturbed	4.26	4.39	0.64	553
SO20	Undisturbed	3.33	2.83	1.45	626
SO20	Disturbed	3.64	5.19	0.14	631
NO20	Undisturbed	4.32	17.46	0.65	672
NO20	Disturbed	4.40	3.04	3.66	697

Table S2.3: List of all 43 retained OTUs identified as being arbuscular mycorrhizal fungi. Best hit (including virtual taxa number (VTX)), family, Genbank accession, alignment length and identity were obtained from the MaarjAM database (Öpik et al., 2011). Elevation range is the elevation differential between the highest and lowest plots in which each OTU was found. Presence refers to the total amount of times each OTU was found across our survey (x samples, out of 144 samples).

OTU	Best hit	Family	Genbank Accession	Alignement length	Identity (%)	Elevation Range (m)	Presence
OTU 1	Glomus sp. VTX00074	Glomeraceae	HF954572	238	100	663	97
OTU 22	Claroideoglomus sp. VTX00057	Claroideoglomeraceae	HF954780	146	96	663	26
OTU 4	Claroideoglomus sp. VTX00193	Claroideoglomeraceae	HF954822	245	100	663	81
OTU 30	Claroideoglomus sp. VTX00056	Claroideoglomeraceae	HF954672	245	100	627	14
OTU 6	Glomus sp. VTX00108	Glomeraceae	HF568295	238	100	688	38
OTU 2	Glomus sp. VTX00113	Glomeraceae	HF568310	238	100	688	78
OTU 8	Glomus sp. VTX00113	Glomeraceae	LN622507	238	100	622	53
OTU 220	Claroideoglomus claroideum VTX00279	Claroideoglomeraceae	HF954736	207	97	663	28
OTU 223	Claroideoglomus Glo G8 VTX00193	Claroideoglomeraceae	HF568513	236	100	663	19
OTU 16	Glomus Goomaral13b Glo 8 VTX00199	Glomeraceae	KF916657	238	100	659	71
OTU 12	Claroideoglomus Glo59 VTX00276	Claroideoglomeraceae	EF041095	244	100	659	17
OTU_258	Glomus sp. VTX00115	Glomeraceae	LN618987	238	99	663	41
OTU_19	Paraglomus sp. VTX00308	Paraglomeraceae	HE613456	238	91	622	18
OTU_7	Glomus sp. VTX00088	Glomeraceae	LN622058	237	100	622	11
OTU_48	Claroideoglomus sp. VTX00225	Claroideoglomeraceae	LN616364	245	99	663	21
OTU_50	Acaulospora sp. VTX00231	Acaulosporaceae	LN621066	240	100	401	6
OTU_96	Acaulospora sp. VTX00015	Acaulosporaceae	LN616396	241	100	0	1
OTU_341	Glomus sp. VTX00115	Glomeraceae	HF954621	238	99	663	23
OTU_369	Glomus LES07 VTX00309	Glomeraceae	JN559815	238	100	431	5
OTU_18	Glomus sp. VTX00149	Glomeraceae	LN621774	237	100	395	6
OTU_63	Glomus sp. VTX00233	Glomeraceae	LN618865	236	100	150	2
OTU_53	Acaulospora sp. VTX00228	Acaulosporaceae	LN621154	240	100	538	2
OTU_39	Claroideoglomus sp. VTX00057	Claroideoglomeraceae	LN621103	245	100	663	8
OTU_71	Glomus sp. VTX00143	Glomeraceae	LN618229	237	100	395	6
OTU_11	Acaulospora sp. VTX00023	Acaulosporaceae	LN621100	240	100	622	15
OTU_40	Acaulospora sp. VTX00030	Acaulosporaceae	KC708350	240	100	659	10
OTU_170	Acaulospora sp. VTX00030	Acaulosporaceae	LN621101	240	99	519	2
OTU_15	Acaulospora Schechter08 Acau2 VTX00021	Acaulosporaceae	EU573760	240	100	459	9
OTU_70	Glomus sp. VTX00166	Glomeraceae	LN621187	237	100	521	4
OTU_267	Glomus sp. VTX00191	Glomeraceae	LN622088	239	100	0	2
OTU_331	Claroideoglomus claroideum VTX00279	Claroideoglomeraceae	AB193051	207	98	478	9
OTU_62	Glomus sp. VTX00114	Glomeraceae	HF954898	238	99	540	7
OTU_44	Glomus QU-Glo5 VTX00166	Glomeraceae	DQ122641	237	100	401	5
OTU_52	Glomus ORVIN GLO3B VTX00223	Glomeraceae	FJ194508	238	100	268	4

