Impacts of soil origin and fire on arbuscular mycorrhizal fungi associated with hawkweeds and tall tussock

Kendall Elva Morman

A thesis submitted to Auckland University of Technology in partial fulfilment of the requirements for the degree of
Master of Science (Research)

Auckland University of Technology
30th September 2021

School of Science

Abstract

New Zealand's tussock grasslands have faced ongoing plant invasions of the hawkweeds *Pilosella officinarum* and *Hieracium lepidulum.* Plant invasions may be facilitated through plant-soil feedbacks, whereby a plants growth can be improved through feedback from the soil, including soil mutualists. Both hawkweed species readily associate with arbuscular mycorrhizal (AM) fungi, which form mutualisms with plant roots and are known to alter the success of invasive plants. Fire activity is also predicted to increase in many areas where hawkweeds are abundant or projected to spread. Fire is believed to have effects on plant and soil communities but also soil carbon through combustion. There is little information on the AM fungal communities and their interactions with native and invasive plants in New Zealand's tussock grasslands. This study aimed to determine the impact of soil origin and simulated fire on AM fungal communities, invasive hawkweeds and soil carbon levels in tussock grasslands. The study had three main objectives: to assess how AM communities are structured in tussock grassland soil and whether they differ by soil origin (plant species) or simulated fire; to assess the roles of plant-soil feedbacks on hawkweed growth under differing soil origins and simulated fire; and to assess the effects of soil origin and simulated fire on soil carbon levels in tussock grasslands.

I collected soil under *P. officinarum, H. lepidulum, Chionochloa macra* plants in the field, or "soil origin" treatment. I then subjected the soils to heat treatments to simulate fire: at 30°C, 45°C and 60°C. I used high throughput sequencing (18S, Illumina MiSeq) to identify AM fungal taxa by origin and heat in the soil. I conducted a growth chamber study to measure the plant growth responses of hawkweeds under differing soil treatments: soil origin and heat. Soil carbon levels were measured by C:N analysis.

My results showed that soil origin was the major driver of AM fungal community structure in terms of composition. The effect of heat on AM fungal richness differed according to soil origin and, surprisingly, had no impact on community composition. In the growth chamber study, hawkweed biomass was influenced by soil origin but not heat. Interestingly, both hawkweed species had the highest plant biomass in *P. officinarum* soils. Although soil carbon was slightly elevated in *C. macra* soils compared to the soils of invasive hawkweeds, soil carbon levels did not differ statistically between different soil origins and heat treatments.

The results support the idea that soil origin is a major driver of plant-soil relationships in New Zealand's tussock grasslands. Further, it suggests that *P. officinarum* invasion may facilitate invasion by *H. lepidulum*. My results indicated that fire has less of an effect on AM communities, hawkweed growth and soil carbon than soil origin in tussock grasslands.

Keywords: Arbuscular mycorrhiza fungi; plant-soil feedbacks; hawkweeds; tussock; fire.

Acknowledgements

I wish to sincerely thank those who have helped and supported me to complete this masters. No doubt, I could not have completed this without all of you. Nicola, thank you so much for taking me on. Your knowledge, patience and commitment to me and my project has been overwhelmingly positive and made me into the aspiring scientist I am today. To Hannah and Colleen, thank you both for all your support, patience and for sharing your wisdom to help me get this completed.

My sincerest thanks to everyone at AUT past and present from the Molecular Biology Research Group, my loyal lab buddies Jack and Kat. Tim Lawrence and Tim Layt, for all your help getting me started in the lab, particularly with all the COVID-19 issues, restrictions and all the helpful technicians and admin team with the School of Science. I would also like to acknowledge the help of Roger Creswell, John Ramana and Cameron Egan for your assistance with technical aspects of this project.

My greatest thanks to the Hellaby Grasslands trust for your financial support of this masters and all the other student projects you have supported in New Zealand's grasslands. Further, I would like to thank the Royal Society of New Zealand for the additional research funding you contributed to this work.

Big thank you to my friends and Whānau for your love and support not asking too many questions about my submission date. Special thanks to the esteemed members of my bubble Ryan, Slinky and Azuki, who have been there day-to-day encouraging me, consoling me, and kept me sane throughout this journey.

Table of Contents

List of Tables

List of Figures

Attestation of authorship

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published by another person (except where explicitly defined), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning.

Kendall Morman

Chapter 1. Introduction

1.1. Plant-soil feedbacks

Soil is the foundation that sustains the functioning of all terrestrial ecosystems (Voroney and Heck 2015). Soils are composed of many different physical components, including organic material, water, and minerals (van der Heijden et al. 1998, van der Putten et al. 2013a, Voroney and Heck 2015). They are home to plants and many macro- and microorganisms that form complex below-ground biological communities that are central for maintaining overall soil and plant health (Van Der Heijden et al. 2008, Aislabie and Deslippe 2013). Soils are comprised of organisms that can positively or negatively impact plant growth, i.e. mutualists (e.g., mycorrhizas) or pathogens (Smith and Read 2008, Aislabie and Deslippe 2013).

The interactions between soil microbial communities and plants are complex, and both are able to alter the growth of the other through plant-soil feedback processes (Bever et al. 2009, 2012, van der Putten et al. 2013). Plants can directly recruit microbes that are beneficial to their growth through chemical signalling (Gaiero et al. 2013, Carbonnel and Gutjahr 2014). They can indirectly recruit soil microorganisms by varying their production of chemical compounds, litter inputs, and transpiration rates, which can cause changes in the soil environment, resulting in changes in microbial community structure and composition (Bennett and Klironomos 2019).

Plant-soil feedback falls into two categories: positive and negative. Positive plant-soil feedback occurs when changes to the soil microbial community enhance a plant species' growth and fitness in its own 'home soil'. Home soil refers to the soil collected

from underneath a plant of the same species from its current range. In contrast to ‘away soil’, which is soil collected outside of the plant’s range or collected from underneath a different species in its current range (Wardle et al. 2004, Bever et al. 2012, Bennett and Klironomos 2019). The growth and fitness benefits of positive plant-soil feedback could be due to the plant accumulating mutualists, such as arbuscular mycorrhizal fungi (AM) (Bennett and Klironomos 2019). The outcome of positive feedback is expected to favour the competitive dominance of the focal plant species and reduce plant diversity (van der Putten et al. 2013, Buerdsell et al. 2021). In contrast, negative soil feedback occurs when soil microbial communities cause declines in growth and/or fitness of the focal plant species, such as accumulating soil pathogens (Wardle et al. 2004, Bever et al. 2009). These changes tend to produce microbial soil communities that do not favour members of the same plant species (Klironomos 2002, Wardle et al. 2004, Bever et al. 2012). Negative plant-soil feedbacks are expected to favour plant species coexistence and increase diversity in a community (Wardle et al. 2004, van der Putten et al. 2013).

Plant-soil feedbacks become important when an ecosystem is experiencing the effects of global change processes such as plant invasion or fire. Many studies have investigated the link between plant-soil feedback and AM fungi (Klironomos 2002, 2003, Reinhart and Callaway 2006, Pringle et al. 2009, Day et al. 2015). Plant invasions can be facilitated through plant-soil feedbacks. This is supported by the enemy release hypothesis, which predicts that a species may do better in a new habitat, where its former enemies (e.g., parasite) are not present (Keane and Crawley 2002). From a plant-soil context, in a new environment, an invasive species could grow better in ‘away soils’, as they can leave behind soil pathogens that existed in their home environment. Thus, negative plant-soil feedbacks that exist between plants and their native soil pathogens in their native range may be left behind. Additionally, invasive plants may do

better in ‘away soils’ due to positive plant-soil feedback in their new host range (Klironomos 2002, Colautti et al. 2004, Inderjit and Cahill 2015). Positive plant-soil feedbacks may occur due to the associations formed between an invasive plant and local mutualists, once it enters a new host range (Inderjit and Cahill 2015). Fire disturbance may also positively or negatively alter plant-soil feedback, depending on the soil microorganisms' response to fire (Beals et al. 2020). Fire may lead to a decline in soil pathogens that would increase the likelihood of a positive plant-soil feedback. Alternatively, it could also result in the decline of important soil mutualists like AM fungi, resulting in a negative plant-soil feedback (Dove and Hart 2017, Pressler et al. 2019, Beals et al. 2020).

Plant-soil feedbacks are also important in the sequestration of carbon into soil. A mechanism in which carbon sequestration can occur is through the allocation of carbon to mycorrhizal fungi into the soil (Bardgett 2011). There is an increased interest in this area as increasing carbon sequestration remains a global challenge, which may help mitigate increased atmospheric carbon dioxide levels associated with climate change (Bardgett 2011). Carbon sequestration has been demonstrated in grassland systems where high plant diversity was linked with enhanced carbon dioxide uptake and belowground allocation to AM fungi and roots (De Deyn et al. 2009, 2011). As important soil mutualists, AM fungi have an important role in mediating plant responses during global change processes, while also being affected by them (Cotton 2018).

1.2. Arbuscular mycorrhizas

An important group of symbionts within soil microbial communities are mycorrhizal fungi. AM fungi are by far the most prevalent mycorrhizal mutualist (Smith and Read 2008) and

are all in subphylum Glomeromycotina (Spatafora et al. 2016, Bruns et al. 2018). These fungi are obligate symbionts that colonise and form a symbiosis within plant roots, where the fungus provides soil nutrients (phosphorus and nitrogen) to the plant in exchange for carbon (Smith and Read 2008). AM symbiosis has many benefits for plants (Smith and Read 2008). AM root colonisation has been shown to enhance plant growth, seedling growth, and seedling establishment through nutrient acquisition (Klironomos 2003, Lekberg and Koide 2005, Hoeksema et al. 2010); improve plant tolerance to environmental stresses such as drought, temperature changes, salinity, and heavy metals (Glassman and Casper 2012, Begum et al. 2019, Fahey et al. 2020); and provide pathogen protection (Newsham et al. 1995, Borowicz 2001, Sikes et al. 2009). This symbiosis is of great importance to plants due to the influence of AM fungi on plant productivity, diversity, and the connections formed to other plants below ground via mycorrhizal networks (Van Der Heijden et al. 2015).

The relationship between plants and AM fungi is ancient (Redecker et al. 2013). It is hypothesised that ancestral AM fungi enabled the colonisation of land by early plants from the ocean nearly 400 million years ago (Redecker et al. 2000). This relationship between AM fungi and their plants is widespread (Wang and Qiu 2006, Smith and Read 2008). It is estimated to occur with upwards of 80% of land plant species across the planet, including plants species from major taxonomic groups such as Bryophytes, Pteridophytes, Gymnosperms and Angiosperms (Wang and Qiu 2006, Smith and Read 2008, Van Der Heijden et al. 2015). Though the majority of land plants benefit from mycorrhizal fungal colonisation, they can also survive without them with, some plant groups having lost them all together (Brundrett 2004, Smith and Read 2008).

The symbiosis between plant and fungus sits on a continuum with variation in the degree of benefit of the relationship between different plant species and families

(Johnson et al. 1997, Klironomos 2003, Wang and Qiu 2006). Some plant species gain significant growth benefits from associating with AM fungal such as *Cerastium vulgatum*, *Poa compressa*, and *Solidago rugosa* (Klironomos 2003, Wang and Qiu 2006). Whereas others in families, such as the Brassicaceae and Chenopodiaceae, are entirely non-mycorrhizal and have evolved alternative strategies for fulfilling their nutritional requirements (Brundrett 2004, Graham et al. 2017, Chen et al. 2018).

AM fungi exist partially within the plant roots and partially within the surrounding soil (Smith and Read 2008). Extraradical hyphae exist outside the plant roots and within the surrounding soil (Souza 2015). They are involved in the colonisation of plant roots, nutrient absorption from the soil, AM fungal reproduction roles, and can connect different plants together through the formation of mycorrhizal networks (Souza 2015, Wipf et al. 2019). AM spores are large asexual reproductive structures of the fungus found within the soil (Smith and Read 2008, Kamel et al. 2017). They contain multiple nuclei per spore and are capable of colonising plant hosts (Smith and Read 2008, Kamel et al. 2017). The dominant structures within the plant roots are intraradical hyphae, vesicles, and arbuscules (Figure 1.1). Intraradical hyphae are found within the plant root cells and are responsible for transferring nutrients, metabolites, and water from extraradical hyphae into the plant root cells (Smith and Read 2008, Souza 2015). The hyphae act as a channel connecting to other structures within the cell including vesicles (Smith and Read 2008, Souza 2015). Vesicles are thick-walled storage structures that store lipids and in some circumstances, act as propagules (Smith and Read 2008, Müller et al. 2017). For two genera, *Scutellospora* and *Gigaspora*, vesicles are absent (Smith and Read 2008, Souza 2015). They instead have auxiliary cells, which are spore-like structures found on extraradical hyphae. These structures are also predicted to have nutrient storage roles (Smith and Read 2008, Souza 2015).

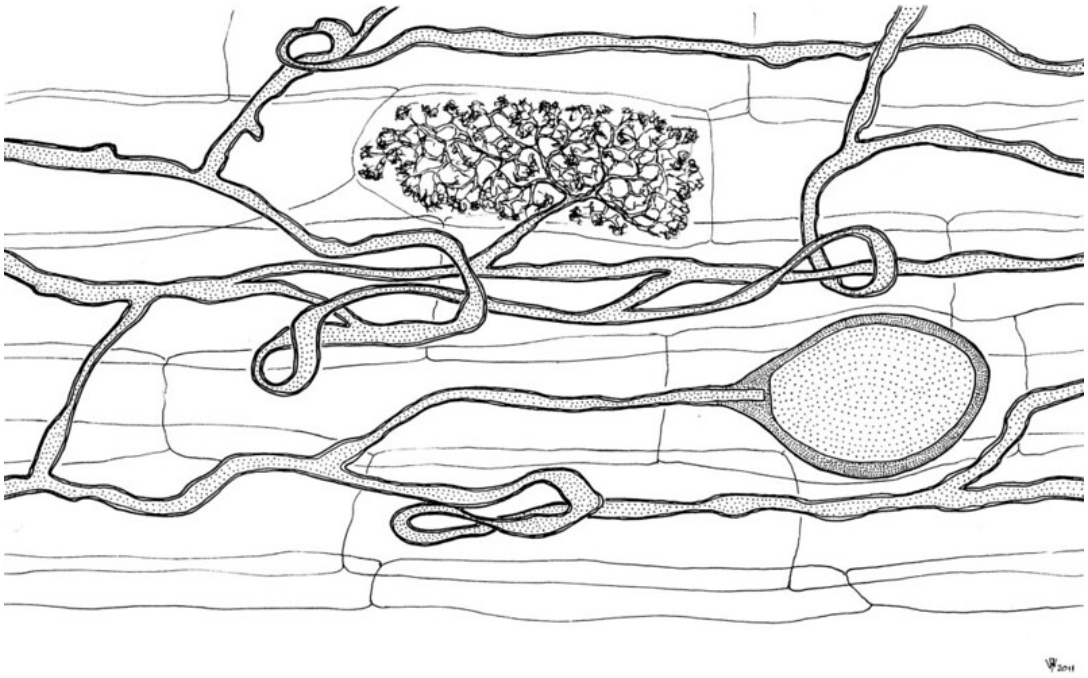

Figure 1.1. This drawing shows the internal structures of an arbuscular mycorrhizal fungus colonising root cells. The root cell consists of oblong brick-like structures colonised by the intra-radical hyphae, a branch-like structure growing throughout these cells. Tree-like branches within a cell indicate Arum type arbuscules, and the circular fingerprint structure indicates vesicles.

Arbuscles are the site of nutrient exchange between the plant and fungus (Souza 2015). Arbuscules are highly branched structures present inside a root cortical cell (Luginbuehl and Oldroyd 2017). There are two types of arbuscules: (1) the "Arum" type, which is characterised by a tree-like structure, and (2) the "Paris" type, which is characterised by a coil like structure (Armstrong and Peterson 2002, Smith and Read 2008, Souza 2015, Luginbuehl and Oldroyd 2017). The arbuscule formed depends on the fungal species (Dickson 2004, Luginbuehl and Oldroyd 2017).

The presence of arbuscules, hyphae, and vesicles is a major diagnostic characteristic for identifying and quantifying AM colonisation of roots (Koide and Mosse 2004, Smith and Read 2008, Stoian et al. 2019). Prior to molecular tools to identify

fungal taxa, AM fungal species were identified via their spore structure and characteristics (Lee et al. 2006, Smith and Read 2008). Though useful techniques, both are low throughput and in particular, spore identification requires specialised knowledge of the spore structure (Chagnon et al. 2013, Crossay et al. 2017). High throughput sequencing platforms such as Illumina have allowed semiquantitative identification of AM fungal taxa in roots and soil samples (Lindahl et al. 2013). This has allowed analysis of AM communities at a much larger throughput than earlier techniques and has subsequently improved our understanding of AM fungi and AM ecology and phylogenetics (Koide and Mosse 2004, Lindahl et al. 2013, Chagnon et al. 2013, Begum et al. 2019).

Though AM fungi are found ubiquitously in soils, there are species-specific variations in the effectiveness of the plant-mycorrhizal relationship (Klironomos 2003, Bever et al. 2009, Öpik et al. 2009, Kiers et al. 2011, Chagnon et al. 2013). For instance, different species of AM fungi have different carbon requirements from their host plant, phosphorus translocation abilities, and differences in biomass investment in intraradical vs extraradical hyphae (Ravnskov and Jakobsen 1995, Hart and Reader 2002, Van Aarle and Olsson 2003, Chagnon et al. 2013). The dynamics of the plant-fungal relationship can be further altered in response to different global changer factors, such as plant invasion (Cotton 2018).

1.3. Exotic plant invasion

Exotic plant invasions are identified as one of the major drivers contributing to global biodiversity loss (Sage 2020, Hulme 2020). Invasions remain a challenge to manage and often continue despite human intervention (Hulme 2006). Exotic 'Invasive' plant

populations begin as a few introduced individuals with, the majority of these species remaining in low numbers (Pyšek et al. 2012). However, some of these exotic species proliferate across landscapes and can have widespread, deleterious effects on plant communities and ecosystems. Some examples of these effects are displacing native plant species through competitive suppression, disrupting trophic networks, altering biochemical nutrient cycles and changing fire regimes (Pringle et al. 2009, Baker and Bode 2016, Sage 2020).

Exotic plant invasions can be a synergistic result of other concurrent global change drivers, such as land transformation, fire, drought and grazing (Keeley and Brennan 2012, Dickie et al. 2017, Sage 2020). Global change drivers such as fire can remove native vegetation biomass and alter the soil microbial environment, thus giving opportunity for an invasive plant species to establish. Once established on the landscape, invasive plants can amplify the negative effects of other global change drivers, which may initiate ecosystem collapse (Sage 2020). An example is the invasion of the grass *Bromus tectorum* in Western North America's sagebrush steppe (Mack 1981). Studies have shown this species was able to become invasive due to being well adapted to the ecosystem and more tolerant of land transformation events occurring at the time (Mack 1981). Fire frequency increased in invaded areas from reoccurring every 60-110 years to every 3-5 years (Mack 1981, Whisenant 1990, Baynes et al. 2012). As an annual grass, *B. tectorum* produces a highly flammable litter, and it is better able to tolerate fire than native species (Mack 1981). This feedback has increased the range of the species facilitating further *B. tectorum* invasion (D'Antonio and Vitousek 1992). Exotic plant invasion can also facilitate further plant invasions through the invasional meltdown hypothesis (Green et al. 2011). This hypothesis predicts that positive feedback between an existing plant invader and a new plant invader may facilitate the proliferation

of the newer invader (Green et al. 2011). Exotic plant invasion can also be facilitated by AM fungi (Pringle et al. 2009). Invasive plants are often mycorrhizal generalists, meaning they are aided in invasion by associating with many AM fungi and not a specific species (Rejmánek 2000, Pringle et al. 2009). Associating with many AM fungi can allow them to take advantage of the different growth benefits provided by different species (Rejmánek 2000).

