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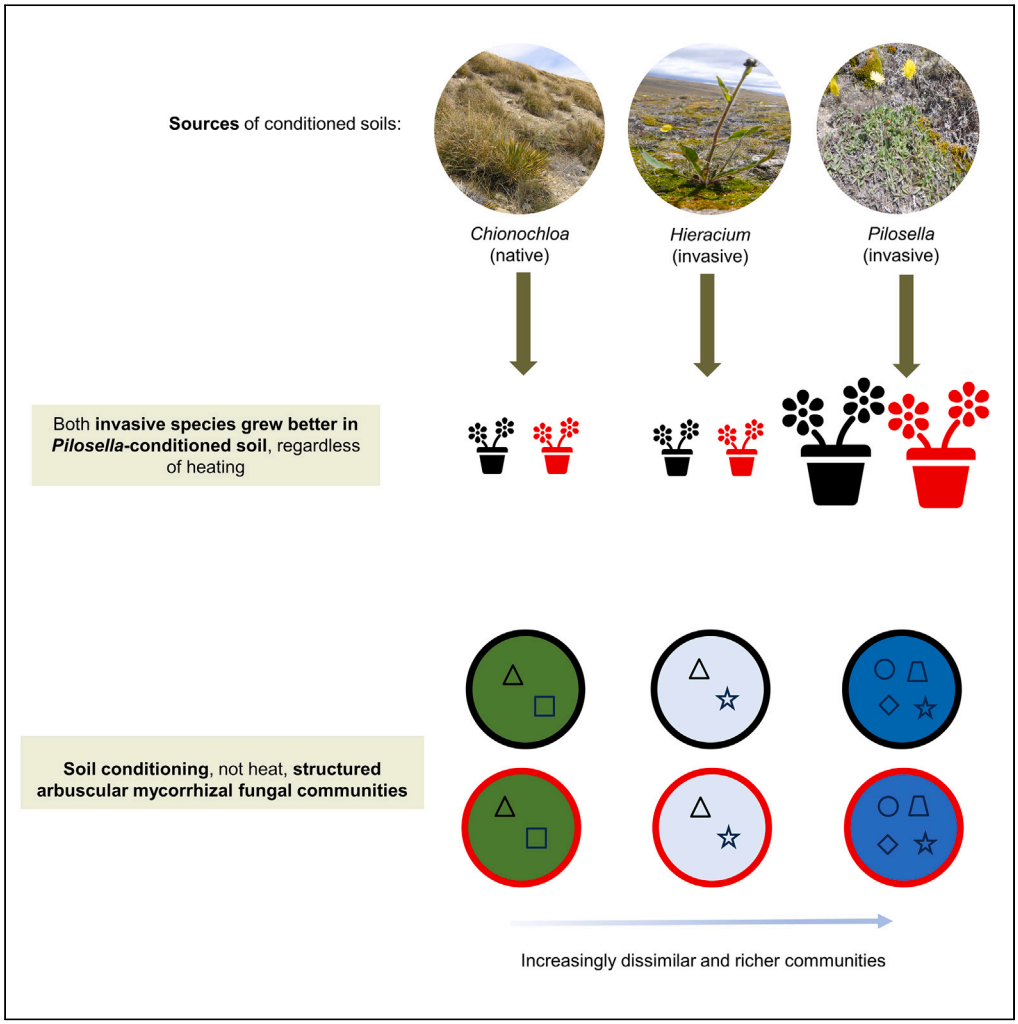

Kendall E. Morman, Hannah L. Buckley, Colleen M. Higgins, Micaela Tosi, Kari E. Dunfield, Nicola J. Day

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Highlights

Experiments showed simulated fire did not alter plant-soil feedback of two species

Both invasive plants grew better in invasive *Pilosella*-conditioned soils

Arbuscular mycorrhizal fungal community structure was not altered by simulated fire

Invasion has had a greater impact on soils than fire

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Simulated fire and plant-soil feedback effects on mycorrhizal fungi and invasive plants

Kendall E. Morman,[1,2,5] Hannah L. Buckley,[1] Colleen M. Higgins,[1] Micaela Tosi,[3] Kari E. Dunfield,[3] and Nicola J. Day[4,6,*]

SUMMARY

Climate change intensifies fires, raising questions about their impacts on plant invasions via changes in soil biota and plant-soil feedback (plants alter soil conditions, changing plant growth and vice-versa). We explored effects of plant-soil feedback and simulated fire (heat) on mutualistic arbuscular mycorrhizal (AM) fungal communities and invasive plant growth. Soils were collected from a dominant native grass (*Chionochloa macra*) and two invasive hawkweeds (*Hieracium lepidulum, Pilosella officinarum*) in a New Zealand grassland and then heated. In our experiment, both hawkweeds exhibited greater biomass in *Pilosella* soils, which also had the highest AM fungal richness. Heat had little effect on plant biomass or AM fungal community composition and richness. Hawkweeds altered AM fungal communities relative to the dominant native grass, and moderate soil heating increased *Hieracium* growth. *Hieracium* plants also grew better in *Pilosella* soils, suggesting the potential for soil-mediated invasional meltdown whereby one invasive species facilitates invasion by another.

INTRODUCTION

Fire activity is increasing in many regions of the world due to climate change, causing changes in plant communities and, sometimes, directly facilitating spread and local abundance of invasive plants.[1] Plants condition their soils over time by altering soil microbial communities, which in turn increase or reduce plant growth, a process known as plant-soil feedback.[2] Soil biota can determine the ability of invasive plants to increase in abundance and spread, often via mutualists such as mycorrhizal fungi that increase plant nutrient acquisition, water relations, stress tolerance, and soil aggregation.[3–5] Since fires can disrupt plant-soil feedback and alter mycorrhizal fungal communities,[6,7] it is predicted that growth of invasive plants that are highly reliant on mycorrhizal fungi and other soil biota would decline after fire. For example, fire could cause declines in particular taxa of mycorrhizal fungi that benefit the growth of a particular invasive species, thereby reducing its invasion potential.