OTU_91	Glomus Wirsel OTU6 VTX00202	Glomeraceae	KF916653	238	100	0	1
OTU_68	Glomus sp. VTX00072	Glomeraceae	HF568259	231	100	394	2
OTU_230	Claroideoglomus Glo G8 VTX00193	Claroideoglomeraceae	FJ875136	236	100	25	2
OTU_150	Scutellospora sp. VTX00052	Gigasporaceae	HQ342701	237	100	232	4
OTU_274	Glomus Shi14b Glo-12 VTX00166	Glomeraceae	LN616639	237	99	0	1
OTU_101	Claroideoglomus Torrecillas12b Glo G7 VTX00402	Claroideoglomeraceae	HG004449	242	97	0	1
OTU_67	Diversispora sp. VTX00062	Diversisporaceae	HF568195	240	100	521	4
OTU_124	Glomus sp. VTX00191	Glomeraceae	LN616419	239	100	262	2
OTU_69	Glomus sp. VTX00088	Glomeraceae	LN622058	237	95	431	4

Table S2.4: Model selection for soil abiotic factors: Coefficients (and their p-values, * *P* ≤ 0.05, ** *P* ≤ 0.01, *** *P* ≤ 0.001) for **a)** pH, **b)** N and **c)** P. Model selection was done by selecting all models with a ΔAICc < 2 from the best model (i.e. Model 1), in this case only one model was retained for each model selection procedure. The factor level that serves as intercept is randomly assigned; other factor levels are compared to this baseline effect.

pH

Model	Intercept (Undisturbed vegetation)	Elevation	Disturbed vegetation	Elevation* Disturbed vegetation	AICc	Δ AICc
1	3.330*** (P<0.001)	0.191· (P=0.053)	1.330*** (P<0.001)	-0.411** (P=0.004)	121.7	0

N

Model	Intercept (Undisturbed vegetation)	Elevation	Disturbed vegetation	AICc	Δ AICc

1	1.460*** (P<0.001)		-2.156*** (P<0.001)	221.2	0
2	1.482*** (P<0.001)	0.321 (P=0.211)	-2.166*** (P<0.001)	222.5	1.3

P

Model	Intercept (Undisturbed vegetation)	Disturbed vegetation	AICc	Δ AICc
1	2.102* (P=0.035)	-1.320** (P=0.002)	181.6	0

Table S2.5: Result distribution of randomized PERMANOVAs for arbuscular mycorrhiza fungal community composition: Results of a thousand PERMANOVAs with arbuscular mycorrhiza fugal community composition as a response variable and elevation as well as disturbance as explanatory variables. For each individual PERMANOVA disturbed and undisturbed vegetations backgrounds samples were randomly selected amongst two to avoid pseudo-replication. Each value represents the upper limit of the corresponding factor for the associated percentage.