1.4. Impacts of fire disturbance on soil, plants, and their associated microbes

Fire is a significant disturbance in many ecosystems. Fire alters soil physical properties in three ways: reducing soil aggregation, reducing moisture content, and altering soil texture (Certini 2005). Fire also alters soil chemical properties by reducing pH, increasing phosphorus availability, and at higher temperatures can cause declines in soil nitrogen and carbon (Certini 2005, Pellegrini et al. 2018, Butler et al. 2018, Ludwig et al. 2018, McLauchlan et al. 2020). Therefore, fire has both direct and indirect impacts on AM communities (Certini et al. 2021). Fire directly impacts AM communities through high soil temperatures and indirectly through damages or mortality to plant hosts caused during a fire (Certini 2005, Pellegrini et al. 2018, Butler et al. 2018, Ludwig et al. 2018, McLauchlan et al. 2020). Both direct and indirect effects have been shown to cause declines in AM richness, abundance and evenness across different taxonomic groups (Xiang et al. 2015, Dove and Hart 2017). Many studies report that AM fungi have low resistance but high resilience to fire, in that mortality is high, but populations can recover quickly after a fire (Xiang et al. 2015, Dove and Hart 2017). In most cases, the mycorrhizal community can recover to pre-fire levels; however, the recovery timeframe varies depending on the initial fire severity and can range from months to decades (Certini 2005, Xiang et al. 2015, Holden et al. 2016, Dove and Hart 2017).

The temperature reached in soil during fire has been shown to greatly impact the soil microbial communities and their recovery; soil temperatures depend on the fire severity and the soils' insulating properties (Pingree and Kobziar 2019). In North American forests, soil temperatures have been reported to be less than 100°C to well exceeding 400°C (Pingree and Kobziar 2019). In grasslands and prairies, there is little information available on soil temperatures during fire; however, temperatures ranging between 20-70°C have been reported (Scotter 1970, Blair 1997, Hill et al. 2017). It is likely soil temperatures are lower during fire in grasslands and prairies compared to forests due to the differences in amount and type of fuel (Pingree and Kobziar 2019, Stavi 2019). In more severe fires, often driven by high fuel loads, higher soil temperatures would be expected. Higher temperatures would thus have greater impacts on the soil microbial community, the ecosystem processes they regulate, and subsequent plant recovery and growth (Holden et al. 2016).

As a component of the soil microbial community, AM fungi are sensitive to fire but show resilience in recovery, depending on the recovery of host plants (Longo et al. 2014, Xiang et al. 2015). As AM fungi are obligate symbionts, they rely on plants for their carbon (Smith and Read 2008). High soil temperatures during a fire may directly cause a reduction in the AM fungal spore bank hyphae due to heat-induced mortality (Bellgard et al. 1994). There have been relatively few studies that have examined the response of AM fungal communities to fire. In burned soil, field studies have reported that measured AM evenness, richness and diversity were lower one year after fire (Longo et al. 2014, Xiang et al. 2015). The death of AM host plants may indirectly reduce AM fungi species/abundance (Begum et al. 2019). For example, Xiang et al. (2015) observed that AM fungal communities in soils 11 years after fire were indistinguishable from those of unburnt soil, and they inferred this was linked to the recovery of the aboveground

vegetation. Earlier studies also showed AM fungal colonisation declined under simulated fire activity (Klopatek et al. 1988, Pattinson et al. 1999). In contrast, Docherty et al. showed that AM fungi were relativity unaffected by fire in experimental field burns in an annual Californian grassland (2012). Changes in the availability of the nutrients in soil, such as an increase in phosphorus or loss of water, can modify the dynamic of the relationship between AM fungi and plants from mutualistic to parasitic (Treseder 2004, Hartnett et al. 2004, Grman 2012). Conversely, given the importance of the role of AM as a mutualist to plant species, a plant with high dependence on symbiosis may be required after disturbances such as fire.

Fire disturbance can alter AM fungal communities through modification of plant species. Fire acts as a biological filter selecting for plant species that are more tolerant to fire or have the ability to rapidly regrow after fire (Archibald et al. 2018). The change in the plant host is expected to affect the AM fungal community composition. These changes may be temporary, and the original community may be restored through succession, but alternatively may be permanent, possibly resulting in a loss of diversity.

Fire causes decline in soil carbon levels. This occurs through processes such as pyrolysis and combustion, which causes losses in the organic layer of the soil, and may also result in the loss of the mineral layer (Certini 2005, McLauchlan et al. 2020, Li et al. 2021). The effects of fire on soil carbon are greater in higher severity fires (Li et al. 2021). Ongoing fire can have legacy effects on soil carbon, where continued fire results in the depletion of carbon in the soil, which is linked to declines in plant regrowth and regeneration (Pellegrini et al. 2018). Losses in soil carbon due to fire are also associated with plant invasion (Nagy et al. 2021).

Figure 1.2. Burnt native shrub *Discaria toumatou* (Matagouri) showing the resprouting of invasive plant *Hieracium lepidulum* with many plants in flower in Ōhau in the Mackenzie Basin, New Zealand (Photo: S. Budha-Magar).

1.5. New Zealand tussock grasslands: plant invasion, fire, microbial communities, and soil carbon

New Zealand's tussock grasslands are unique ecosystems comprising many native and endemic species including plants, invertebrates, reptiles, and birds (Mark et al. 2013). New Zealand's tussock grasslands are classified into two types: tall tussock grasslands, which are dominated by *Chionochloa* species, and short tussock grasslands, dominated by *Festuca novae-zelandiae* and *Poa* species (Mark and McLennan 2005). As indigenous ecosystems, New Zealand's tussock grasslands provide many important ecosystem services such as water retention, soil carbon storage and carbon

sequestration (Mark and Dickinson 2008, Mark et al. 2013). Tussock grasslands are believed to have existed in small landscape pockets in pre-human times; they have become widespread after the arrival of Polynesian and European settlers who burned and harvested forests, then maintained by pastoral farming (McGlone 1989, Mark et al. 2013, Perry et al. 2014). The area of tussock grasslands have declined, and in some places, have disappeared, mainly due to increased agricultural land use and exotic plant invasion (Mark and McLennan 2005). Fire was a commonly used farming practice to open the tussock canopy to improve plant palatability for the grazing of sheep (Treskonova 1991). Over time, these practices have led to the decline in tussocks and the native inter-tussock plant communities (Treskonova 1991).

The degradation of tussock grasslands has also coincided with the invasion of hawkweeds, but there is mixed opinion on whether hawkweeds are the cause (Treskonova 1991, Fan and Harris 1996). There are three invasive hawkweed species commonly found in tussock grasslands: *Pilosella officinarum* (formerly *Hieracium pilosella*), *Hieracium lepidulum* and *Hieracium praealtum* (Wiser and Allen 2000, Day and Buckley 2011). All species have originated from Europe and are believed to have arrived in New Zealand as a contaminant in pasture seed (Makepeace 1980). Hawkweeds are broad-leaved perennial herbs that are arranged as a basal rosette (Makepeace 1980, Wiser and Allen 2000). *Hieracium lepidulum* reproduces apomictically and has wind-dispersed seeds (Wiser and Allen 2000). *Pilosella officinarum* is also wind-dispersed but can also be spread by stolons (Makepeace 1980). These stolons allow *P. officinarum* to form characteristic hawkweed mats commonly found dominating inter-tussock plant communities, as shown in Figure 1.3 (Makepeace 1980). Both *P. officinarum* have characteristic yellow flowers (Figure 1.2, Figure 1.3, and Figure 1.4).

Figure 1.3. *Pilosella officinarum* invasion in tussock grasslands showing vegetative mat-like structure formed by the plants (Photo: N. Day).

Pilosella officinarum has been present in New Zealand the longest of all the hawkweeds. Its invasion history consisted of a long lag phase over several decades where populations were at lower numbers, then later increased drastically in population size and distribution (Treskonova 1991, Groves 2006). This species is now widespread across South Island tussock grasslands (Day and Buckley 2011). *Hieracium lepidulum* is the newest invader of the three, but has also begun to increase its range in areas across the South Island high country (Day and Buckley 2011). *Hieracium lepidulum* possibly has a wider niche than *P. officinarum* because it is better able to tolerate shade than the light-demanding *P. officinarum* and is even observed colonising beech forests (Wiser and Allen 2000, Spence et al. 2011).

Figure 1.4. *Hieracium lepidulum* plants in flower, growing in inter-tussock space within a 1 m by 1 m quadrat in *Chionochloa macra* dominated tussock grasslands, approximately two years after fire in Deep stream, Otago. (Photo: S. Budha-Magar).

Both *P. officinarum* and *H. lepidulum* readily form symbiotic associations with AM fungi (Wiser and Allen 2000, Downs and Radford 2005, Wang and Qiu 2006, Höpfner et al. 2015). Previous work by Downs and Radford (2005) showed that for *H. lepidulum*, the presence of AM fungal was associated with higher biomass and better plant establishment in unfertilised pots but not in fertilised pots. A similar result was observed for *P. officinarum,* where root biomass decreased in the presence of fertiliser, but increased in the presence of AM fungi (Höpfner et al. 2015). Both these results suggest that AM facilitation play an important role in nutrient acquisition for both species. AM

fungi help plants with phosphorus uptake, so when fertilised hawkweeds do not need to form associations with the fungi.

Fire causes declines in soil carbon levels and plant biomass. In tussock grasslands, periodic fire causes the loss of tussock biomass (Payton and Pearce 2009, Pearce et al. 2009). The effects on tussock biomass and soil carbon are greater when conditions are drier and cause short to medium-term loss of tussock dominance (Payton and Pearce 2009, Pearce et al. 2009). The effects on carbon are higher as severe fires have greater impacts on carbon (Li et al. 2021). As soil carbon sequestration is linked to plant diversity, ecosystems that experience continued fire are likely to have a reduction in carbon sequestration (Pellegrini et al. 2018). Interestingly, there is some evidence that New Zealand's tussock grasslands contain high pools of carbon in the soil, and were shown to have higher soil carbon than neighbouring beech forests (Ross et al. 1996, Mark et al. 2013, Krna 2015). Hence, fire could have disruptive effects on this soil carbon sequestration in tussock grasslands.

New Zealand's tussock grasslands on the eastern South Island are increasingly at risk of fire disturbance due to climate change (Pearce et al. 2011). Recent fires in the Lammerlaw Ranges, Otago, Lake Ōhau and Lake Pukaki in Canterbury have caused extensive damage to the tussock grassland vegetation (Perry 2019, Frykberg 2020, Holden et al. 2020). The effect of this increased fire regime on hawkweed invasion, the AM fungi associated with hawkweeds, and soil carbon implications are not clear. This thesis addresses this research gap by experimentally testing the interactions among AM fungi, hawkweeds, fire and soil carbon.

This thesis aimed to quantify the relative effects of soil origin (soil collected under different plant species) and simulated fire effects (heat) on the community structure of

tussock grassland and AM fungal communities associated with hawkweeds (*H. lepidulum* and *P. officinarum*) and native tussock (*C. macra*); hawkweed growth (plant biomass and root-shoot ratio); and soil carbon.

The first research question was: Does soil origin and simulated fire affect AM community structure (species richness and composition)? I hypothesised that: **Hypothesis 1 (H1)** — AM fungal species richness and community composition would differ by soil origin due to plant-soil feedbacks (van der Putten et al. 2013, Inderjit and Cahill 2015), whereby different hawkweeds will be associated with different AM fungal communities (Pringle et al. 2009, Yang et al. 2012, Torrecillas et al. 2012). and that: **H2** AM fungal species richness would decrease in response to simulated fire activity soils because fire kills fungi (Dove and Hart 2017, Pressler et al. 2019),

The second research question was: Do invasive hawkweeds experience positive or negative plant-soil feedbacks and is this altered by simulated fire? I hypothesised that: **H3** – Plant biomass would not differ between soils of different origins because invasive hawkweeds are generalists and are therefore expected to be able to associate with many species of AM fungi present in soil (Pringle et al. 2009, Lekberg et al. 2013), that: **H4** – Plant biomass would be lower in soil treatments experiencing fire activity because the mycorrhizal richness had declined (Dove and Hart 2017, Pressler et al. 2019), and that: **H5** – root-shoot ratios would be lower after heating because of higher nutrient availably in the soil after fire (Nguyen et al. 2019).

The third research question was: Does soil carbon differ by soil origin and/or under simulated fire activity? I hypothesised that: **H6** – Soil carbon would decline in heated soils because of combustion (Stavi 2019, McLauchlan et al. 2020), and that: **H7** – Soil carbon would be higher in soil associated with *C. macra* than that of

invasive hawkweeds because tussocks are long-lived plant species that are known to effectively accumulate soil carbon (Mark et al. 2013, Krna 2015).

Chapter 2. Methods and Materials

2.1. Soil and seed collection

In February 2020, soil was collected from one location at Porters Ski field, Canterbury, New Zealand. The dimensions of the soil samples collected were 10 cm by 10 cm at a 5 cm depth, which made up a total ~1-2 L volume (Q2). Soil samples were collected directly underneath three target plant species: *P. officinarum, H. lepidulum*, and *C. macra*. An effort was made to collect soil at least 5 m apart for each species. Equipment was disinfected between the collection of each sample using bleach wipes. A total of ten samples per species were collected for the growth chamber study and the DNA and carbon analysis. Each soil sample was split into three: one subsample was used for potting in the growth chamber, one for sequencing, and one for soil carbon testing. Samples were stored at -20°C until use. Seeds of *H. lepidulum* and *P. officinarum* were collected from the Porters Ski field area. Additional *P. officinarum* seeds were collected from Deep Stream, Otago.

2.2. Molecular analysis of soils

2.2.1. Soil treatments

To test hypotheses regarding the community structure of tussock grassland AM fungal communities associated with hawkweeds (H1, H2), an experiment was set up as shown

in Figure 2.1A. Soil origin refers to the plant species the soil was collected underneath (*H. lepidulum*, *P. officinarum*, or *C. macra*). For each soil origin, there were ten biological replicates which were separated into four heat treatments. The heat treatments indicate the temperature that the soil was exposed to for 10 minutes: unheated (UH), 30°C, 45°C, or 60°C. There were ten biological replicates per soil origin-heat combination and ten replicates per species for a total of 120 samples (40 experimental units for each soil origin; Figure 2.1A.).

To date, the soil temperature reached during fire in tussock grasslands is unknown. Based on work by Pearce et al. (2009), soil temperatures during a tussock grassland fire did not reach 69°C. Estimates of soil temperatures in grasslands were used to determine the temperature used to simulate fire (Scotter 1970, Blair 1997, Hill et al. 2017). The heating time was based on rapid cool down of surface soil reported by Pearce et al. (2009) during a controlled burn.

Soil samples were randomly selected for heating and were processed in batches across different days, where all treatments were applied each day. A subsample of each soil sample was placed into an autoclaved aluminium dish measuring 2 cm deep by 6 cm diameter. These were covered using autoclaved aluminium foil and left at room temperature for 10 minutes. Samples were then heated in a drying oven for 10 minutes. The temperature of the oven was confirmed with a glass bulb thermometer. Following heating, samples were left to cool at room temperature for 30 minutes then left on ice overnight. Unheated samples were left at room temperature for 10 minutes then left on ice overnight.

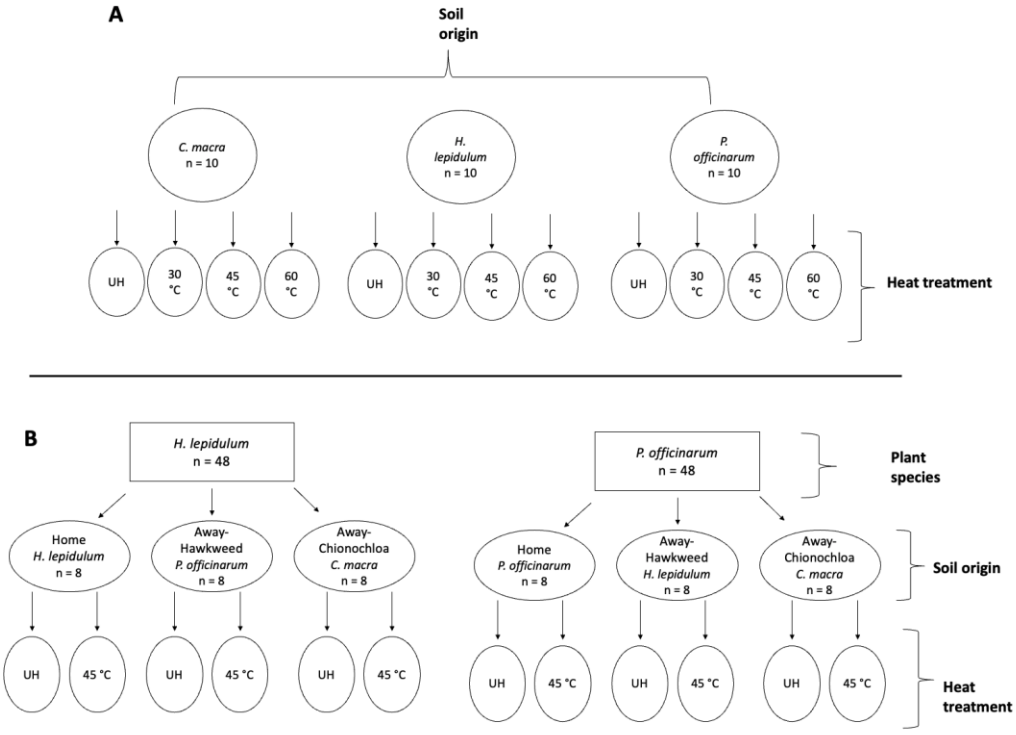

Figure 2.1. Outline of the experimental design of the three experiments; DNA sequencing of soils analyses, hawkweed plant growth study, and soil carbon measurement. A) shows the setup of the DNA sequencing of the soils experiment and soil carbon analyses. Soil origin refers to the soil collected underneath target plant species. Heat treatment refers to the heat treatment applied on the soils under laboratory conditions. There were four heat treatments, Unheated (UH) 30°C, 45°C and 60°C. N = 120. B) This shows the set-up of the plant growth study of two hawkweeds; *Pilosella officinarum* and *Hieracium lepidulum*. Soil origin refers to the soil collected underneath target plant species. Heat treatment refers to the heat treatment applied on the soils under laboratory conditions. For this experiment, there were two heat treatments, UH and 45°C. N = 96

2.2.2. Soil molecular analyses

Soil DNA extraction occurred on the day following heating. DNA was extracted from 250 mg of soil using the Qiagen DNeasy PowerSoil kit following the manufacturer's instructions, and the Vortex-2-Genie bench vortex was used for the lysis step. The concentration and purity of the DNA was estimated using a NanoVue UV-visible

spectrometer (GE Healthcare). During the initial DNA extraction optimisation, a subset of samples were visualised on a 1.0 % agarose gel made with 1X Tris/borate/EDTA and stained with 2.5 µL of 10 mg/mL ethidium bromide. Electrophoresis was carried out at 75 volts for 60 minutes.

Prior to PCR, all DNA extracts were standardised to 5 ng/µL by diluting with nuclease-free water. Glomeromycotina DNA was amplified from the DNA extracts using the small ribosomal subunit (18S) rRNA primers WANDA (5'-CAG CCG CGG TAA TTC CAG CT-3'; Dumbrell et al. 2011) and AML2 (5'- GAA CCC AAA CAC TTT GGT TTC C-3'; Lee et al. 2008). These primers were modified to include Illumina flow cell adaptor sequences. Each reaction consisted of 12.5 µL of Kappa HIFI HotStart Readymix (Kapa Biosystems, Wilmington, MA, United States), 5 µL primers at 1 µM concentration, and 2.5 µL of genomic DNA. The total volume of the PCR was 25 µL, with all PCRs performed on 96 well plates. The PCR conditions consisted of an initial denaturation at 95°C for 2 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes (Lekberg et al. 2018). AM DNA acquired from John Ramana at the University of Canterbury was used as a positive control. The PCR products were cleaned using AMPure XP beads (Beckman-Coulter, Brea, CA, United States) following the protocol available from (Jackson 2016).

Illumina MiSeq libraries were prepared and sequenced as per the manufacturer's protocol (Metagenomic Sequencing Library Preparation Part # 15044223 Rev. B; Illumina, San Diego, CA, USA). DNA in PCR products was quantified using the Qubit dsDNA High Sensitivity assay kit (Invitrogen, Waltham, MA, USA). Index adaptors for Illumina MiSeq sequencing were added via a second PCR step. Each reaction had 12.5 µL of Kappa HIFI HotStart Readymix, 2.5 µL of each forward and reverse indexing

primers at 1 µM, 2.5 µL of DNA template and 5 µL of nuclease-free water. These PCR conditions consisted of an initial denaturation at 95 °C for 3 minutes, then 8 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes. Following PCR, libraries on each plate were pooled into one tube. These were cleaned twice using AMPure XP beads, then quantified using the Qubit dsDNA High Sensitivity assay kit. The sequence quality and length were assessed by running samples on a bioanalyzer (Agilent, Santa Clara, CA, USA).

2.2.3. Bioinformatics

Raw sequence amplicons for the Glomeromycotina 18S rRNA gene were processed using the Divisive Amplicon Denoising Algorithm (DADA2) pipeline in R 4.0.4 (Callahan et al. 2016, R Core Team 2020). Due to merging issues, only the forward reads were used. Sequence data was first processed through the initial steps of the DADA2 ITS pipeline to remove ambiguous bases (Ns). Primer sequences were removed using cutadapt v 3.3 (Martin 2011). For the remaining steps, reads were processed following the DADA2 v 1.4 tutorial (Callahan n.d.). Briefly, forward reads were trimmed to 280 bp and filtered by removing ambiguous reads that exceed a maximum expected error of two. The remaining reads were used to train an error model, and unique sequences were obtained through dereplication. The trained error model was used on the dereplicated sequences to infer sequence variants. Chimeric sequences were removed from the remaining sequence variants and used to build an ASV counts table. In total, 2,337,080 reads and 11,278 ASVs were generated following DADA2 processing for the 120 samples and controls.

Taxonomy was assigned to the ASVs using the Ribosomal Database Project classifier, where bootstrapping was set to a minimum of 80 (Wang and Qiu 2006). A compiled FASTA file of the MaarjAM database (Accessed 2019) provided by Dr Cameron Egan from Okanagan College, Canada, was used as the reference database because of difficulties obtaining it directly from the database curator (Öpik et al. 2010). Positive controls were all identified as arbuscular mycorrhizae and were removed from subsequent analyses. Negative controls were checked for PCR contaminants using the R package decontam (Davis et al. 2018). This compares the abundance of sequences between the libraries of true samples and PCR controls and removes samples that are likely contaminants. No contaminants were detected using this method. ASVs that were not identified in the Subphylum Glomeromycotina were removed, leaving a total of 5,164 ASVs.

Due to the large variation in sequencing depth among samples (Figure AB.1), samples were rarefied to 2,500 reads per sample using the ‘Rarefy’ function from GUniFrac (Chen 2021). This number was chosen to maintain a reasonable sample read depth. Following rarefaction, ASVs present in fewer than two samples were removed.

2.3. Plant growth chamber experiment of invasive hawkweeds *P. officinarum* and *H. lepidulum*

A plant growth experiment was set up to test hypotheses regarding the growth of H. lepidulum and P. officinarum (H3-H5) (Figure 2.1B). The experimental design was fully factorial with two treatments: soil origin and heat. Soil origin had three levels: 'Home'; hawkweeds species grown in their own soil; 'Away-H'; hawkweeds grown in the other hawkweed species soil, and 'Away-C'; hawkweeds grown in *C. macra* soil. Heat treatment had two levels, including unheated and 45°C. Due to growth chamber space restrictions, only two of the heat treatments were tested for this experiment. Soil samples were pooled by origin prior to heating (described below). Altogether, my study had eight individual plants per origin-heat combination per species (i.e., replicates) for 48 experimental units for each plant species and 96 plants in total Figure 2.1B.

Seedlings were pre-germinated in the following manner. Hawkweed seeds of *H. lepidulum* and *P. officinarum* were surface disinfected with 1% bleach for 5 minutes and rinsed with Millipore water. Surface-disinfection trials were conducted to determine the appropriate time as discussed (Appendix A; Table A1.1). The seeds were grown in an autoclaved (2x 121°C for 30 minutes) sand-pumice mix for three weeks before transplantation into their respective soil-heat treatment. During this time, the seedlings were watered with Millipore water twice weekly and grown underneath 55 W Starlite 6400K twin fluorescent tubes for a 16/8 day/night photo period at 21°C.

The experiment was set up as follows. All potting equipment was soaked in diluted bleach for a minimum of 20 minutes, rinsed and air dried to avoid cross contamination. Further, the workspace was scrubbed and disinfected with Trigene 1:20 between preparation and potting of each soil inoculum. Soil was thawed for 72 hours at 4°C. Individual soil samples from each origin were then pooled and sieved (5 mm) to

remove large debris. These samples were halved and placed into one of the two heat treatments. Soil was then placed in aluminium trays at a 5 mm depth and covered with tin foil. The soil heat treatment consisted of 10 minutes at 45°C in a drying oven. Unheated soil was left at room temperature. All soil was rested at room temperature overnight before potting.

For potting, soil of each origin-heat treatment combination was mixed in a 2:1:1 ratio with autoclaved pumice and sand and added to pots (300 mL) on saucers. Individual seedlings were transplanted into individual pot of their respective soil origin treatment. Pots were randomly allocated using a random number generator in R and were re-randomised four times during the 12-week experiment. Plants were watered with Millipore water twice weekly and grown underneath 55 W Starlite 6400K twin fluorescent tube for a 16/8 day/night photo period with an air temperature of 21°C.

At the end of the growth chamber study at 12 weeks, I observed 100% seedling survival. Seedlings were then destructively harvested and the roots and shoots separated. The roots were then cleaned by washing with tap water and patting dry with paper towels. Roots and shoots were dried in a drying oven (65°C, 48 hours). All roots and shoots were weighed on a four decimal place balance.

2.4. Carbon measurement of tussock grassland soils under simulated fire

To test hypotheses regarding grassland soil carbon levels (H6-H7), an experiment was set up, as shown in Figure 2.1B. Soil was then heated, as explained in section 2.2.1. In preparation for the carbon analysis, soils were air dried for 48 hours, then ground and sieved (< 2 mm). Carbon (%) in soil was measured using Vario-Max CN elemental analyser (Elementar GmbH, Germany) at Lincoln University. This method measures total soil carbon as a percentage, including carbon present in organic matter, charcoal and carbonate.

2.5. Statistical data analyses

All statistical analyses were performed in R 4.0.4, and graphs were made using ggplot2 (Wickham 2016, R Core Team 2020). The relative abundance (percentage of reads) of AM fungi in each order was calculated for each sample. These were averaged per soil origin-heat combination to give a mean relative abundance. The results were visualised as a stacked bar graph.

A generalised linear model was constructed to test if soil origin and simulated fire impacted AM fungal species richness (number of ASVs). Soil origin and heat treatment were used as predictors, and AM fungal ASV richness was specified as a Poisson response because it is a count variable. An interaction term was specified between soil origin and heat. The emmeans R package was used to perform *post hoc* pairwise comparisons of the soil origin-heat combinations (Lenth 2021). Predicted values and confidence intervals were computed using the ggeffects package (Lüdecke 2018).

To test if soil origin and simulated fire impacted AM fungal (ASV) community composition, a principal coordinate analysis (PCoA) with Bray-Curtis distances was performed. The Bray-Curtis dissimilarity matrix was created using the 'vegdist' function from the R package vegan (Oksanen et al. 2020), and the 'pco' function in the labdsv R package was used to perform the PCoA (Roberts 2019). Effects of soil origin and heat on AM fungal community composition were tested by conducting a permutational multivariate analysis of variance (PERMANOVA) using soil origin and heat treatment as predictor variables. The 'adonis' function was used, specifying 999 permutations on a Bray Curtis dissimilarity matrix (Oksanen et al. 2020).

Linear models were used to assess the impacts of soil origin and heat treatment on plant growth of the two hawkweed species. I ran separate models for each species

for two plant variables: plant biomass and root-shoot ratio. An interaction term was specified between the predictor's soil origin and heat treatment. *P*-values of the models were corrected for multiple models with the same replicates using the false discovery method. Model assumptions were evaluated using diagnostic plots and Levene's test (Fox and Weisberg 2019). Plant biomass for *P. officinarum* was log-transformed to meet assumptions of normality. Predicted values and confidence intervals were computed using the ggeffects package (Lüdecke 2018). Significant differences in plant growth responses between treatments were determined using pairwise comparisons with Tukey's *P*-value adjustment using functions within the emmeans package (Lenth 2021).

Differences in soil carbon under differing soil origin and/or under simulated fire activity were tested using a linear model. An interaction term was specified between predictors soil origin and heat treatment. The assumptions of the model were checked using diagnostic plots and Levene's test (Fox and Weisberg 2019). Predicted soil carbon values and associated confidence intervals were computed using the ggeffects package (Lüdecke 2018). The model was investigated further using pairwise comparisons with a Tukey adjustment (Lenth 2021).

Chapter 3. Results

3.1. Arbuscular mycorrhizal fungal communities

3.1.1. Bioinformatics results

There were two main issues that I faced processing my Illumina MiSeq sequences through dada2. The first was having no samples retained after merging. I attribute the first issue to the large amplicon size that was amplified using the WANDA /AML2 primer pair (550 bp +- 5bp), including the primers' sequence (Lee et al. 2008, Dumbrell et al. 2011, Egan 2017). Poor sequence quality at the 3’ end of the reverse reads required trimming of between 50-100 bp to maintain the recommended quality score of 30 (Callahan et al. 2016). Trimming this much of the reverse reads did not allow merging in later steps. I also tried not trimming my reads but instead reducing the maximum number of expected errors, anticipating that this would only allow sequences of good quality through that would be large enough to merge. This produced merged reads of variable size (Figure 3.1). My target region for sequencing was the small ribosomal subunit (18S), which is a conserved size compared to other fungal metabarcoding regions such as the internal transcribed spacer (Thiéry et al. 2016). The distribution in the sizes of merged reads appears inappropriate for analysis of the 18S region and prompted my decision only to process the forward reads as presented in my thesis (Figure 3.1).

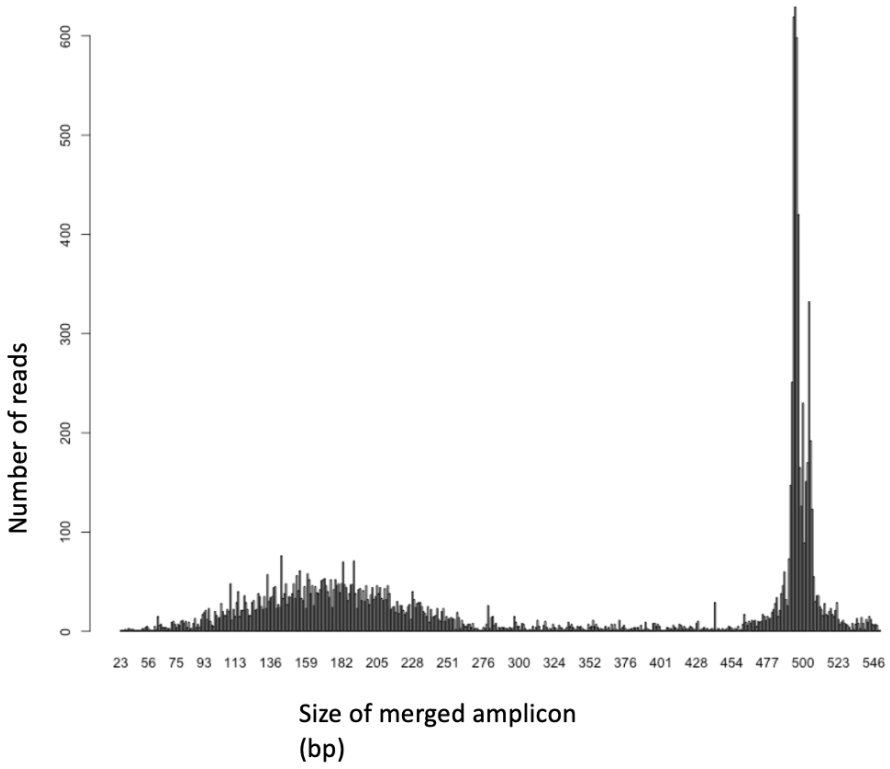

Figure 3.1.Distribution of merged reads of arbuscular mycorrhizal sequencing data from MiSeq Illumina (18S) when reads were not trimmed.

The rarefaction results of the samples resulted in the loss of 14 samples that did not meet the criterion of 2500 reads per sample depth (Figure 3.2 and Table 3.1). Rarefaction and the removal of low abundant ASVs also reduced the number of ASVs in the dataset to 792. The remaining dataset used for subsequent statistical analyses comprised of 792 ASVs and 106 samples.

Table 3.1. Number of samples and ASVs retained after rarefaction and removal of low abundant ASVs (those present in < 2 samples).

Soil origin	Heat treatment				Total samples
	Unheated	30°C	45°C	60°C	
C. macra	10	9	10	9	38
H. lepidulum	6	9	7	9	31
P. officinarum	10	9	8	10	37
Total samples	26	27	25	28	106

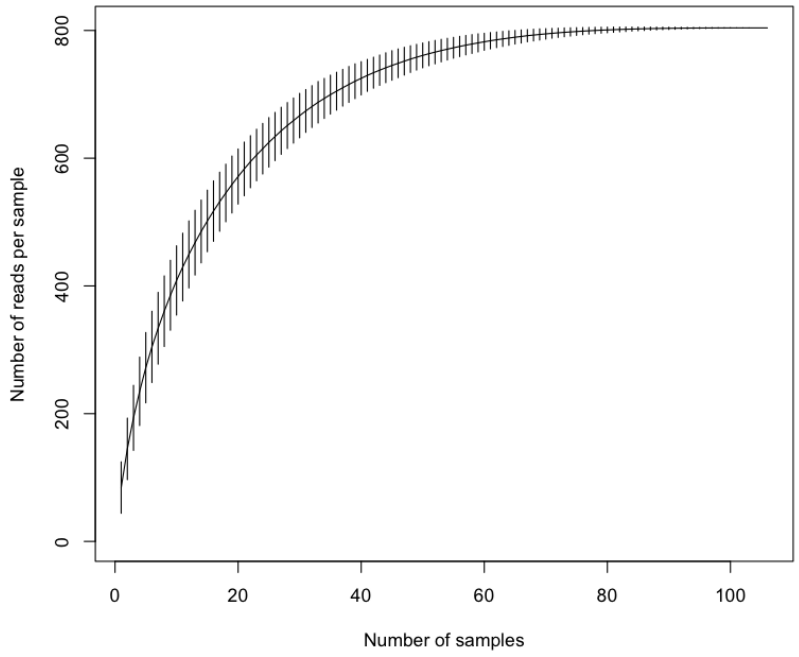

Figure 3.2. ASV accumulation curve of 18S AMF soil samples rarefied to 2500 reads per sample = Number of samples = 106.

3.1.2. Description of AM fungi communities

Of the 792 ASVs, at least 70% for each soil origin heat combination could not be classified as belonging to any of the three AM fungal taxonomic orders, as shown in (Figure 3.3). In terms of abundances of different orders, *Archaeosporales* was in the highest abundance in soils of *P. officinarum* and lowest in soils of *C. macra*. ASVs classified as *Glomerales* and *Diversisporales* appeared consistent in number across all soil origins. *Paraglomerales* occurred in < 1% abundance in all soil origin-heat treatment combinations but appeared slightly higher in soils of *P. officinarum*. There did not appear to be differences in taxonomy based on heat treatment.

The mean AM fungal ASV richness per sample was 84 (SD = 20, range = 34 - 137). Soil origin and fire had differential effects on AM fungal ASV richness (Figure 3.4). There were differences in richness between soil origin of plant species, and in some cases, significance varied depending on heat treatment. In *C. macra* soils, AM fungal richness was significantly lower at 60°C compared to the three other soil temperatures, but there were no differences in richness between the Unheated, 30°C, and 45°C soils. In *H. lepidulum* soils, there was no difference in richness between any of the heat treatments. In *P. officinarum* soils, there was no difference between Unheated, 45 °C and 60 °C heat treatments; however, at 30 °C, richness was significantly lower than the other heat treatments.

Results from the PCoA and PERMANOVA showed AM fungal community composition differed by soil origin but not by heat treatment (Figure 3.5, Table 3.2). For the PCoA, samples were clustered in ordination space based on soil origin. There were compositional differences among the samples of different soil origins but not among the heat treatments (Figure 3.5). The PERMANOVA showed soil origin explained 13% of the

variation in AM fungal composition (Table 3.2). Heat treatment had no effect on AM fungal composition.

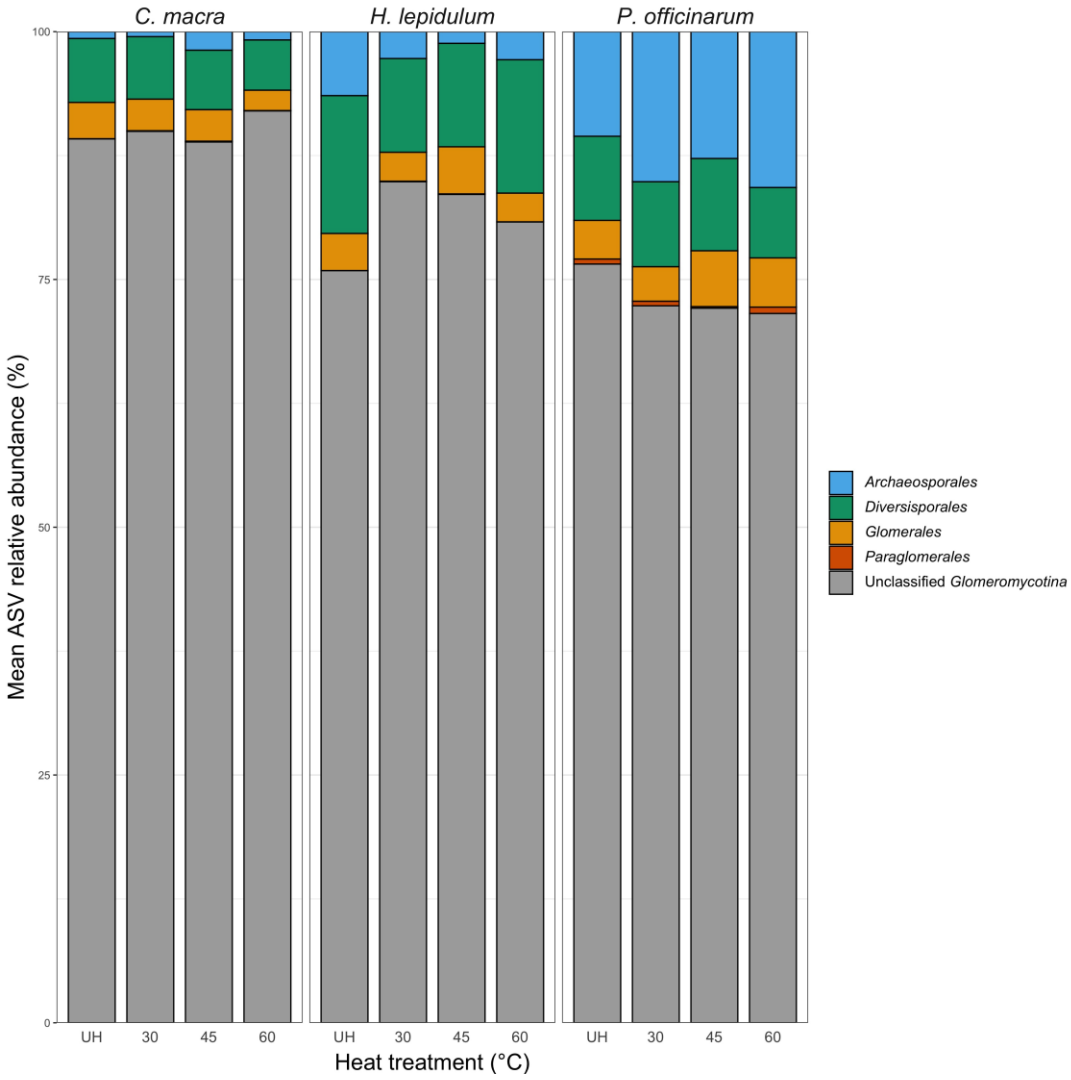

Figure 3.3. Mean relative abundance (percentage of reads) of different soil AM fungal ASVs grouped by taxonomic order (fill colours) separated by heat and soil origin experimental treatments. UH refers to unheated soil.