	0%	1%	5%	10%	40%	50%	90%	95%	99%	100%
R^2 Elevation	0.017	0.019	0.021	0.022	0.026	0.027	0.036	0.039	0.043	0.053
P-value Elevation	0.020	0.079	0.157	0.228	0.485	0.537	0.766	0.807	0.880	0.951
R^2 Disturbance	0.021	0.027	0.031	0.033	0.043	0.046	0.058	0.064	0.073	0.083
P-value Disturbance	0.001	0.002	0.005	0.009	0.049	0.067	0.322	0.430	0.593	0.832
R^2 Interaction	0.020	0.022	0.025	0.026	0.032	0.034	0.046	0.050	0.058	0.064
P-value Interaction	0.003	0.008	0.028	0.059	0.240	0.286	0.605	0.681	0.767	0.895

Figure S2.1 Pictures of disturbed roadside taken across our three studied roads (R1, R2 and R3 with their transect name and the elevation of each transect.

R3-2 - 50 m

R2-11 - 373 m

R1-14 - 404 m

R2-20 - 672 m

R3-10 - 320 m

R3-13 - 411 m

Figure S2.2 Soil pH variation with elevation in the disturbed (DB) and undisturbed (UB) vegetation.

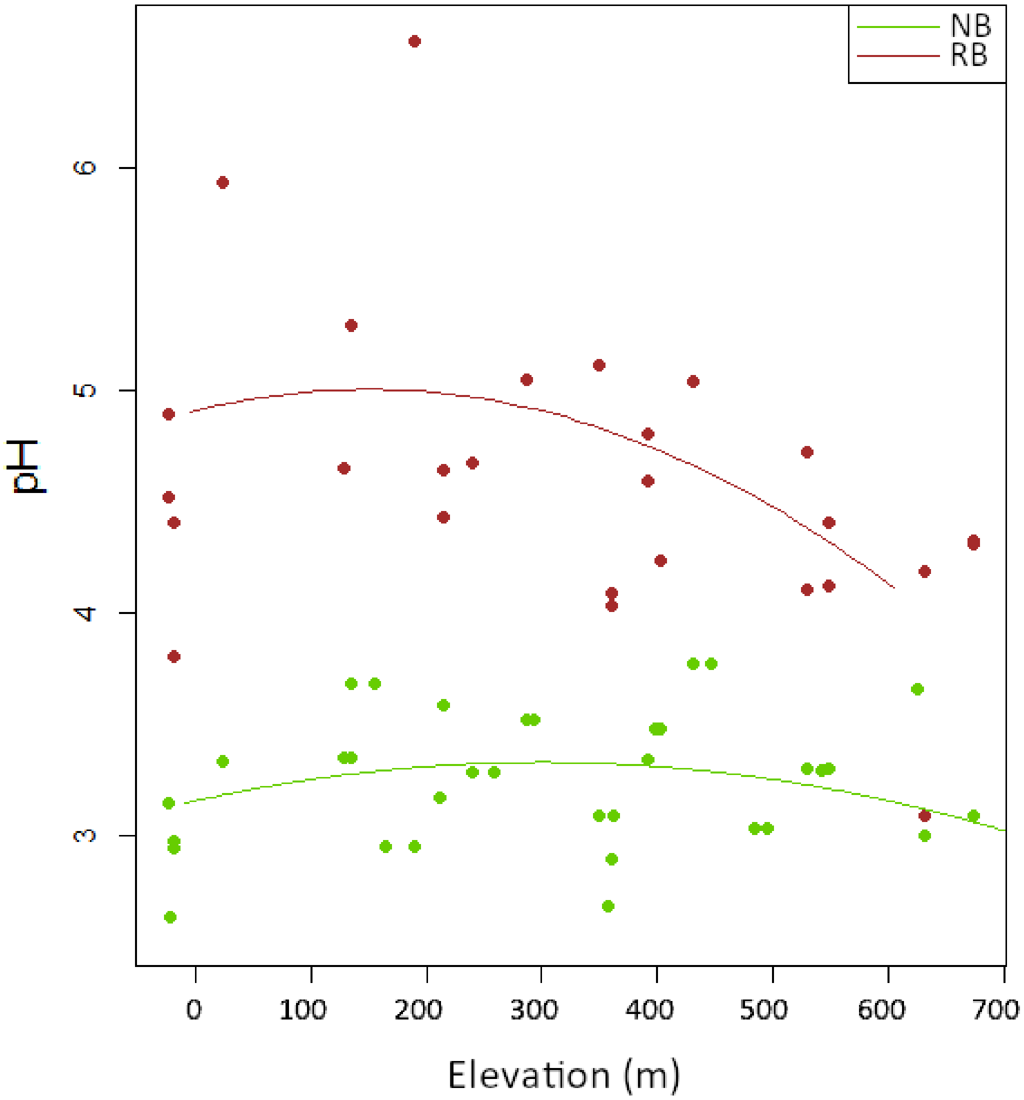

Table S3.1: Selected models explaining percentage of vegetation cover associated with a certain mycorrhizal type: Coefficients and their p-values (between brackets) for each type of mycorrhizal association: arbuscular mycorrhizas (AM), ecto-mycorrhizas (EcM), ericoid mycorrhizas (ErM) and non-mycorrhizal (NM). Model selection was performed by selecting all models with a ΔAICc < 2 from the best model (i.e. Model 1). Explanatory variables were: scaled elevation, disturbance