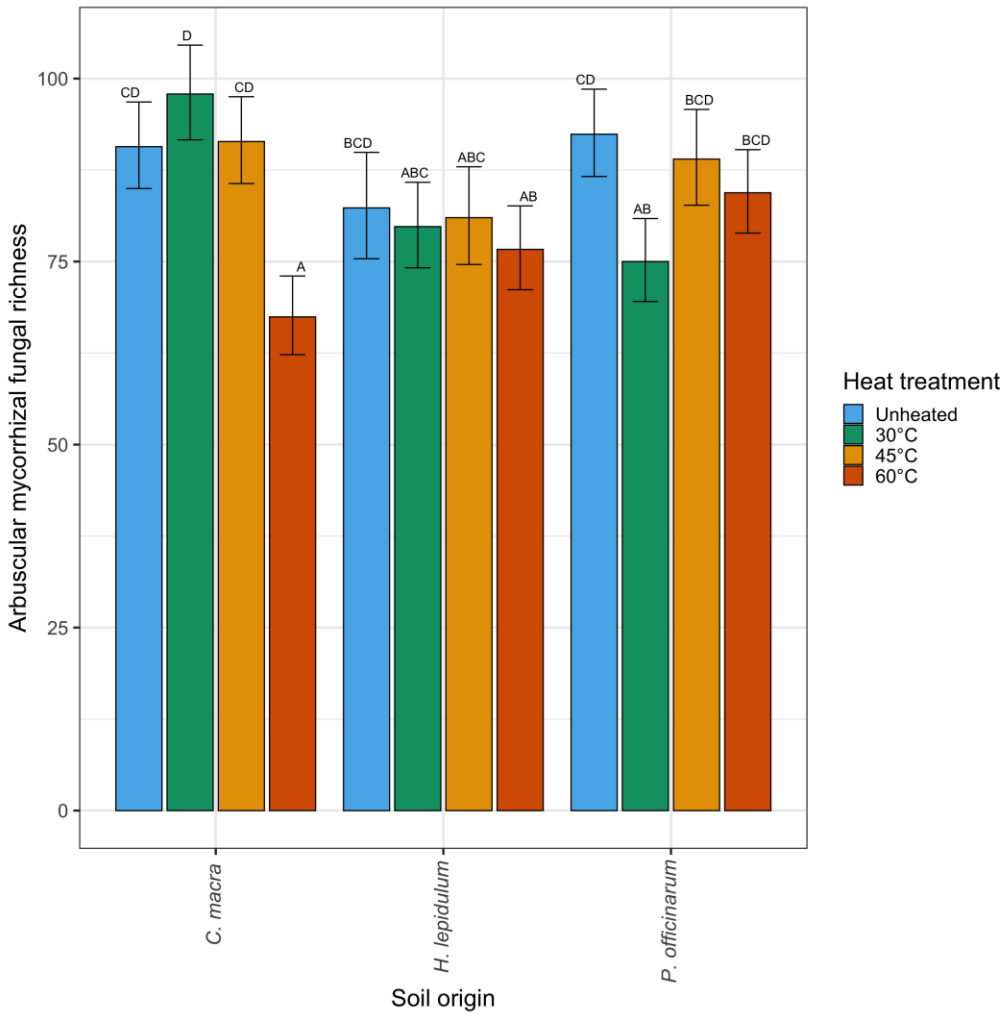

Figure 3.4 Bar graph of the values of soil arbuscular mycorrhizal fungal richness (number of ASVs) by soil origin from (*Chionochloa macra, Hieracium lepidulum and Pilosella officinarum*) and heat treatments (Unheated, 30°C, 45°C, and 60°C). Values on the y-axis represent predicted richness values from a generalised linear model with 95% confidence intervals represented by error bars. Heat treatment is represented by colour fill. Letters above bars indicate statistically significant differences based on the Tukey Post hoc comparison *P*-value < 0.05. N = 120.

Table 3.2. Permutational multivariate analysis of variance (PERMANOVA) table for arbuscular mycorrhizal fungal communities. Predictors are soil origin (*Chionochloa macra*, *Hieracium lepidulum* and *Pilosella officinarum*) and heat treatment (Unheated, 30°C, 45°C, and 60°C). Significant sources of variation are bolded and based on 999 permutations. df: degrees of freedom; SS: sums of squares; MS: mean sum of squares.

Source of variation	Variation explained (%)	df	SS	MS	Pseudo F	P
Soil origin	**13**	**2**	**4.45**	**2.22**	**7.69**	**0.001**
Heat treatment	2	3	0.67	0.22	0.77	0.933
Soil origin x Heat treatment	3	6	1.14	0.19	0.65	1.000
Residuals	81	94	27.30	0.29		
Total	100	105	33.51			

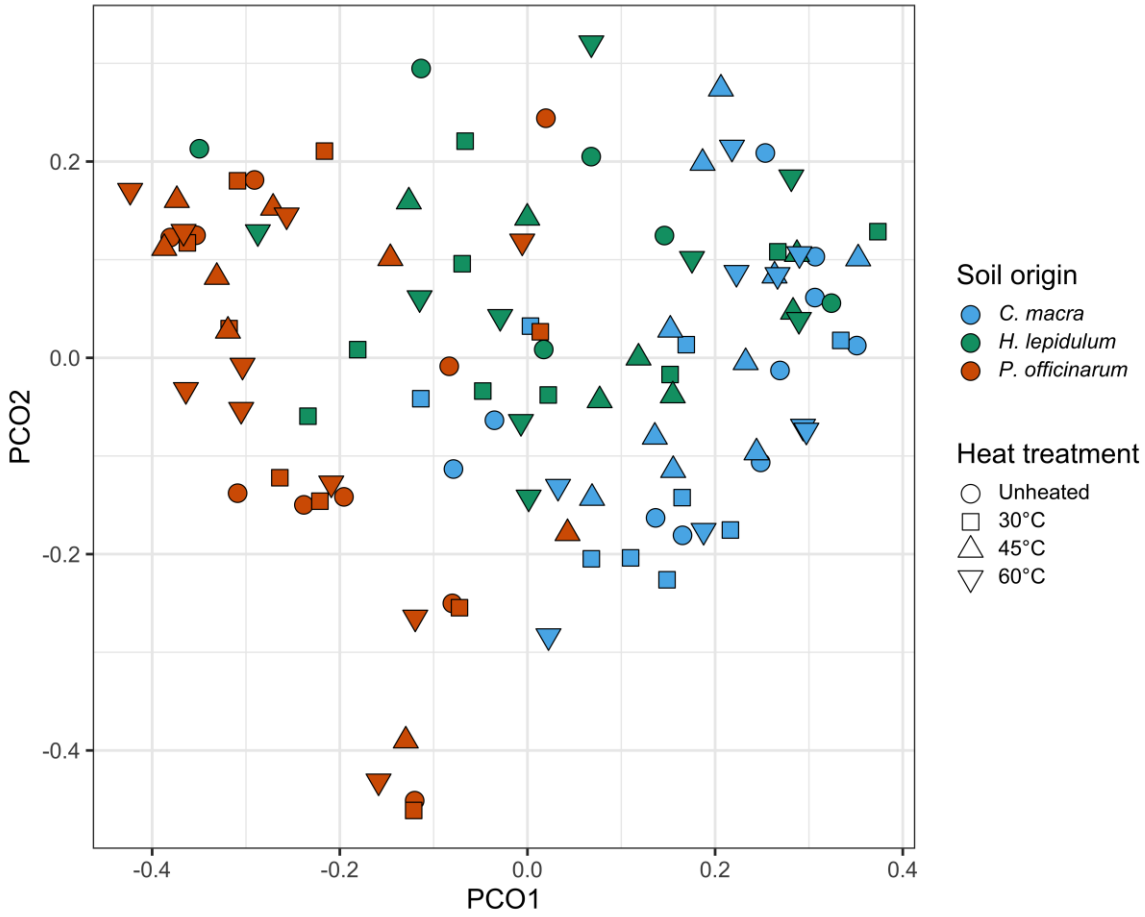

Figure 3.5. Principal coordinate analysis plot showing the community composition of soil arbuscular mycorrhizal fungi by different soil origins (*Chionochloa macra*, *Hieracium lepidulum* and *Pilosella officinarum*) and heat treatments (Unheated, 30°C, 45°C, and 60°C). Colour indicates the soil origin and shape indicates heat treatment.

3.2. Hawkweed plant growth chamber study

For the plant growth study, in most instances, linear model results and *post hoc* pairwise comparisons showed that soil origin was the only important factor influencing plant biomass and root-shoot ratios of *H. lepidulum* and *P. officinarum*. Both species grew better in *P. officinarum* soil. For *H*. *lepidulum*, soil origin, but not heat, had a significant influence on plant biomass (Soil origin: F-value = 17.75, *P*-value = 0.006; Heat treatment: F-value = 0.744, *P*-value = 0.456; Figure 3.6a, Table AB.2). The interaction between soil and heat was not significant (Soil origin:Heat F-value = 2.943, *P*-value = 0.1908,). *Post hoc* pairwise comparisons showed plant biomass was consistently higher in *H. lepidulum* grown in away-H (*P. officinarum*) 45°C soil compared to those grown in Home unheated and 45°C soils (*P*-value < 0.05). *Hieracium lepidulum* that was grown in away-H 45°C soil also had higher biomass than seedlings grown in Away-*C. macra* unheated soil (*P*-value < 0.01) but not Away-*C* 45 °C soil (*P*-value > 0.05). *Hieracium lepidulum* grown in Away-*C* 45 °C soil had consistently higher biomass than those grown in Home 45 °C soil (*P*-value < 0.5), but not Home Unheated soil (*P*-value > 0.05). There were no significant differences in root-shoot ratios of *H. lepidulum* in the different heat treatments (Figure 3.6c; *P*-value > 0.05).

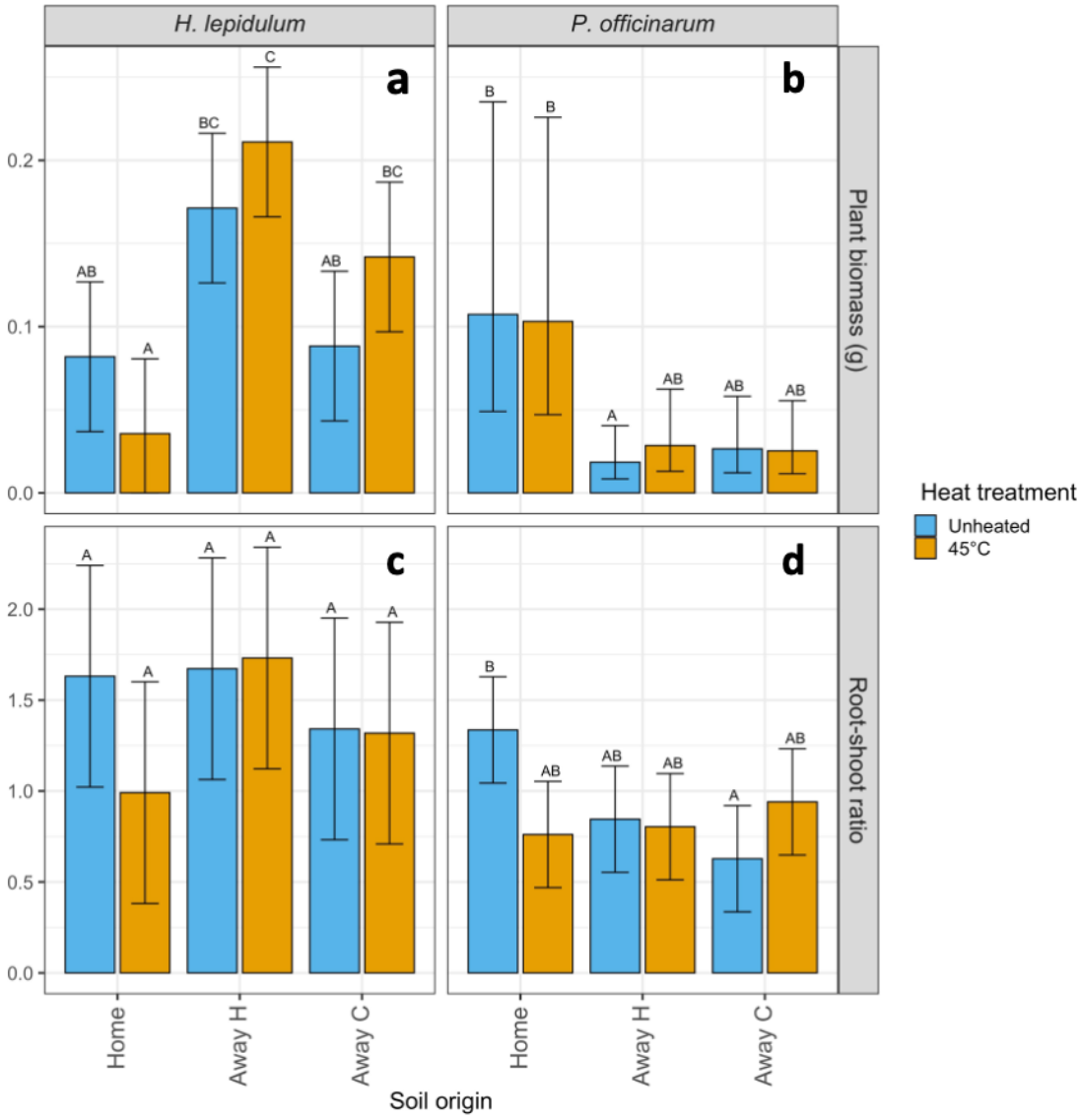

Figure 3.6. Total plant biomass and root-shoot ratios of *Hieracium lepidulum* and *Pilosella officinarum.* Soil origin had three levels: 'Home': plant species grown in their own soil; 'Away-H': hawkweeds grown in the other hawkweed species' soil; and 'Away-C': hawkweeds grown in *C. macra* soil. Heat treatment had two levels, including unheated and 45°C and is represented by colour fill. Values on the y-axis represent predicted values of either plant biomass (a,b) or root-shoot ratios (c,d) from a linear model with 95% confidence intervals represented by error bars. Lower case letters in the right-hand corner of the graphs indicate the different measures of plant biomass and root-shoot ratios for the two plant species. Upper case letters above bars indicate statistically significant differences based on the Tukey *Post hoc* comparison *P*-value < 0.05. N = 96.

Similar to *H. lepidulum*, plant biomass of *P. officinarum* differed by soil origin but not heat (Soil origin: *F*-value = 9.489, *P*-value = 0.002; Heat treatment: *F*-value = 0.133, *P*-value = 0.779; Figure 3.6b). Additionally, the interaction between soil and heat was not significant (Soil origin:Heat treatment *F*-value = 0.251, *P*-value = 0.779). *Post hoc* pairwise comparisons showed *P. officinarum* seedlings had the highest plant biomass in Home soils. Plant biomass in Away-H unheated soils were significantly lower than Home unheated (*P*-value = 0.03) and Home 45 °C (*P*-value = 0.04) soils. However, there was no difference in biomass between Home soils and Away-H 45 °C (*P*-value > 0.05), Away-C unheated (*P*-value > 0.05), and Away-C 45 °C soils (*P*-value > 0.05). For root-shoot ratios, there was a significant interaction between soil origin and heat that indicated that the impacts of soil depended on heat (F-value = 4.76, *P*-value = 0.014). However, *post hoc* pairwise comparisons revealed that only Home unheated soils were significantly higher than Away-C unheated soils (Figure 3.6d; *P*-value = 0.015).

3.3. Soil carbon

Percent soil carbon ranged from 1.41 – 8.85% of the total volume of soil. There were no significant differences in total soil carbon among different soil origins or heat treatments (Figure 3.7), and although total soil carbon under *C. macra* was marginally higher than both hawkweeds, this was not statistically significant.

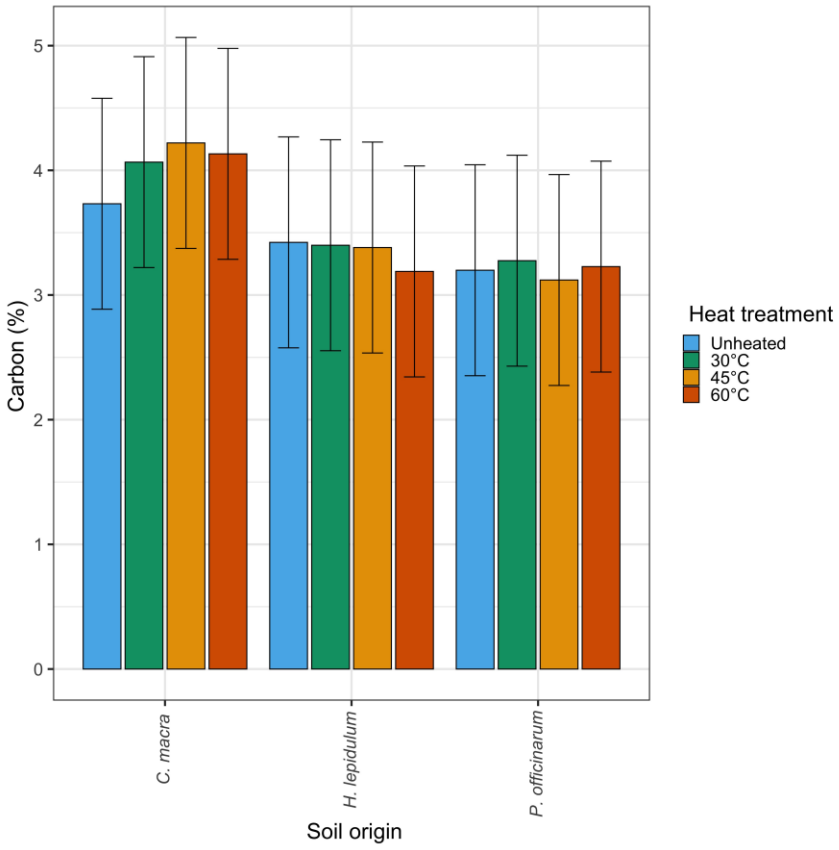

Figure 3.7. Bar graph of soil carbon (%) by soil origin and heat treatment. Soil origin indicates the origin of the soil collected under plants (*Chionochloa macra, Hieracium lepidulum* and *Pilosella officinarum*) and heat treatments indicate soil heat treatments (Unheated, 30°C, 45°C, and 60°C). Values on the y-axis are predicted values based on a linear model with 95% confidence intervals represented by error bars. Heat treatments are represented by colour fill. N = 120.

Chapter 4. Discussion

The findings from my study were consistent with my hypotheses regarding soil AM fungal richness and community composition. Soil origin was the major driver of AM fungal richness and community composition (H1), and soil richness was reduced under some heat treatments (H2). Contrary to my hypothesis that the biomass of hawkweeds would not differ between different soil origins (H3), I found that both *H. lepidulum* and *P. officinarum* seedlings had the highest biomass in *P. officinarum* soil. Soil heating had surprisingly little impact on both above and below-ground responses of invasive hawkweeds (H4, H5). This indicates that the invasion of *H. lepidulum* may be facilitated by the existing invasion of *P. officinarum* in tussock grasslands. Interestingly, my results were inconsistent with my last two hypotheses and showed that soil carbon levels did not differ by soil origin or heat treatment (H6, H7). The results from this study suggest that soil origin is a major driver of plant-soil interactions in New Zealand's tussock grasslands. These results have implications for the ecology of hawkweed invasion in tussock grasslands and AM research in New Zealand.

4.1. Arbuscular mycorrhizal richness and community composition

Results from the PCoA and PERMANOVA supported my first hypothesis (H1) that AM fungal community composition differed with soil origin. I originally predicted this would occur due to plant-soil feedbacks (Bever et al. 2009). Throughout the literature, there is strong evidence of selectivity in the relationships between plants and their soil microbial communities (van der Heijden et al. 1998, Bever et al. 2009, 2012, Pringle et al. 2009, Yang et al. 2012, Torrecillas et al. 2012). Heat did not appear to influence composition

and only impacted richness in some cases. In general, our understanding of the impacts of fire on AM fungal richness and composition is varied.

My results suggest that AM fungi associated with hawkweeds and native tussock are differentially affected by fire. This partially supports my hypothesis that there would be lower AM richness at higher temperatures (H2). In *C*. *macra* soils, AM fungal richness declined significantly at the highest heat treatment (60°C) but not at the two lower heat treatments (30 and 45°C). This is in contrast to oils collected under *H. lepidulum* plants, where AM fungal richness did not vary significantly in response to heat. Finally, soils of *P. officinarum* origin had lower AM richness in the 30°C heat treatment. This was significantly lower compared to the unheated, 45°C, and 60°C soil heat treatments, which did not vary significantly from each other. These results suggest that the AM fungal communities associated with the three plant species had differential responses to heat. In *C. macra* soils, AM fungal richness began to decline at the highest heat treatment (60°C) but remained unaffected at the lower temperatures (30 and 45°C). This could be because AM fungal mortality began to occur at this temperature (Bellgard et al. 1994, Dove and Hart 2017).

A recent meta-analysis investigating the effects of fire on mycorrhizal fungi showed that fire caused an initial decline in the richness of AM fungi but was able to recover completely after 11 years (Dove and Hart 2017). These declines were thought to result from heat death but could also be from potential losses of host vegetation (Xiang et al. 2015, Dove and Hart 2017). Most literature has shown that AM communities are capable of recovering to pre-fire levels over a decade or more (Xiang et al. 2015, Dove and Hart 2017). The timeframe for recovery varies and is linked to the regeneration of the pre-existing plant communities (Xiang et al. 2015, Dove and Hart 2017). In comparison to *C. macra*, the AM communities in the soils of the hawkweeds appeared less sensitive to the

soil heat treatment. These results have important implications for invasion in tussock grasslands and suggest that the AM fungi associated with native tussock *C.macra* are more sensitive to fire than those of the invasive hawkweeds *P. officinarum* and *H. lepidulum*.

4.2. Plant biomass

Hawkweed biomass differed by soil origin but not heat in my growth chamber study (H3). Interestingly, both hawkweeds had the highest plant biomass when grown in *P. officinarum* soils. This is in contrast to my hypothesis that hawkweeds would have similar plant biomass in all soil origins and supports the idea that both species may have been getting positive plant soil feedbacks in *P*. *officinarum* soils (Bever et al. 2012). Considering both hawkweeds are highly mycorrhizal species, these benefits could be coming from AM fungi in *P. officinarum* soil (Downs and Radford 2005, Wang and Qiu 2006, Roberts et al. 2009, Höpfner et al. 2015).

The result that *H. lepidulum* had higher plant biomass in *P. officinarum* soils compared to its own soils suggests two potential scenarios. The first could be that *H. lepidulum* is experiencing positive plant-soil feedback in the soils of *P. officinarum*. This is a plausible scenario when you consider the invasion history of both species in New Zealand's tussock grasslands. *Hieracium lepidulum,* which was first identified in New Zealand in the 1940s and become widely distributed by the 1970s is a more recent invader (Wiser and Allen 2000, Day and Buckley 2011). A most recent survey by Day and Buckley (2011), showed it has increased its range across tussock grasslands. This is compared to *P. officinarum,* which has been present in tussock grasslands since 1864, but did not draw significant attention as an invasive species until the late 1960s to early 1970s (Treskonova 1991, Espie 2001, French 2021). Hence over time, one might

expect it to have evolved strategies that have allowed it to persist in this environment (Treskonova 1991, Espie 2001, Day and Buckley 2011, Steer and Norton 2013). One mechanism could involve the formation of relationships with soil mutualists, including AM fungi (Pringle et al. 2009). As a more recent invader to tussock grasslands, *H. lepidulum* may be benefiting from mutualists of *P. officinarum* (Wiser and Allen 2000). Though *P. officinarum* is no longer placed in the genus *Hieracium,* both plants remain biologically similar (French 2021); therefore, it is not unreasonable to assume that *H. lepidulum* could gain growth benefits associating with mutualists of *P. officinarum* (Pringle et al. 2009). The scenario of potential shared symbionts supports the idea of invasional meltdown (Simberloff and Holle 1999), whereby initial invasion by *P. officinarum* could have facilitated subsequent invasion by *H. lepidulum*. Spreading of *H. lepidulum* into areas where *P. officinarum* has been present for decades supports this idea (Day and Buckley 2011). Alternatively, *H. lepidulum* might be an AM fungal generalist, which is a plant species that benefits from associating with a range of AM fungal taxa (Pringle et al. 2009).

Another scenario is perhaps *P. officinarum* plants are changing the soil's abiotic conditions to those that support the growth of both species. There is some evidence to support the theory that hawkweeds modify the abiotic conditions of the soil (Scott 1975, McIntosh et al. 1995, Knicker et al. 2000). Soil pH underneath *P. officinarum* plant mats was significantly lower than the neighbouring tussock or pasture species(Scott 1975, McIntosh et al. 1995). Soil pH can have significant effects on plant growth, soil microbial growth and is one of the largest drivers of soil microbial community composition (Alguacil et al. 2016, Neina 2019). Lower soil pH can reduce the availability of cations, which affects the availability of essential plant nutrients such as phosphorus (Scott 1975).

There have been earlier studies that looked into the allelopathic potential of *P. officinarum* plants (Makepeace 1980, Henn et al. 1988). Two phytotoxic compounds, umbelliferon and apigenin-glucoside, were discovered in the roots and rhizomes of *P. officinarum* plants (Makepeace 1980, Henn et al. 1988). However, these compounds were undetectable in the soil. Suggesting more work would be needed to ascertain a link between these chemical changes and *P. officinarum*, and whether or not they provide growth or competitive benefits to *P. officinarum* or related species. It is also unclear whether these compounds have an impact on AM fungi.

Another potential explanation for biomass differences is *H. lepidulum* could be experiencing negative plant-soil feedback in its own soil. A well-known cause of negative plant-soil feedback is the accumulation of pathogens (Bennett and Klironomos 2019). This could not be ruled out in this study as pathogen species were not specifically investigated. However, this scenario does seem less likely as *H. lepidulum* is a newer invader to tussock grasslands; therefore, according to the enemy release hypothesis, it would not be expected to accumulated pathogens as rapidly as *P. officinarum* (Keane and Crawley 2002, Day et al. 2015). Nevertheless, this could be a possible explanation for the observations in this study. Further investigation would be needed to confirm this.

4.3. Fire and hawkweed invasion in tussock grasslands

My study showed plant biomass of hawkweeds was not affected by heating soil (H4). This is interesting as it supports the idea that both hawkweeds would not have problems establishing after a fire (Treskonova 1991, Bosch et al. 1996). Fire kills or damages existing vegetation (McLauchlan et al. 2020). Though tussocks are capable of resprouting after fire, other alpine species are more sensitive (Gitay et al. 1992). Resprouting tussocks also have to compete with invasive hawkweeds, which are faster

growing than native tussock, are already in high abundance in most tussock grasslands and tolerate the high-country conditions well (Makepeace 1985, Treskonova 1991, Moen and Meurk 2001, Day and Buckley 2011). They reproduce annually and do not rely on infrequent masting events that are a feature of tussock reproduction (Makepeace 1980, Schauber et al. 2002, Turnbull et al. 2012). The fact that both hawkweeds have wind-dispersed seeds is also an advantage in a high-country environment. This will facilitate seeds travelling to burnt areas after fire (Makepeace 1985, Steer and Norton 2013). *Pilosella officinarum*'s ability to form stolons that result in vegetative mats will also aid its spread in burnt areas (Makepeace 1985).

It was surprising that my results showed that heating soil did not cause declines in soil carbon (H6). Generally, fire is associated with declines in soil carbon due to pyrolysis and combustion. Reasons could include soil heat treatments did not reach high enough temperatures to cause declines in soil carbon. There is little information about the effect of fire on soil in tussock grasslands.

Carbon content from soil collected from *C. macra* compared to soil from hawkweeds showed little difference. While the average carbon content in C.macra was higher, this was not statistically significant (H7). These results contrast those from Scott et al., who found soil carbon levels were higher under *Hieracium* species compared to neighbouring tussock grasslands (2001). There is evidence that tussock grasslands hold important carbon reserves. (Ross et al. 1996, Wakelin et al. 2013). The differences seen in carbon levels between my study and Scott et al. could indicate that carbon levels in tussock grassland are modified by hawkweed invasion. Both studies have highlighted changes in carbon sequestration between different species in tussock grasslands.

4.4. Strengths, limitations, and recommendations of this study

My study is the first to examine the impacts of soil origin and heat on the plant-soil relationship between invasive hawkweeds and AM fungi. Combining both a sequencing component and a glasshouse study is powerful as it gives us a better understanding of the plant-soil relationship between invasive hawkweeds and AM fungi and how they may be affected by fire. This study also addressed the effects of multiple global change factors, plant invasion and fire, on the plant-soil relationship. A recent paper by Rillig et al. highlighted the importance of addressing multiple factors, as the combinations of the process occurring tended to have different directional effects on soil process and properties (2019).

One consideration that applies to the sequencing data is that AM communities were sequenced from soil and not roots. AM fungal communities from roots originate from internal AM structures such as intraradical hyphae, arbuscules and vesicles (Smith and Read 2008). This gives indications of the species that are colonising plants. In contrast, AM communities from soil can originate from all AM structures but likely originate from external AM structure such as extraradical hyphae and spores which exist outside of the root (Smith and Read 2008). This gives indications of the AM fungi that exist in the rhizosphere of the plant but not necessarily those colonising the plant. I did attempt to sequence the roots of the hawkweeds from the growth chamber study to determine what AM species were colonising them, unfortunately the sequences were of poor quality (Appendix C). This would have provided information on which AM fungi were colonising the roots of the hawkweeds in the growth chamber study and whether or not they might be affected by heat.

Previous studies have shown that different species of AM fungi have different colonization strategies (Hart and Reader 2002; Maherali and Klironomos 2007). These will likely be affected by heat. For example AM fungi in the family Glomeraceae tend to be faster root colonisers than those from Gigasporaceae, which have been shown to allocate higher biomass to extraradical hyphae and spores (Hart and Reader 2002; Maherali and Klironomos 2007). AM fungi in the family Glomeraceae can increase after disturbances, which could include fire, compared to Acaulosporaceae and Gigasporaceae (Chagnon et al. 2013). Work from Longo et al. (2014) supports this, showing that Glomeraceae species had a significantly higher abundance of spores in field sites after fire compared to AM fungi in families Gigasporaceae and Acaulosporaceae whose spore abundance declined after fire. This is somewhat different to the pattern I observed in my results where the most abundant AM fungi detected were in order Diversporales in *C. macra* and *H. lepidulum* soils of which Gigasporaceae belong to and Archaeosporales in *P. officinarum* soils. Glomerales appeared in similar abundance across the different soil origins. Heat did not appear to influence the relative abundance of taxa between the different soil origins.

The methods I used to assess the effects of soil origin and heat on AM fungal communities could not discriminate between DNA from dead AM fungi (extracellular DNA) and DNA from living AM fungi. Extracellular DNA can account for an average of 40% of DNA observed of prokaryotes and fungi in soil using sequencing techniques (Carini et al. 2017). The presence of extracellular DNA was shown to inflate richness by up to 55% and caused misestimations of taxon relative abundances (Carini et al. 2017). Despite this issue DNA metabarcoding is widely used to assess the difference between different ecological states between different microbial communities (Longo et al 2014; Day et al. 2019; Grossman et al. 2019; Yang et al. 2019), including studies assessing

impacts of fire on soil microbes (Longo et al 2014; Day et al. 2019). Moreover, many of those studies have shown differences in soil microbial communities due to fire using DNA-based techniques (Glassman et al. 2016; Longo et al 2014). Therefore, it was not unreasonable for me to expect to see a difference using these same methods in my study. Taking approaches to remove extracellular DNA using propidium iodide dye prior to heating of the soil could have reduced potentially sequencing extracellular DNA (Carini et al. 2017). Alternatively, applying newer techniques such as RNA sequencing could give a better indication of AM fungi that are alive and subsequently affected by heat (Romero-Olivares et al. 2019; Bang-Andreasen et al. 2020); however, it is difficult to determine taxonomic identities using RNA sequencing.

Since I only sequenced AM fungi, I cannot conclusively say that AM fungi are responsible for the effects of soil origin in the growth chamber study. Soil origin is a complex predictor and consists of many components that were not addressed in my study. For example, other biological variables were not measured and may be responsible for the plant growth effects, such as bacterial communities or general fungal communities (Van Der Heijden et al. 2008). Similarly, other soil environmental variables might have differed by soil origin, such as phosphorus or pH, which also affect plant growth (Scott 1975, McIntosh et al. 1995, Scott et al. 2001). Further analyses should include testing for these biotic and abiotic variables, which could provide insight into soil dynamics underneath hawkweed species.

I chose to test the effects of three soil heat temperatures of 30°C, 45°C and 60°C on AM richness and composition, and one soil heat treatment 45°C on plant biomass of hawkweeds. Interestingly, soil heating did not alter soil AM fungal community composition or hawkweed biomass. These soil temperatures are similar to those expected from other studies in grasslands and prairie ecosystems (Scotter 1970, Hill et

al. 2017). I was limited because there are no studies that have concretely assessed how hot soil gets in tussock grassland fires. Pearce et al. showed that soils at 2.5 and 5 cm depth did not heat above 69°C in controlled burns, so I did not heat my soils above that (2009, 2011). There is also no information on how long that soil remains at that temperature in tussock grasslands. Still, it is known that fire spreads quickly, and likely, the soil is only briefly exposed and cools quickly (Payton and Pearce 2001, 2009b, Pearce et al. 2009). Therefore I predicted that soil temperature would not exceed the tested temperature for longer than 10 minutes. More research is needed into the fire dynamics in tussock grasslands, particularly since fires are predicted to increase (Pearce et al. 2011). Future research should focus on the effects of fire on soils, as there are many gaps on the effects of fire on soil communities and their biological processes. However, my study gives some indication on what effects may be expected on soil in the field during a fire.

4.4.1. Critique of methods

Overall, AM fungal richness for all treatments was relatively high when compared to similar studies (Longo et al. 2014, Xiang et al. 2015, Lekberg et al. 2018, Chaudhary et al. 2020) and resembles richness found by (Day et al. 2019, Phillips et al. 2020) for total soil fungi. One factor contributing to the high AM fungal richness observed could be the high diversity of AM fungal species present in New Zealand's tussock grasslands. To date, few studies have examined AM fungal species colonising New Zealand's plants in tussock grasslands (Crush 1973, Spence et al. 2011). Considering the diversity of plants in New Zealand and within tussock grasslands (Winkworth et al. 2005), one might also expect to find many unique AM fungal mutualists. AM fungi were identified using a

taxonomy file complied from sequences in the MaarjAM database (Öpik et al. 2010). The complied taxonomy file was dominated by sequences from the northern hemisphere, which may explain why so few ASVs in our study were able to be identified to order level. Using other tools such as Basic local alignment searches (BLAST) available on the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov) may have benefited my study as it may have allowed further identification of AM fungi.

4.5. Conclusions

Overall, this thesis has explored the effects of fire and soil origin on plant invasion, AM fungi and soil carbon. The findings support the idea that soil origin is a major driver of plant-soil relationships in New Zealand's tussock grasslands and highlight the importance of plant-mycorrhizal relationships in plant invasion.

My results show that soil origin was the major driver of AM fungal community structure in terms of composition. The effect of heat on AM fungal richness differed according to soil origin and, surprisingly, had no impact on community composition. In the growth chamber study, hawkweed biomass was influenced by soil origin but not heat. Interestingly, both hawkweed species had the highest plant biomass in *P. officinarum* soils. Although soil carbon was slightly elevated in *C. macra* soils compared to the soils of invasive hawkweeds, soil carbon levels did not differ statistically between different soil origins and heat treatments.

My results supported my hypothesis that soil origin is a major driver of plant-soil relationships in New Zealand's tussock grasslands. Further, it suggests that *P. officinarum* invasion may facilitate invasion by *H. lepidulum*. My results indicate that fire

has less effect on AM communities, hawkweed growth and soil carbon than soil origin in tussock grasslands.

References

Aislabie, J., and J. R. Deslippe. 2013. Soil microbes and their contribution to soil services. Pages 143–161 *in* J. R. Dymond, editor. Ecosystem services in New Zealand : conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand.

Alguacil, M. del M., M. P. Torres, A. Montesinos-Navarro, and A. Roldán. 2016. Soil characteristics driving arbuscular mycorrhizal fungal communities in semiarid Mediterranean soils. Applied and Environmental Microbiology 82:3348–3356.

Archibald, S., et al. 2018. Biological and geophysical feedbacks with fire in the Earth system. Environmental Research Letters 13: [https://doi.org/10.1088/1748-9326/aa9ead].

Armstrong, L., and R. L. Peterson. 2002. The interface between the arbuscular mycorrhizal fungus *Glomus intraradices* and root cells of *Panax quinquefolius* : A *Paris*-type mycorrhizal association. Mycologia 94:587–595.

Baker, C. M., and M. Bode. 2016. Placing invasive species management in a spatiotemporal context. Ecological Applications 26:712–725.

Bang-Andreasen, T., M. Z. Anwar, A. Lanzén, R. Kjøller, R. Rønn, F. Ekelund, and C. S. Jacobsen. 2020. Total RNA sequencing reveals multilevel microbial community changes and functional responses to wood ash application in agricultural and forest soil. FEMS Microbiology Ecology 96:fiaa016.

Bardgett, R. D. 2011. Plant-soil interactions in a changing world. F1000 Biology Reports 3: [https://doi.org/10.3410/B3-16].

Baynes, M., G. Newcombe, L. Dixon, L. Castlebury, and K. O'Donnell. 2012. A novel plant–fungal mutualism associated with fire. Fungal Biology 116:133–144.

Beals, K. K., J. A. M. Moore, S. N. Kivlin, S. L. J. Bayliss, C. Y. Lumibao, L. C. Moorhead, M. Patel, J. L. Summers, I. M. Ware, J. K. Bailey, and J. A. Schweitzer. 2020. Predicting plant-soil feedback in the field: meta-analysis reveals that competition and environmental stress differentially influence PSF. Frontiers in Ecology and Evolution 8:191.

Begum, N., C. Qin, M. A. Ahanger, S. Raza, M. I. Khan, M. Ashraf, N. Ahmed, and L. Zhang. 2019. Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Frontiers in Plant Science 10:1068.

Bellgard, S. E., R. J. Whelan, and R. M. Muston. 1994. The impact of wildfire on vesicular-arbuscular mycorrhizal fungi and their potential to influence the re-establishment of post-fire plant communities. Mycorrhiza 4:139–146.

Bennett, J. A., and J. Klironomos. 2019. Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. New Phytologist 222:91–96.

Bever, J. D., T. G. Platt, and E. R. Morton. 2012. Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annual Review of Microbiology 66:265–283.

Bever, J. D., S. C. Richardson, B. M. Lawrence, J. Holmes, and M. Watson. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. Ecology Letters 12:13–21.

Blair, J. M. 1997. Fire, N availability, and plant response in grasslands: A test of the transient maxima hypothesis. Ecology 78:2359–2368.

Borowicz, V. A. 2001. Do arbuscular mycorrhizal fungi alter plant–pathogen relations? Ecology 82:3057–3068.

Bosch, O. J. H., W. J. Allen, K. F. O'Connor, A. H. Ensor, A. J. Jopp, H. Cameron, and B. E. Allan. 1996. Developing a better understanding of *Hieracium* invasion in the

New Zealand high country: a participatory approach. Proceedings of the New Zealand Grassland Association:161–165.

Brundrett, M. 2004. Diversity and classification of mycorrhizal associations. Biological Reviews 79:473–495.