as a three-level plot factor (1: 52 to 102 m from the road, 2: 2 to 52 m from the road, 3: 0 to 2 m from the road) and the interaction between elevation and disturbance. Green and orange fields are respectively positive and negative correlations. Blank spaces represent explanatory variables that were not retained in a given model.

Model	Intercept	Elevation	Disturbance	Disturbance * Elevation
AM				
1	1.7236 (p<0.001)	-0.0238 (p=0.502)	0.167 (p=0.005)	-0.1031 (p<0.001)
EcM				
1	-3.3647 (p<0.001)	0.0746 (p=0.061)	-0.2543 (p=0.092)	
2	-3.3634 (p<0.001)	0.0597 (p=0.151)	-0.2530 (p=0.009)	0.0382 (p=0.217)
3	-3.3630 (p<0.001)		-0.2541 (p=0.009)	
ErM				
1	-4.2885 (p<0.001)	0.1404 (p<0.001)	-0.1828 (p=0.023)	
2	-4.2868 (p<0.001)	0.1262 (p<0.001)	-0.1807 (p=0.024)	-0.0329 (p=0.228)
NM				
1	-2.3348 (p<0.001)	-0.0328 (p=0.349)	0.1157 (p=0.110)	-0.1014 (p<0.001)

Figure S3.1: Examples of diverse mountain roads for which adjacent plant distribution was monitored using the MIREN protocol. **a)** High elevation gravel roads in the Argentinian Andes **b)** Lowland gravel service road in the Norwegian Scandes **c)** High elevation asphalt road in Yellowstone, USA **d)** Lowland asphalt road in Chile with strongly invaded roadside vegetation **e)** High elevation gravel road in Tenerife **f)** Lowland snowed-in gravel road in the Norwegian Scandes.