Bruns, T. D., N. Corradi, D. Redecker, J. W. Taylor, and M. Öpik. 2018. Glomeromycotina: what is a species and why should we care? New Phytologist 220:963–967.

Buerdsell, S. L., B. G. Milligan, and E. A. Lehnhoff. 2021. Invasive plant benefits a native plant through plant-soil feedback but remains the superior competitor. NeoBiota 64:119–136.

Butler, O. M., J. J. Elser, T. Lewis, B. Mackey, and C. Chen. 2018. The phosphorus-rich signature of fire in the soil–plant system: a global meta-analysis. Ecology Letters 21:335–344.

Callahan, B. J., (n.d.). A DADA2 workflow for Big Data (1.4 or later). https://benjjneb.github.io/dada2/bigdata.html.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods 13:581–583.

Carbonnel, S., and C. Gutjahr. 2014. Control of arbuscular mycorrhiza development by nutrient signals. Frontiers in Plant Science 5: [https://doi.org/10.3389/fpls.2014.00462]

Carini, P., P. J. Marsden, J. W. Leff, E. E. Morgan, M. S. Strickland, and N. Fierer. 2017. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nature Microbiology 2:16242.

Certini, G. 2005. Effects of fire on properties of forest soils: a review. Oecologia 143:1–10.

Certini, G., D. Moya, M. E. Lucas-Borja, and G. Mastrolonardo. 2021. The impact of fire on soil-dwelling biota: A review. Forest Ecology and Management 488:118989.

Chagnon, P.-L., R. L. Bradley, H. Maherali, and J. N. Klironomos. 2013. A trait-based framework to understand life history of mycorrhizal fungi. Trends in Plant Science 18:484–491.

Chaudhary, V. B., S. Nolimal, M. A. Sosa-Hernandez, C. Egan, and J. Kastens. 2020. Trait-based aerial dispersal of arbuscular mycorrhizal fungi. New Phytologist 228:238–252.

Chen, J. 2021. GUniFrac: generalized UniFrac distances and distance-based multivariate analysis of variance. R package version 1.2. https://CRAN.R-project.org/package=GUniFrac.

Chen, M., M. Arato, L. Borghi, E. Nouri, and D. Reinhardt. 2018. Beneficial services of arbuscular mycorrhizal fungi – from ecology to application. Frontiers in Plant Science 9:[doi:10.3389/fpls.2018.01270].

Colautti, R. I., A. Ricciardi, I. A. Grigorovich, and H. J. MacIsaac. 2004. Is invasion success explained by the enemy release hypothesis? Ecology Letters 7:721–733.

Cotton, T. A. 2018. Arbuscular mycorrhizal fungal communities and global change: an uncertain future. FEMS Microbiology Ecology 94:[doi:10.1093/femsec/fiy179].

Crossay, T., C. Antheaume, D. Redecker, L. Bon, N. Chedri, C. Richert, L. Guentas, Y. Cavaloc, and H. Amir. 2017. New method for the identification of arbuscular mycorrhizal fungi by proteomic-based biotyping of spores using MALDI-TOF-MS. Scientific Reports 7:[doi: 10.1038/s41598-017-14487-6].

Crush, J. R. 1973. Significance of endomycorrhizas in tussock grassland in Otago, New Zealand. New Zealand Journal of Botany 11:645–660.

D'Antonio, C. M., and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23:63–87.

Davis, N. M., D. M. Proctor, S. P. Holmes, D. A. Relman, and B. J. Callahan. 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6:[doi: 10.1186/s40168-018-0605-2].

Day, N. J., and H. L. Buckley. 2011. Invasion patterns across multiple scales by *Hieracium* species over 25 years in tussock grasslands of New Zealand's South Island. Austral Ecology 36:559–570.

Day, N. J., K. E. Dunfield, and P. M. Antunes. 2015. Temporal dynamics of plant-soil feedback and root-associated fungal communities over 100 years of invasion by a non-native plant. Journal of Ecology 103:1557–1569.

Day, N. J., K. E. Dunfield, J. F. Johnstone, M. C. Mack, M. R. Turetsky, X. J. Walker, A. L. White, and J. L. Baltzer. 2019. Wildfire severity reduces richness and alters composition of soil fungal communities in boreal forests of western Canada. Global Change Biology 25:2310–2324.

De Deyn, G. B., H. Quirk, and R. D. Bardgett. 2011. Plant species richness, identity and productivity differentially influence key groups of microbes in grassland soils of contrasting fertility. Biology Letters 7:75–78.

De Deyn, G. B., H. Quirk, Z. Yi, S. Oakley, N. J. Ostle, and R. D. Bardgett. 2009. Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting soil fertility. Journal of Ecology 97:864–875.

Dickie, I. A., N. Bolstridge, J. A. Cooper, and D. A. Peltzer. 2010. Co-invasion by *Pinus* and its mycorrhizal fungi. New Phytologist 187:475–484.

Dickie, I. A., J. L. Bufford, R. C. Cobb, M. Desprez-Loustau, G. Grelet, P. E. Hulme, J. Klironomos, A. Makiola, M. A. Nuñez, A. Pringle, P. H. Thrall, S. G. Tourtellot, L. Waller, and N. M. Williams. 2017. The emerging science of linked plant–fungal invasions. New Phytologist 215:1314–1332.

Dickson, S. 2004. The *Arum–Paris* continuum of mycorrhizal symbioses. New Phytologist 163:187–200.

Docherty, K. M., T. C. Balser, B. J. M. Bohannan, and J. L. M. Gutknecht. 2012. Soil microbial responses to fire and interacting global change factors in a California annual grassland. Biogeochemistry 109:63–83.

Dove, N. C., and S. C. Hart. 2017. Fire reduces fungal species richness and In Situ mycorrhizal colonization: A meta-analysis. Fire Ecology 13:37–65.

Downs, T. M., and I. J. Radford. 2005. Arbuscular mycorrhizal fungal colonisation of Hieracium lepidulum roots in experimental and field soil inoculated media. New Zealand Journal of Botany 43:843–850.

Dumbrell, A. J., P. D. Ashton, N. Aziz, G. Feng, M. Nelson, C. Dytham, A. H. Fitter, and T. Helgason. 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytologist 190:794–804.

Egan, C. P. 2017. Community structure of arbuscular mycorrhizal fungi along an altitudinal gradient. Thesis, The University of British Columbia, British Columbia, Canada.

Espie, P. R. 2001. *Hieracium* in New Zealand: ecology and management. AgResearch. Mosgiel, New Zealand.

Fahey, C., A. Koyama, P. M. Antunes, K. Dunfield, and S. L. Flory. 2020. Plant communities mediate the interactive effects of invasion and drought on soil microbial communities. The ISME Journal 14:1396–1409.

Fan, J., and W. Harris. 1996. Effects of soil fertility level and cutting frequency on interference among *Hieracium pilosella, H. praealtum, Rumex acetosella,* and *Festuca novae-zelandiae*. New Zealand Journal of Agricultural Research 39:1–32.

Fox, J., and S. Weisberg. 2019. An R companion to applied regression. 3rd edition. Sage publications, Thousand Oaks, CA, USA.

French, K. 2021. Invasion by hawkweeds. Biological Invasions.[doi: 10.1007/s10530-021-02604-6].

Frykberg, E. 2020. Lake Ōhau fire: Former firefighter says he warned DOC for years over potential blaze. RNZ. New Zealand. https://www.rnz.co.nz/news/national/427980/lake-ohau-fire-former-firefighter-says-he-warned-doc-for-years-over-potential-blaze

Gaiero, J. R., C. A. McCall, K. A. Thompson, N. J. Day, A. S. Best, and K. E. Dunfield. 2013. Inside the root microbiome: Bacterial root endophytes and plant growth promotion. American Journal of Botany 100:1738–1750.

Gitay, H., W. G. Lee, R. B. Allen, and J. B. Wilson. 1992. Recovery of *Chionochloa rigida* tussocks from fires in South Island, New Zealand. Journal of Environmental Management 35:249–259.

Glassman, S. I., and B. B. Casper. 2012. Biotic contexts alter metal sequestration and AMF effects on plant growth in soils polluted with heavy metals. Ecology 93:1550–1559.

Graham, S. W., V. K. Y. Lam, and V. S. F. T. Merckx. 2017. Plastomes on the edge: the evolutionary breakdown of mycoheterotroph plastid genomes. New Phytologist 214:48–55.

Green, P. T., D. J. O'Dowd, K. L. Abbott, M. Jeffery, K. Retallick, and R. Mac Nally. 2011. Invasional meltdown: Invader–invader mutualism facilitates a secondary invasion. Ecology 92:1758–1768.

Grman, E. 2012. Plant species differ in their ability to reduce allocation to non-beneficial arbuscular mycorrhizal fungi. Ecology 93:711–718.

Groves, R. H. 2006. Are some weeds sleeping? Some concepts and reasons. Euphytica 148:111–120.

Hart, M. M., and R. J. Reader. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytologist 153:335–344.

Hartnett, D. C., A. F. Potgieter, and G. W. T. Wilson. 2004. Fire effects on mycorrhizal symbiosis and root system architecture in Southern African savanna grasses. African Journal of Ecology 42:328–337.

van der Heijden, M. G. A., J. N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and I. R. Sanders. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72.

Henn, H., D. Petit, and P. Vernet. 1988. Interference between *Hieracium pilosella* and *Arrhenatherum elatius* in Colliery Spoils of North of France. Allelopathy or Competition? Oecologia 76:268–272.

Hill, K. C., J. D. Bakker, and P. W. Dunwiddie. 2017. Prescribed fire in grassland butterfly habitat: Targeting weather and fuel conditions to reduce soil temperatures and burn severity. Fire Ecology 13:24–41.

Hoeksema, J. D., V. B. Chaudhary, C. A. Gehring, N. C. Johnson, J. Karst, R. T. Koide, A. Pringle, C. Zabinski, J. D. Bever, J. C. Moore, G. W. T. Wilson, J. N. Klironomos, and J. Umbanhowar. 2010. A meta-analysis of context-dependency

in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13:394–407.

Holden, J., S. Walton, J. Guildford, A. Williams, and S. Mohanlall. 2020. Weather forecast looks promising for firefighters battling Lake Pukaki blaze. Stuff. New Zealand. https://www.stuff.co.nz/national/122610766/policeled-convoys-lead-campers-to-safety-through-lake-pukaki-fire-zone

Holden, S. R., B. M. Rogers, K. K. Treseder, and J. T. Randerson. 2016. Fire severity influences the response of soil microbes to a boreal forest fire. Environmental Research Letters 11:035004.

Höpfner, I., W. Beyschlag, M. Bartelheimer, C. Werner, and S. Unger. 2015. Role of mycorrhization and nutrient availability in competitive interactions between the grassland species *Plantago lanceolata* and *Hieracium pilosella*. Plant Ecology 216:887–899.

Hulme, P. E. 2006. Beyond control: wider implications for the management of biological invasions: Wider implications for managing invasions. Journal of Applied Ecology 43:835–847.

Hulme, P. E. 2020. Plant invasions in New Zealand: global lessons in prevention, eradication and control. Biological Invasions 22:1539–1562.

Inderjit, and J. F. Cahill. 2015. Linkages of plant-soil feedbacks and underlying invasion mechanisms. Annals of Botany 7:plv022–plv022.

Jackson, B. 2016. Clean-up using AMPure XP beads. https://www.protocols.io/view/clean-up-using-ampure-xp-beads-f3ebqje.

Johnson, N. C., J. H. Graham, and F. A. Smith. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. New Phytologist 135:575–585.

Kamel, L., M. Keller-Pearson, C. Roux, and J. Ané. 2017. Biology and evolution of arbuscular mycorrhizal symbiosis in the light of genomics. New Phytologist 213:531–536.

Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17:164–170.

Keeley, J. E., and T. J. Brennan. 2012. Fire-driven alien invasion in a fire-adapted ecosystem. Oecologia 169:1043–1052.

Kiers, E. T., et al. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882.

Klironomos, J. N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417:67–70.

Klironomos, J. N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301.

Klopatek, C. C., L. F. Debano, and J. M. Klopatek. 1988. Effects of simulated fire on vesicular-arbuscular mycorrhizae in pinyon-juniper woodland soil. Plant and Soil 109:245–249.

Knicker, H., S. Saggar, R. Bäumler, P. D. McIntosh, and I. Kögel-Knabner. 2000. Soil organic matter transformations induced by *Hieracium pilosella* L. in tussock grassland of New Zealand. Biology and Fertility of Soils 32:194–201.

Koide, R. T., and B. Mosse. 2004. A history of research on arbuscular mycorrhiza. Mycorrhiza 14:145–163.

Krna, M. A. 2015. Productivity, decomposition and carbon sequestration of *Chionochloa* species across altitudinal gradients in montane tussock grasslands of New Zealand. Thesis, Massey University, Manawatū, New Zealand.

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35:1547–1549.

Lee, J., S. Lee, and J. P. W. Young. 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi: PCR primers for arbuscular mycorrhizal fungi. FEMS Microbiology Ecology 65:339–349.

Lee, J.-K., S.-H. Park, and A.-H. Eom. 2006. Molecular identification of arbuscular mycorrhizal fungal spores collected in Korea. Mycobiology 34:7–13.

Lekberg, Y., S. M. Gibbons, S. Rosendahl, and P. W. Ramsey. 2013. Severe plant invasions can increase mycorrhizal fungal abundance and diversity. The ISME Journal 7:1424–1433.

Lekberg, Y., and R. T. Koide. 2005. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytologist 168:189–204.

Lekberg, Y., M. Vasar, L. S. Bullington, S.-K. Sepp, P. M. Antunes, R. Bunn, B. G. Larkin, and M. Öpik. 2018. More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers? New Phytologist 220:971–976.

Lenth, R. 2021. emmeans: Estimated marginal means, aka least-Squares Means. R package version 1.6.0. https://CRAN.R-project.org/package=emmeans.

Li, J., J. Pei, J. Liu, J. Wu, B. Li, C. Fang, and M. Nie. 2021. Spatiotemporal variability of fire effects on soil carbon and nitrogen: A global meta-analysis. Global Change Biology 27:4196–4206.

Lindahl, B. D., R. H. Nilsson, L. Tedersoo, K. Abarenkov, T. Carlsen, R. Kjøller, U. Kõljalg, T. Pennanen, S. Rosendahl, J. Stenlid, and H. Kauserud. 2013. Fungal

community analysis by high-throughput sequencing of amplified markers – a user's guide. The New Phytologist 199:288–299.

Longo, S., E. Nouhra, B. T. Goto, R. L. Berbara, and C. Urcelay. 2014. Effects of fire on arbuscular mycorrhizal fungi in the Mountain Chaco Forest. Forest Ecology and Management 315:86–94.

Lüdecke, D. 2018. ggeffects: Tidy data frames of marginal effects from regression models. Journal of Open Source Software 3:772.

Ludwig, S. M., H. D. Alexander, K. Kielland, P. J. Mann, S. M. Natali, and R. W. Ruess. 2018. Fire severity effects on soil carbon and nutrients and microbial processes in a Siberian larch forest. Global Change Biology 24:5841–5852.

Luginbuehl, L. H., and G. E. D. Oldroyd. 2017. Understanding the arbuscule at the heart of endomycorrhizal symbioses in plants. Current Biology 27:R952–R963.

Mack, R. N. 1981. Invasion of Bromus tectorum L. into Western North America: An ecological chronicle. Agro-Ecosystems 7:145–165.

Maherali, H., and J. N. Klironomos. 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748.

Makepeace, W. 1980. Ecological studies of *Hieracium pilosella* and *H. praealtum*. University of Canterbury, Christchurch, New Zealand.

Makepeace, W. 1985. Growth, reproduction, and production biology of mouse-ear and king devil hawkweed in eastern South Island, New Zealand. New Zealand Journal of Botany 23:65–78.

Mark, A. F., B. I. P. Barratt, and E. Weeks. 2013. Ecosystem services in New Zealand's indigenous tussock grasslands: conditions and trends. Page 33 *in* J. R. Dymond, editor. Ecosystem services in New Zealand – conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand.

Mark, A. F., and K. J. Dickinson. 2008. Maximizing water yield with indigenous non-forest vegetation: a New Zealand perspective. Frontiers in Ecology and the Environment 6:25–34.

Mark, A. F., and B. McLennan. 2005. The conservation status of New Zealand's indigenous grasslands. New Zealand Journal of Botany 43:245–270.

Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12.

McGlone, M. S. 1989. The polynesian settlement of New Zealand in relation to environmental and biotic changes. New Zealand Journal of Ecology 12:115–129.

McIntosh, P. D., M. Loeseke, and K. Bechler. 1995. Soil changes under mouse-ear hawkweed (*Hieracium pilosella*) 19:29–34.

McLauchlan, K. K., et al. 2020. Fire as a fundamental ecological process: Research advances and frontiers. Journal of Ecology 108:2047–2069.

Moen, J., and C. D. Meurk. 2001. Competitive abilities of three indigenous New Zealand plant species in relation to the introduced plant *Hieracium pilosella*. Basic and Applied Ecology 2:243–250.

Müller, A., B. Ngwene, E. Peiter, and E. George. 2017. Quantity and distribution of arbuscular mycorrhizal fungal storage organs within dead roots. Mycorrhiza 27:201–210.

Nagy, R. C., E. J. Fusco, J. K. Balch, J. T. Finn, A. Mahood, J. M. Allen, and B. A. Bradley. 2021. A synthesis of the effects of cheatgrass invasion on US Great Basin carbon storage. Journal of Applied Ecology 58:327–337.

Neina, D. 2019. The role of soil pH in plant nutrition and soil remediation. Applied and Environmental Soil Science 2019:1–9.

Newsham, K. K., A. H. Fitter, and A. R. Watkinson. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. The Journal of Ecology 83:991–1000.

Nguyen, T. D., T. R. Cavagnaro, and S. J. Watts-Williams. 2019. The effects of soil phosphorus and zinc availability on plant responses to mycorrhizal fungi: a physiological and molecular assessment. Scientific Reports 9:14880.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, H. H. Stevens, E. Szoecs, and H. Wagner. 2020. vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan.

Öpik, M., M. Metsis, T. J. Daniell, M. Zobel, and M. Moora. 2009. Large-scale parallel 454 sequencing reveals host ecological group specificity of arbuscular mycorrhizal fungi in a boreonemoral forest. New Phytologist 184:424–437.

Öpik, M., A. Vanatoa, E. Vanatoa, M. Moora, J. Davison, J. M. Kalwij, Ü. Reier, and M. Zobel. 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytologist 188:223–241.

Pattinson, G. S., K. A. Hammill, B. G. Sutton, and P. A. Mcgee. 1999. Simulated fire reduces the density of arbuscular mycorrhizal fungi at the soil surface. Mycological Research 103:491–496.

Payton I. J. and G. Pearce. 2001. Does fire deplete the physical and biological resources of tall-tussock (*Chionochloa*) grasslands? The latest attempt at some answers. In: G. Pearce and L. Lester (eds.). Bushfire 2001. Joint Bushfire/FRFANZ Conference; 3–6 July 2001; Christchurch, New Zealand: New Zealand Forest Research Institute. p. 243–249.

Payton, I. J., and H. G. Pearce. 2009. Fire-induced changes to the vegetation of tall-tussock (*Chionochloa rigida*) grassland ecosystems. Department of Conservation / Te Papa Atawhai, Wellington, New Zealand.

Pearce, H. G., S. A. J. Anderson, and I. J. Payton. 2009. Fire behaviour as a determinant of fire effects in tussock grasslands: Comparison of fire behaviour associated with experimental burns conducted to assess fire effects. Scion, Rural Fire Research Group, Christchurch, New Zealand.

Pearce, H. G., J. Kerr, A. Clark, D. Ackerley, T. Carey-Smith, and E. Yang. 2011. Improved estimates of the effect of climate change on NZ fire danger. Scion, Rural Fire Research Group, Christchurch, New Zealand.

Pellegrini, A. F. A., A. Ahlström, S. E. Hobbie, P. B. Reich, L. P. Nieradzik, A. C. Staver, B. C. Scharenbroch, A. Jumpponen, W. R. L. Anderegg, J. T. Randerson, and R. B. Jackson. 2018. Fire frequency drives decadal changes in soil carbon and nitrogen and ecosystem productivity. Nature 553:194–198.