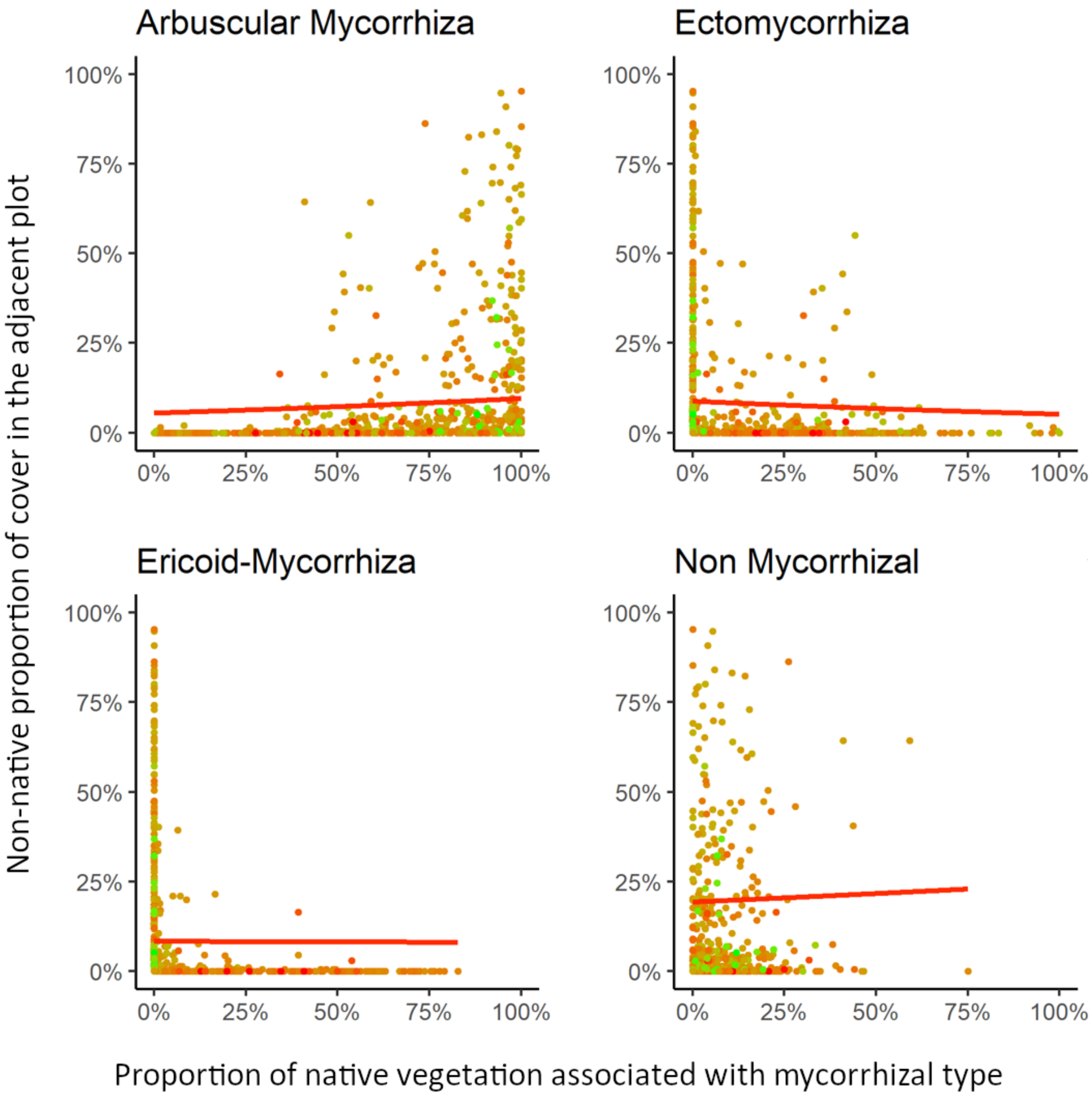

Figure S3.2: Relationship between native mycorrhizal associations and cover of non-native species in the undisturbed vegetation. For each mycorrhizal-type association, the proportion of the vegetation cover in 50 m plots perpendicular to the road (52 m to 102 m from the road) that was of non-native origin was plotted against the proportion of the native vegetation in the same plots which was associated with a specific mycorrhizal type. Each dot represents an individual plot. Regression lines were plotted using the results of generalized linear models (see methods) and individual dots were colored according to the relative elevation of each plot from low elevation (green) to high elevation (red).