Perry, E. 2019. Threat to Dunedin water supply after massive fire destroys main catchment area. NZ Herald. New Zealand.

Perry, G. L. W., J. M. Wilmshurst, and M. S. McGlone. 2014. Ecology and long-term history of fire in New Zealand. New Zealand Journal of Ecology 38:157–176.

Phillips, M. L., E. L. Aronson, M. R. Maltz, and E. B. Allen. 2020. Native and invasive inoculation sources modify fungal community assembly and biomass production of a chaparral shrub. Applied Soil Ecology 147: [doi: 10.1016/j.apsoil.2019.103370].

Pingree, M. R. A., and L. N. Kobziar. 2019. The myth of the biological threshold: A review of biological responses to soil heating associated with wildland fire. Forest Ecology and Management 432:1022–1029.

Pressler, Y., J. C. Moore, and M. F. Cotrufo. 2019. Belowground community responses to fire: meta-analysis reveals contrasting responses of soil microorganisms and mesofauna. Oikos 128:309–327.

Pringle, A., J. D. Bever, M. Gardes, J. L. Parrent, M. C. Rillig, and J. N. Klironomos. 2009. Mycorrhizal symbioses and plant invasions. Annual Review of Ecology, Evolution, and Systematics 40:699–715.

Pyšek, P., V. Jarošík, P. E. Hulme, J. Pergl, M. Hejda, U. Schaffner, and M. Vilà. 2012. A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. Global Change Biology 18:1725–1737.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ravnskov, S., and I. Jakobsen. 1995. Functional compatibility in arbuscular mycorrhizas measured as hyphal P transport to the plant. New Phytologist 129:611–618.

Redecker, D., R. Kodner, and L. E. Graham. 2000. Glomalean Fungi from the Ordovician. Science 289:1920–1921.

Redecker, D., A. Schüßler, H. Stockinger, S. L. Stürmer, J. B. Morton, and C. Walker. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23:515–531.

Reinhart, K. O., and R. M. Callaway. 2006. Soil biota and invasive plants. New Phytologist 170:445–457.

Rejmánek, M. 2000. Invasive plants: approaches and predictions: Invasive plants. Austral Ecology 25:497–506.

Rillig, M. C., M. Ryo, A. Lehmann, C. A. Aguilar-Trigueros, S. Buchert, A. Wulf, A. Iwasaki, J. Roy, and G. Yang. 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. Science 366:886–890.

Roberts, A. E., I. J. Radford, and D. A. Orlovich. 2009. Do alterations of arbuscular mycorrhizal fungal communities change interactions between an invader *Hieracium lepidulum* and two co- occurring species? A glasshouse study. Australasian Mycologist 28:29–35.

Roberts, D. W. 2019. labdsv: Ordination and Multivariate Analysis for Ecology. R package version 2.0-1. https://CRAN.R-project.org/package=labdsv.

Romero-Olivares, A. L., G. Meléndrez-Carballo, A. Lago-Lestón, and K. K. Treseder. 2019. Soil metatranscriptomes under long-term experimental warming and drying: Fungi allocate resources to cell metabolic maintenance Rather than decay. Frontiers in Microbiology 10:1914.

Ross, D. J., K. R. Tate, and C. W. Feltham. 1996. Microbial biomass, and C and N mineralization, in litter and mineral soil of adjacent montane ecosystems in a southern beech (*Nothofagus*) forest and a tussock grassland. Soil Biology and Biochemistry 28:1613–1620.

Sage, R. F. 2020. Global change biology: A primer. Global Change Biology 26:3–30.

Schauber, E. M., D. Kelly, P. Turchin, C. Simon, W. G. Lee, I. J. Payton, P. R. Wilson, P. E. Cowan, and R. E. Brockie. 2002. Masting by eighteen New Zealand plant species: The role of temperature as a synchronizing cue. Ecology 83:1214–1225.

Scott, D. 1975. Variation in soil pH under tussock grassland species. New Zealand Journal of Experimental Agriculture 3:143–145.

Scott, N. A., S. Saggar, and P. D. McIntosh. 2001. Biogeochemical impact of *Hieracium* Invasion in New Zealand's grazed tussock grasslands: sustainability implications. Ecological Applications 11:1311–1322.

Scotter, D. R. 1970. Soil temperatures under grass fires. Soil Research 8:273–279.

Sikes, B. A., K. Cottenie, and J. N. Klironomos. 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. Journal of Ecology 97:1274–1280.

Simberloff, D., and B. V. Holle. 1999. Positive interactions of nonindigenous species: Invasional meltdown? Biological Invasions 1:21–32.

Smith, S. E., and D. J. Read. 2008. Mycorrhizal symbiosis. Third Edition. Elsevier Ltd, New York

Souza, T. 2015. AMF's Main Structures. Pages 43–63 *in* T. Souza, editor. Handbook of arbuscular mycorrhizal fungi. Springer International Publishing, Switzerland.

Spatafora, J. W., et al. 2016. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108:1028–1046.

Spence, L. A., I. A. Dickie, and D. A. Coomes. 2011. Arbuscular mycorrhizal inoculum potential: a mechanism promoting positive diversity–invasibility relationships in mountain beech forests in New Zealand? Mycorrhiza 21:309–314.

Stavi, I. 2019. Wildfires in grasslands and shrublands: A review of impacts on vegetation, soil, hydrology, and geomorphology. Water 11:1042.

Steer, M., and D. Norton. 2013. Factors influencing abundance of invasive hawkweeds, *Hieracium* species, in tall tussock grasslands in the Canterbury high country. New Zealand Journal of Botany 51:61–70.

Stoian, V., R. Vidican, I. Crişan, C. Puia, M. Şandor, V. A. Stoian, F. Păcurar, and I. Vaida. 2019. Sensitive approach and future perspectives in microscopic patterns of mycorrhizal roots. Scientific Reports 9:10233.

Thiéry, O., M. Vasar, T. Jairus, J. Davison, C. Roux, P.-A. Kivistik, A. Metspalu, L. Milani, Ü. Saks, M. Moora, M. Zobel, and M. Öpik. 2016. Sequence variation in nuclear ribosomal small subunit, internal transcribed spacer and large subunit

regions of *Rhizophagus irregularis* and *Gigaspora margarita* is high and isolate-dependent. Molecular Ecology 25:2816–2832.

Torrecillas, E., M. M. Alguacil, and A. Roldán. 2012. Host preferences of arbuscular mycorrhizal fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies. Applied and Environmental Microbiology 78:6180–6186.

Treseder, K. K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytologist 164:347–355.

Treskonova, M. 1991. Changes in the structure of tall tussock grasslands and infestation by species of Hieracium in the Mackenzie country, New Zealand. New Zealand Journal of Ecology 15:65–78.

Turnbull, M. H., R. P. Pharis, L. V. Kurepin, M. Sarfati, L. N. Mander, and D. Kelly. 2012. Flowering in snow tussock (*Chionochloa spp.*) is influenced by temperature and hormonal cues. Functional Plant Biology 39:38.

Van Aarle, I. M., and P. A. Olsson. 2003. Fungal lipid accumulation and development of mycelial structures by two arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 69:6762–6767.

Van Der Heijden, M. G. A., R. D. Bardgett, and N. M. Van Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.

Van Der Heijden, M. G. A., F. M. Martin, M. Selosse, and I. R. Sanders. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist 205:1406–1423.

van der Putten, W. H., R. D. Bardgett, J. D. Bever, T. M. Bezemer, B. B. Casper, T. Fukami, P. Kardol, J. N. Klironomos, A. Kulmatiski, J. A. Schweitzer, K. N.

Suding, T. F. J. Van de Voorde, and D. A. Wardle. 2013. Plant-soil feedbacks: the past, the present and future challenges. Journal of Ecology 101:265–276.

Voroney, R. P., and R. J. Heck. 2015. The soil habitat. Pages 15–39 *in* E. A. Paul, editor. Soil microbiology, ecology and biochemistry. Fourth edition. Academic press, San Diego, USA.

Wakelin, S. A., B. I. P. Barratt, E. Gerard, A. L. Gregg, E. L. Brodie, G. L. Andersen, T. Z. DeSantis, J. Zhou, Z. He, G. A. Kowalchuk, and M. O'Callaghan. 2013. Shifts in the phylogenetic structure and functional capacity of soil microbial communities follow alteration of native tussock grassland ecosystems. Soil Biology and Biochemistry 57:675–682.

Wang, B., and Y.-L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. Van Der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–1633.

Whisenant, S. G. 1990. Changing fire frequencies on Idaho's Snake River plains: ecological and management implications. Pages 4–10 Symposium on cheatgrass invasion, shrub die off and other aspects of shrub biology and management. Intermountain Research Station, Las Vegas, Nervada, USA.

Wickham, H. 2016. ggplot2 - elegant graphics for data analysis. Springer-Verlag New York.

Winkworth, R., S. J. Wagstaff, D. Glenny, and P. J. Lockhart. 2005. Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. Organisms Diversity & Evolution 5:237–247.

Wipf, D., F. Krajinski, D. van Tuinen, G. Recorbet, and P.-E. Courty. 2019. Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. New Phytologist 223:1127–1142.

Wiser, S., and R. Allen. 2000. *Hieracium lepidulum* invasion of indigenous ecosystems. Department of Conservation / Te Papa Atawhai, Wellington, New Zealand.

Xiang, X., S. M. Gibbons, J. Yang, J. Kong, R. Sun, and H. Chu. 2015. Arbuscular mycorrhizal fungal communities show low resistance and high resilience to wildfire disturbance. Plant and Soil 397:347–356.

Yang, H., Y. Zang, Y. Yuan, J. Tang, and X. Chen. 2012. Selectivity by host plants affects the distribution of arbuscular mycorrhizal fungi: evidence from ITS rDNA sequence metadata. BMC Evolutionary Biology 12:50.

Yang, F., K. Niu, C. G. Collins, X. Yan, Y. Ji, N. Ling, X. Zhou, G. Du, H. Guo, and S. Hu. 2019. Grazing practices affect the soil microbial community composition in a Tibetan alpine meadow. Land Degradation & Development 30:49–59.

Appendix A. Seed surface disinfection trial on hawkweed seeds

I conducted an experiment to ensure my seed disinfection method limited microbial growth. Seeds were surface disinfected by immersing them in 1% bleach within a pre-bleached tea strainer. Three different disinfection times were tested 0, 5, 10 and 20 minutes. Following disinfection, seeds were rinsed in Millipore water to remove residual bleach. Ten seeds were placed on potato dextrose agar supplemented with chloramphenicol. Plates were sealed with parafilm and left for two weeks at room temperature to germinate. Following the two weeks, the 0-minute plates for both species showed clear fungal contamination. My trial indicated that the 5-minute surface disinfection time was the most appropriate because both plant species had at least one seed out of ten on the plate germinate, and there was also no fungal contamination present (Table A1.1).

Table A1.1. The number of seedlings of *P. officinarum* and *H. lepidulum* that had germinated during 1% bleach surface disinfection trial for different times.

Plant	Surface disinfection time	Replicate	Number of seeds germinated	Fungal contamination
P. officinarum	0	1	0	Yes
P. officinarum	5	1	1	No
P. officinarum	5	2	0	No
P. officinarum	10	1	2	No
P. officinarum	10	2	2	No
P. officinarum	20	1	0	No
P. officinarum	20	2	0	No
H. lepidulum	0	1	0	Yes
H. lepidulum	5	1	1	No
H. lepidulum	5	2	0	No
H. lepidulum	10	1	0	No
H. lepidulum	10	2	0	No
H. lepidulum	20	1	0	No
H. lepidulum	20	2	0	No

Appendix B. Statistical analyses of soils and hawkweeds

This appendix contains graphs and tables resulting from statistical analyses conducted during the sequencing and plant growth experiments

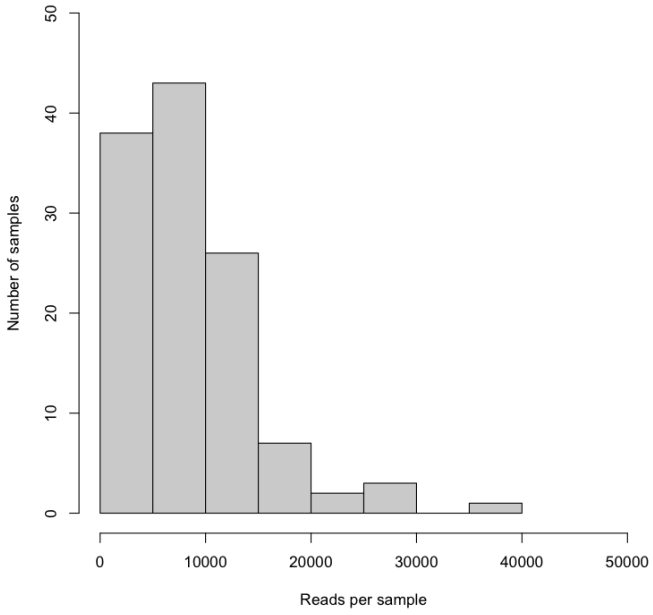

Figure AB.1.Histogram showing the distribution of reads per sample for all 18S AM fungal soil samples processed through DADA2. N = 120.

AB.1. Tukey *post hoc* pairwise comparisons of soil origin and heat treatments for a generalised linear model of AM fungal ASV richness. Treatment comparisons include soil origin from (*Chionochloa macra, Hieracium lepidulum and Pilosella officinarum*) and heat treatments (Unheated, 30°C, 45°C, and 60°C). Standard errors are shown in brackets. Statistically significant effects are shown in bold.

Treatment comparisons	Estimate	Z ratio	*P*-value
C. macra UH - *H. lepidulum* UH	0.10 (0.06)	1.73	0.854
C. macra UH - *P. officinarum* UH	0.10 (0.05)	-0.40	1.000
C. macra UH - *C. macra* 30°C	0.10 (0.05)	-1.61	0.905
C. macra UH - *H. lepidulum* 30°C	0.10 (0.05)	2.57	0.298
C. macra UH **- *P. officinarum*** 30°C	**0.10 (0.05)**	**3.74**	**0.010**
C. macra UH - *C. macra* 45°C	0.10 (0.05)	-0.16	1.000
C. macra UH - *H. lepidulum* 45°C	0.10 (0.05)	2.11	0.614
C. macra UH - *P. officinarum* 45°C	0.10 (0.05)	0.38	1.000
C. macra UH **- *C. macra*** 60°C	**0.10 (0.05)**	**5.65**	**< 0.001**
C. macra UH **- *H. lepidulum*** 60°C	**0.10 (0.05)**	**3.33**	**0.042**
C. macra UH - *P. officinarum* 60°C	0.10 (0.05)	1.51	0.940
H. lepidulum UH - *P. officinarum* UH	0.10 (0.05)	-2.07	0.645
H. lepidulum UH - *C. macra* 30°C	0.10 (0.06)	-3.08	0.087
H. lepidulum UH - *H. lepidulum* 30°C	0.10 (0.06)	0.54	1.000
H. lepidulum UH - *P. officinarum* 30°C	0.10 (0.06)	1.58	0.918
H. lepidulum UH - *C. macra* 45°C	0.10 (0.06)	-1.87	0.778
H. lepidulum UH - *H. lepidulum* 45°C	0.10 (0.06)	0.27	1.000
H. lepidulum UH - *P. officinarum* 45°C	0.10 (0.06)	-1.33	0.975
H. lepidulum UH **- *C. macra*** 60°C	**0.10 (0.06)**	**3.29**	**0.046**
H. lepidulum UH - *H. lepidulum* 60°C	0.10 (0.06)	1.21	0.988
H. lepidulum UH - *P. officinarum* 60°C	0.10 (0.06)	-0.44	1.000
P. officinarum UH - *C. macra* 30°C	0.10 (0.05)	-1.23	0.987
P. officinarum UH - *H. lepidulum* 30°C	0.10 (0.05)	2.95	0.123
P. officinarum UH **- *P. officinarum*** 30°C	**0.10 (0.05)**	**4.12**	**0.002**
P. officinarum UH - *C. macra* 45°C	0.10 (0.05)	0.23	1.000
P. officinarum UH - *H. lepidulum* 45°C	0.10 (0.05)	2.47	0.360
P. officinarum UH - *P. officinarum* 45°C	0.10 (0.05)	0.75	1.000
P. officinarum UH **- *C. macra*** 60°C	**0.10 (0.05)**	**6.03**	**< 0.001**
P. officinarum UH **- *H. lepidulum*** 60°C	**0.10 (0.05)**	**3.71**	**0.011**
P. officinarum UH - *P. officinarum* 60°C	0.10 (0.05)	1.90	0.758
C. macra 30°C **- *H. lepidulum*** 30°C	**0.10 (0.05)**	**4.07**	**0.003**
C. macra 30°C **- *P. officinarum*** 30°C	**0.10 (0.05)**	**5.21**	**< 0.001**
C. macra 30°C - *C. macra* 45°C	0.10 (0.05)	1.45	0.953
C. macra 30°C **- *H. lepidulum*** 45°C	**0.10 (0.05)**	**3.52**	**0.022**
C. macra 30°C - *P. officinarum* 45°C	0.10 (0.05)	1.89	0.766

C. macra 30°C **-** ***C. macra*** 60°C	**0.10 (0.05)**	**7.06**	**< 0.001**
C. macra 30°C **-** ***H. lepidulum*** 60°C	**0.10 (0.05)**	**4.81**	**< 0.001**
C. macra 30°C - *P. officinarum* 60°C	0.10 (0.05)	3.08	0.087
H. lepidulum 30°C - *P. officinarum* 30°C	0.10 (0.05)	1.15	0.992
H. lepidulum 30°C - *C. macra* 45°C	0.10 (0.05)	-2.73	0.213
H. lepidulum 30°C - *H. lepidulum* 45°C	0.10 (0.06)	-0.27	1.000
H. lepidulum 30°C - *P. officinarum* 45°C	0.10 (0.05)	-2.07	0.646
H. lepidulum 30°C - *C. macra* 60°C	0.10 (0.06)	3.05	0.096
H. lepidulum 30°C - *H. lepidulum* 60°C	0.10 (0.05)	0.75	1.000
H. lepidulum 30°C - *P. officinarum* 60°C	0.10 (0.05)	-1.11	0.994
P. officinarum 30°C **-** ***C. macra*** 45°C	**0.10 (0.05)**	**-3.90**	**0.006**
P. officinarum 30°C - *H. lepidulum* 45°C	0.10 (0.06)	-1.35	0.972
P. officinarum 30°C - *P. officinarum* 45°C	0.10 (0.05)	-3.19	0.064
P. officinarum 30°C - *C. macra* 60°C	0.10 (0.06)	1.90	0.761
P. officinarum 30°C - *H. lepidulum* 60°C	0.10 (0.05)	-0.41	1.000
P. officinarum 30°C - *P. officinarum* 60°C	0.10 (0.05)	-2.29	0.486
C. macra 45°C - *H. lepidulum* 45°C	0.10 (0.05)	2.26	0.506
C. macra 45°C - *P. officinarum* 45°C	0.10 (0.05)	0.53	1.000
C. macra 45°C **-** ***C. macra*** 60°C	**0.10 (0.05)**	**5.81**	**< 0.001**
C. macra 45°C **-** ***H. lepidulum*** 60°C	**0.10 (0.05)**	**3.49**	**0.025**
C. macra 45°C - *P. officinarum* 60°C	0.10 (0.05)	1.67	0.882
H. lepidulum 45°C - *P. officinarum* 45°C	0.10 (0.06)	-1.67	0.881
H. lepidulum 45°C - *C. macra* 60°C	0.10 (0.06)	3.14	0.074
H. lepidulum 45°C - *H. lepidulum* 60°C	0.10 (0.06)	0.97	0.998
H. lepidulum 45°C - *P. officinarum* 60°C	0.10 (0.05)	-0.76	1.000
P. officinarum 45°C **-** ***C. macra*** 60°C	**0.10 (0.06)**	**5.02**	**< 0.001**
P. officinarum 45°C - *H. lepidulum* 60°C	0.10 (0.05)	2.79	0.183
P. officinarum 45°C - *P. officinarum* 60°C	0.10 (0.05)	1.04	0.997
C. macra 60°C - *H. lepidulum* 60°C	0.10 (0.06)	-2.30	0.474
C. macra 60°C **-** ***P. officinarum*** 60°C	**0.10 (0.05)**	**-4.21**	**0.002**
H. lepidulum 60°C - *P. officinarum* 60°C	0.10 (0.05)	-1.87	0.777

Table AB.2. ANOVA tables for linear models of plant biomass and root-shoot ratios of *P. officinarum* seedlings. *P*-values corrected using the false discovery method. Statistically significant predictors are shown in bold.

	Plant biomass				
Predictor	Df	Sum Sq	Mean Sq	F-value	*P*-value
Soil origin	**2.00**	**22.93**	**11.47**	**9.49**	**0.002**
Heat treatment	1.00	0.16	0.16	0.13	0.779
Soil origin: Heat treatment	2.00	0.61	0.30	0.25	0.779
Residuals	42.00	50.74	1.21		
	Root-shoot ratios				
Predictor	Df	Sum Sq	Mean Sq	F-value	*P*-value
Soil origin	2.00	0.65	0.32	1.94	0.314
Heat treatment	1.00	0.12	0.12	0.73	0.594
Soil origin: Heat treatment	**2.00**	**1.60**	**0.80**	**4.77**	**0.042**
Residuals	42.00	7.04	0.17		

Table AB.3. ANOVA tables for linear models of plant biomass and root-shoot ratios of *H. lepidulum* seedlings. *P*-values corrected using the false discovery method. Statistically significant predictors are shown in bold.

	Plant biomass				
Predictor	Df	Sum Sq	Mean Sq	F-value	*P*-value
Soil origin	**2.00**	**0.14122**	**0.07061**	**17.756**	**0.006**
Heat treatment	1.00	0.00296	0.00296	0.744	0.456
Soil origin: Heat treatment	2.00	0.02341	0.01171	2.943	0.191
Residuals	42.00	0.16702	0.00398		
	Root-shoot ratios				
Predictor	Df	Sum Sq	Mean Sq	F-value	*P*-value
Soil origin	2.00	1.555	0.7777	1.066	0.456
Heat treatment	1.00	0.487	0.487	0.668	0.456
Soil origin: Heat treatment	2.00	1.168	0.5841	0.801	0.456
Residuals	42.00	30.628	0.7292		

Table AB.4. Tukey *post hoc* pairwise comparisons of soil origin and heat treatments for linear models of plant biomass and root-shoot ratios of hawkweeds; *P. officinarum* and *H. lepidulum* plants. Treatment comparisons include soil origin from (Home; Hawkweed grown in its own soil, Away-H; hawkweed grown in the others soil and Away-C; hawkweed grown in *C. macra* soil) and heat treatments (Unheated, and 45°C). Standard errors are shown in brackets. Statistically significant comparisons are shown in bold. N = 96.

Plant species	*P. officinarum*						*H. lepidulum*					
Treatment combination	Plant biomass			Root-shoot ratio			Plant biomass			Root-shoot ratio		
	Estimate	*T*-value	*P*-value	Estimate	*T*-value	P-value	Estimate	*T*-value	*P*-value	Estimate	*T*-value	*P*-value
Home UH x Away C UH	1.40(0.55)	2.54	0.130	**0.71(0.21)**	**3.46**	**0.015**	-0.01(0.03)	-0.2	1.000	0.29 (0.43)	0.68	0.983
Home UH x Away H UH	**1.76(0.55)**	**3.2**	**0.030**	0.49(0.21)	2.4	0.18	-0.09(0.03)	-2.84	0.071	-0.04 (0.43)	-0.10	1.000
Home UH x Home 45°C	0.04(0.55)	0.07	1.000	0.58(0.21)	2.81	0.076	0.05(0.03)	1.47	0.687	0.64 (0.43)	1.50	0.667
Home UH x Away C 45°C	1.44(0.55)	2.63	0.110	0.40(0.21)	1.93	0.397	-0.06(0.03)	-1.9	0.415	0.31 (0.43)	0.73	0.977
Home UH x Away H 45°C	1.33(0.55)	2.41	0.170	0.53(0.21)	2.6	0.12	**-0.13(0.03)**	**-4.1**	**0.002**	-0.10 (0.43)	-0.24	1.000
Away C UH x Away H UH	0.36(0.55)	0.66	0.990	-0.22(0.21)	-1.06	0.894	-0.08(0.03)	-2.63	0.112	-0.33 (0.43)	-0.78	0.970
Away C UH x Home 45°C	-1.36(0.55)	-2.47	0.160	-0.13(0.21)	-0.65	0.986	0.05(0.03)	1.67	0.559	0.35 (0.43)	0.82	0.962
Away C UH x Away C 45°C	0.05(0.55)	0.09	1.000	-0.31(0.21)	-1.53	0.649	-0.05(0.03)	-1.7	0.540	0.02 (0.43)	0.05	1.000
Away C UH x Away H 45°C	-0.07(0.55)	-0.13	1.000	-0.18(0.21)	-0.86	0.954	**-0.12(0.03)**	**-3.89**	**0.004**	-0.39 (0.43)	-0.91	0.941
Away H UH x Home 45°C	**-1.72(0.55)**	**-3.13**	**0.040**	0.08(0.21)	0.41	0.998	**0.14(0.03)**	**4.3**	**0.001**	0.68 (0.43)	1.60	0.606
Away H UH x Away C 45°C	-0.32(0.55)	-0.57	0.990	-0.10(0.21)	-0.47	0.997	0.03(0.03)	0.93	0.936	0.35 (0.43)	0.83	0.960
Away H UH x Away H 45°C	-0.43(0.55)	-0.79	0.970	0.04(0.21)	0.2	1	-0.04(0.03)	-1.26	0.804	-0.06 (0.43)	-0.14	1.000
Home 45°C x Away C 45°C	1.40(0.55)	2.56	0.130	-0.18(0.21)	-0.88	0.95	**-0.11(0.03)**	**-3.37**	**0.019**	-0.33 (0.43)	-0.77	0.972
Home 45°C x Away H 45°C	1.29(0.55)	2.34	0.200	-0.04(0.21)	-0.21	1	**-0.18(0.03)**	**-5.56**	**0.001**	-0.74 (0.43)	-1.73	0.518
Away C 45°C x Away H 45°C	-0.12(0.55)	-0.22	1.000	0.14(0.21)	0.67	0.985	-0.07(0.03)	-2.19	0.263	-0.41 (0.43)	-0.97	0.926

Appendix C. Sequencing of hawkweed roots

This section outlines methods I undertook to identify AM fungi that had colonised roots of *H. lepidulum* and *P. officinarum* in my growth chamber study. Ultimately, I did not use this data because there was a high abundance of non-AM fungal reads, and when I removed these, I had many samples that had read depths too low to be considered feasible for further analysis.

During the harvesting of hawkweeds in the growth chamber study, I collected fresh root tissue (100 mg) from four randomly selected plants for each treatment. Roots were dried gently with paper towels, and the total fresh root weight and sample root weights were recorded. The sampled roots were cut into 1-2 cm pieces and placed in a sterile 2 mL Eppendorf tube which were stored at -20°C for a maximum of three weeks prior to DNA extraction. In total, this equated to 48 samples for both species (Figure 2.1).

Dried root weights were adjusted for the removal of root tissue for DNA extraction. This was done using the following calculation, which provides an estimate of the total dried root biomass based on the weights available. Dry root mass = dry mass + ((fresh root mass – fresh mass after subsample taken) / fresh mass) * dry mass). This adjusted weight was used to calculate the total plant biomass and root-shoot ratios.

Root DNA was extracted from 100 mg (fresh weight) of root tissue. A sterile stainless-steel bead (5 mm) was added to 2 mL Eppendorf tubes containing frozen root tissue. The tubes were immersed in liquid nitrogen for 30 seconds, then placed into the FastPrep-24 and run for 30 seconds at 4.5 m/z. One round was sufficient to turn the root tissue of both hawkweeds into a fine powder. DNA was extracted using the Qiagen plant mini kit, following the manufacturer's instructions. The concentration and purity of the

DNA from root extractions was estimated using a GE NanoVue UV-visible spectrometer. A subset of samples were visualised on a 1.0 % agarose gel stained with 2.5µL of ethidium bromide during the initial DNA extraction optimisations. These were run at 75 volts for 1 hour.

Root DNA was amplified via polymerase chain reaction using the WANDA and AML2 primers in the same manner as soil DNA extracts as described in section 2.2.2. These extracts were processed and sequenced in the same manner as described in section 2.2.2. Root and soil samples were all sequenced on the same Illumina MiSeq run.

Samples were processed via the DADA2 pipeline together with the soil DNA sequences in the same manner as described in section 2.2.3. After processing through the DADA2 bioinformatics pipeline, sequences that were not identified as AM fungi using the RDP classifier were removed. Root and soil sequencing projects were separated. Following DADA2 processing, there were 93,158 reads of the 48 samples in the root sequencing data. There were two highly abundant amplicon sequence variants identifying as AM fungi in the root sequencing data. These two amplicon sequence variants were present in very high abundance compared to other ASVs. One of the two amplicon sequence variants was associated with roots from *H. lepidulum,* and one was associated with roots of *P. officinarum*. Additional investigations were conducted to determine their identity. Both amplicon sequence variants were initially identified as AM from the MaarjAM database, but I wanted to identify them further.

I did a preliminary BLAST nucleotide database with default settings National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). For both amplicon sequence variants, the highest match was *Pilosella officinarum* isolate DM380 small

subunit ribosomal RNA gene GenBank number MT796508.1. This match was a direct submission to NCBI without a publication reference. This raised doubts on identifying these amplicons as AM fungi and prompted me to conduct further phylogenetic analyses.

I constructed a multiple sequence alignment of small ribosomal subunit sequences spanning the region of my primers using MEGA X (Kumar et al. 2018). This was used to construct a maximum likelihood phylogenetic tree. The Tamura and Nei model (TN93) with Gamma distributed invariant sites (G + I) was used with 1000 bootstraps. I included reference sequences of all AM families from my complied FASTA file of AM fungi. I also included plant sequences and non-AM fungi obtained from NCBI to construct this tree. The GenBank numbers of the sequences are included in the phylogenetic tree.

The results of the phylogenetic tree showed that the two amplicon sequence variants were grouping with the plant sequences, which had high bootstrapping support (Figure AC.1). The AM fungi were grouping roughly in their expected families with good bootstrapping support. The non-AM fungi were grouping with *Paraglomus* AM fungi which was unexpected. However, the purpose of this tree was to figure out whether the two amplicon sequence variants in high sequence abundance were plant or fungi. Results of the phylogenetic tree provided good evidence that they were, in fact, plant and not AM fungi. Therefore, it was decided to remove these two amplicon sequence variants from both the soil and root sequencing data.

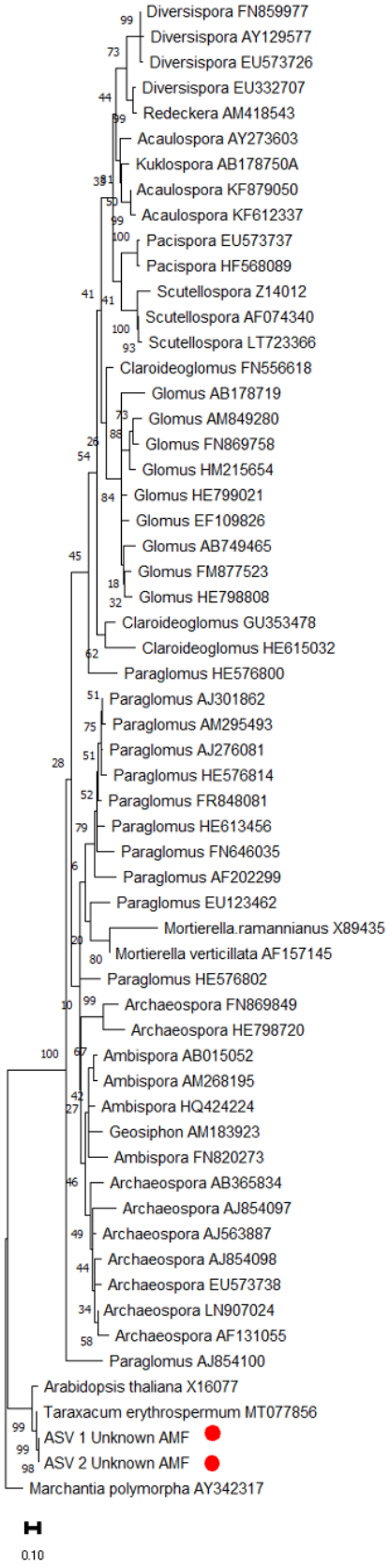
Diversispora FN859977
Diversispora AY129577
Diversispora EU573726
Diversispora EU332707
Redeckera AM418543
Acaulospora AY273603
Kuklospora AB178750A
Acaulospora KF879050
Acaulospora KF612337
Pacispora EU573737
Pacispora HF568089
Scutellospora Z14012
Scutellospora AF074340
Scutellospora LT723366
Claroideoglomus FN556618
Glomus AB178719
Glomus AM849280
Glomus FN869758
Glomus HM215654
Glomus HE799021
Glomus EF109826
Glomus AB749465
Glomus FM877523
Glomus HE798808
Claroideoglomus GU353478
Claroideoglomus HE615032
Paraglomus HE576800
Paraglomus AJ301862
Paraglomus AM295493
Paraglomus AJ276081
Paraglomus HE576814
Paraglomus FR848081
Paraglomus HE613456
Paraglomus FN646035
Paraglomus AF202299
Paraglomus EU123462
Mortierella.ramannianus X89435
Mortierella verticillata AF157145
Paraglomus HE576802
Archaeospora FN869849
Archaeospora HE798720
Ambispora AB015052
Ambispora AM268195
Ambispora HQ424224
Geosiphon AM183923
Ambispora FN820273
Archaeospora AB365834
Archaeospora AJ854097
Archaeospora AJ563887
Archaeospora AJ854098
Archaeospora EU573738
Archaeospora LN907024
Archaeospora AF131055
Paraglomus AJ854100
Arabidopsis thaliana X16077
Taraxacum erythrospermum MT077856
ASV 1 Unknown AMF
ASV 2 Unknown AMF
Marchantia polymorpha AY342317
0.10

Figure AC.1. Maximum likelihood phylogenetic tree of partial small ribosomal subunit nucleotide sequences. Based on a Tamura and Nei model (TN93) with Gamma distributed invariant sites (G + I). Numbers at tree nodes represent bootstrap support values (1,000 replications). Reference arbuscular mycorrhizal sequences, plants and fungi have a GenBank number next to the name. Amplicon sequence variants obtained from my study that were tested are indicated by red circles.

I attempted to rarefy my root sequencing data in the same manner as I had done for the soil. Unfortunately, I had very low sample read depth for my root sequences. Out of the 48 root samples sequenced, 18 had a library size of fewer than 1000 reads per sample (Table AC.1). I decided that 1000 reads per sample would be the lowest value that we would rarefy to. However, this would mean the loss of 30 samples which is more than half of those sequenced. Rarefying to less than 1000 reads per sample would make the root samples incomparable to the soil. Due to time restraints, it was decided not to explore this dataset any further for my masters.

Table AC.1 Number of reads per sample obtained for hawkweed root sequencing data.

Sample ID	Plant	Soil origin	Heat treatment	Replicate	Number of reads per sample
H. lepidulum_Away-C_UH_2	*H. lepidulum*	Away-C	UH	2	790
H. lepidulum_Away-C_UH_6	*H. lepidulum*	Away-C	UH	6	197
H. lepidulum_Away-C_UH_7	*H. lepidulum*	Away-C	UH	7	731
H. lepidulum_Away-C_UH_8	*H. lepidulum*	Away-C	UH	8	623
H. lepidulum_Away-C_45°C_2	*H. lepidulum*	Away-C	45°C	2	4387
H. lepidulum_Away-C_45°C_3	*H. lepidulum*	Away-C	45°C	3	1030
H. lepidulum_Away-C_45°C_4	*H. lepidulum*	Away-C	45°C	4	1268
H. lepidulum_Away-C_45°C_8	*H. lepidulum*	Away-C	45°C	8	684
H. lepidulum_Home_UH_2	*H. lepidulum*	Home	UH	2	452
H. lepidulum_Home_UH_3	*H. lepidulum*	Home	UH	3	1638
H. lepidulum_Home_UH_4	*H. lepidulum*	Home	UH	4	460
H. lepidulum_Home_UH_5	*H. lepidulum*	Home	UH	5	790
H. lepidulum_Home_45°C_4	*H. lepidulum*	Home	45°C	4	11073
H. lepidulum_Home_45°C_5	*H. lepidulum*	Home	45°C	5	1035
H. lepidulum_Home_45°C_6	*H. lepidulum*	Home	45°C	6	825
H. lepidulum_Home_45°C_7	*H. lepidulum*	Home	45°C	7	1316
H. lepidulum_Away-H_UH_2	*H. lepidulum*	Away-H	UH	2	1079
H. lepidulum_Away-H_UH_4	*H. lepidulum*	Away-H	UH	4	2607
H. lepidulum_Away-H_UH_5	*H. lepidulum*	Away-H	UH	5	1019
H. lepidulum_Away-H_UH_7	*H. lepidulum*	Away-H	UH	7	1227
H. lepidulum_Away-H_45°C_1	*H. lepidulum*	Away-H	45°C	1	1444
H. lepidulum_Away-H_45°C_6	*H. lepidulum*	Away-H	45°C	6	1109
H. lepidulum_Away-H_45°C_7	*H. lepidulum*	Away-H	45°C	7	1208
H. lepidulum_Away-H_45°C_8	*H. lepidulum*	Away-H	45°C	8	1608
P. officinarum_Away-C_UH_3	*P. officinarum*	Away-C	UH	3	1327
P. officinarum_Away-C_UH_4	*P. officinarum*	Away-C	UH	4	2281
P. officinarum_Away-C_UH_5	*P. officinarum*	Away-C	UH	5	1088
P. officinarum_Away-C_UH_7	*P. officinarum*	Away-C	UH	7	310
P. officinarum_Away-C_45°C_2	*P. officinarum*	Away-C	45°C	2	642
P. officinarum_Away-C_45°C_3	*P. officinarum*	Away-C	45°C	3	323

P. officinarum_Away-C_45°C_5	*P. officinarum*	Away-C	45°C	5	1589
P. officinarum_Away-C_45°C_8	*P. officinarum*	Away-C	45°C	8	922
P. officinarum_Away-H_UH_3	*P. officinarum*	Away-H	UH	3	5402
P. officinarum_Away-H_UH_4	*P. officinarum*	Away-H	UH	4	7887
P. officinarum_Away-H_UH_7	*P. officinarum*	Away-H	UH	7	10461
P. officinarum_Away-H_UH_8	*P. officinarum*	Away-H	UH	8	317
P. officinarum_Away-H_45°C_2	*P. officinarum*	Away-H	45°C	2	749
P. officinarum_Away-H_45°C_3	*P. officinarum*	Away-H	45°C	3	2566
P. officinarum_Away-H_45°C_4	*P. officinarum*	Away-H	45°C	4	1635
P. officinarum_Away-H_45°C_6	*P. officinarum*	Away-H	45°C	6	446
P. officinarum_Home_UH_2	*P. officinarum*	Home	UH	2	1657
P. officinarum_Home_UH_3	*P. officinarum*	Home	UH	3	674
P. officinarum_Home_UH_5	*P. officinarum*	Home	UH	5	2053
P. officinarum_Home_UH_8	*P. officinarum*	Home	UH	8	493
P. officinarum_Home_45°C_5	*P. officinarum*	Home	45°C	5	4509
P. officinarum_Home_45°C_6	*P. officinarum*	Home	45°C	6	1741
P. officinarum_Home_45°C_7	*P. officinarum*	Home	45°C	7	1545
P. officinarum_Home_45°C_8	*P. officinarum*	Home	45°C	8	3941
Total number of reads					93158