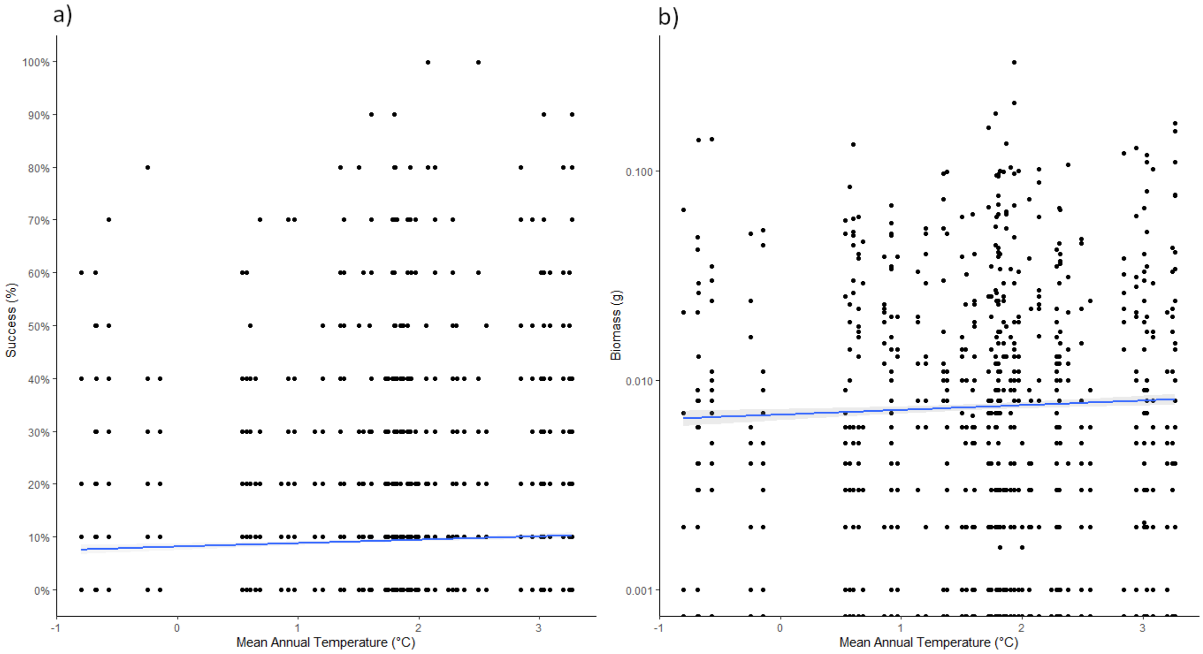

Figure S4.1: Relation between mean annual temperature and non-native plants' success and growth. Percentage of non-native plant seeds that successfully germinated after one year (a) and total biomass produced by these seeds (b) in function of the mean annual temperature of the plots they were planted in as measured by either TMS sensors or HOBO sensors. The biomass axis was transformed using a log transformation for better visualization.

Table S5.1. Highest elevation of non-native species across years: Highest elevation (meters above sea level) observed for all non-native plant species found in the Northern Scandes across three years of vegetation surveys along mountain roads. NA values correspond to years where a given species was not observed in any of the plots of the survey.

	Achillea millefolium	*Aquilegia sp.*	*Cerastium fontanum*	*Equisetum variegatum*	*Festuca rubra*
2012	426	NA	690	591	642
2017	554	NA	690	598	684
2022	426	18	704	591	635

	Lotus corniculatus	*Matricaria discoidea*	*Phleum pratense*	*Picea abies*	*Plantago major*
2012	500	NA	464	496	174
2017	500	NA	684	88	166
2022	426	166	605	598	415

	Poa annua	*Poa trivialis*	*Polygonatum vulgare*	*Ranunculus acris*	*Ranunculus repens*
2012	415	NA	NA	704	115
2017	415	NA	NA	684	226
2022	447	447	18	704	690

	Rumex acetosella	*Stellaria graminea*	*Stellaria media*	*Trifolium pratense*	*Trifolium repens*
2012	635	86	18	429	557
2017	635	94	171	429	557
2022	571	171	18	429	557

	Tussilago farfara	*Urtica dioica*	*Vicia cracca*
2012	415	NA	NA
2017	415	18	256
2022	415	18	256