Invasive species can have detrimental impacts on ecosystems,[8] so understanding mechanisms underlying the spread of invasive plant species is key to managing the current biodiversity crisis. Invasive plants can experience positive plant-soil feedback, where a given species grows better in soils conditioned by its own species compared to that of another species.[9] This response is due to reductions in pathogens or accumulation of a particular suite of mutualists. In negative plant-soil feedback, plant growth is reduced in soils conditioned by the same species and increased in soils conditioned by other species, thought to be due to either accumulation of pathogens or reductions of mutualists in their own soils. Whereas positive plant-soil feedback reinforces growth, abundance, and local establishment of a given plant species,[10] negative plant-soil feedback could favor the species to disperse and colonize new areas. A few studies have suggested a role of soil biota in invasional meltdown, where invasion by one species changes conditions to facilitate invasion by other invasive species.[11] For example, a reciprocal pot study showed that soil conditioned by a non-native plant altered soil microbial communities and enhanced growth of multiple other non-native species over native species.[12] Other studies in field conditions[13,14] and in controlled soil conditioning experiments[15] suggest that changes in soil abiotic or biotic conditions by exotic plants can facilitate subsequent invaders, suggesting that at least in some cases plant-soil feedback could be a key mechanism driving invasional meltdown.

An estimated 72% of plant species form mutualisms with arbuscular mycorrhizal (AM) fungi.[16] Differences in AM fungal community structure can cause host-specific differences in plant growth; these differences play a key role in at least some successful plant invasions.[3,5] Plant invasions often correlate with declines in AM fungal richness and altered AM fungal community composition.[5] Invasive plants that grow well without mycorrhizal fungi or are generalists in their ability to form mycorrhizas with a broad range of fungal taxa experience fewer barriers to successfully spreading and establishing in new environments.[3] In the absence of mediating factors, invasive plants that change AM fungal

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communities to increase their own growth and experience positive plant-soil feedback are expected to lead to successful invasions.[17] Thus, factors that disrupt plant-soil feedback or alter AM fungal communities will impact growth of invasive species.

Heat from fires directly alters abiotic and biotic components of soils,[18] and knowledge of its impact is increasingly important for understanding plant-soil feedback and plant community structure as fire activity increases with climate change.[19,20] Because AM fungal richness and root colonization rates often decline after fires,[6] invasive plants that are highly dependent on AM fungi may experience reduced growth after fire. Further, differences in heat tolerance among AM fungal taxa could lead to convergence in AM fungal community composition after fire,[21] impacting plant growth. Although grassland fires tend to be fast moving with little penetration of heat into soils for more than a few minutes,[22–24] these have been shown to alter AM fungal communities[21,25] and plant-soil feedback.[7] Knowledge of the role of fire in determining mycorrhizal fungi-mediated invasions is poorly understood, but likely to become increasingly important as climate change progresses.

In this study, we assessed how plant-soil feedback and simulated fire, via soil heating, impacted AM fungal communities and growth of two invasive species. We collected soils from under individual plants at a site where a native dominant grass, *Chionochloa macra* Zotov, has coexisted with invasive hawkweeds, *Hieracium lepidulum* (Stenstr.) Omang and *Pilosella officinarum* Vaill. at fine spatial scales (0.25 m^2) for at least 40 years[26] and, being perennial species, is likely to have conditioned the soil. All three species form associations with AM fungi.[27–29] We subjected soil samples to heat treatments in the lab to simulate temperature experienced in grassland fires. We predicted the following patterns: (1) both hawkweeds would experience positive plant-soil feedback, and the strength and/or direction of this feedback would be altered by soil heating; (2) AM fungal communities in soils conditioned by the two hawkweed species would be more similar to each other than those conditioned by *Chionochloa*; and (3) AM fungal community structure (richness, composition) would be altered by heating, and there would be increases in a distinct group of taxa.

RESULTS

Both invasive hawkweeds had greater biomass in soils conditioned by *Pilosella*, regardless of heat

Using soils from a site where *Chionochloa*, *Hieracium*, and *Pilosella* had been present for at least 40 years, the growth chamber study showed both hawkweed species had significantly greater biomass after 12 weeks of growth in soils conditioned by *Pilosella*, compared to those conditioned by either *Chionochloa* or *Hieracium*; these patterns were largely consistent whether soils were heated to 45°C or not (Figure 1; Tables S1 and S2). *Hieracium* plants also had greater biomass in *Chionochloa*-heated soils compared to *Hieracium*-heated soils. Root-shoot ratios of both *Hieracium* and *Pilosella* plants did not significantly differ among soil conditioning (*Chionochloa*, *Hieracium*, *Pilosella*) or heat treatments (unheated, 45°C).

AM fungal community structure was related to soil conditioning, not heat

We used four heat treatments to assess the impacts of simulated heat from fire on community structure of AM fungi in field-collected soils conditioned by each plant species (*Chionochloa*, *Hieracium*, *Pilosella*): Unheated, 30°C, 45°C, or 60°C (10 biological replicates per soil conditioning-heat combination minus one sample that failed to sequence; $n = 119$). The MiSeq Illumina sequencing (small ribosomal subunit [SSU]) of soils resulted in a total of 16,690,523 reads, of which 110,620 reads were AM fungi according to the MaarjAM database.[30] Our final dataset contained a total of 178 AM fungal amplicon sequence variants (ASVs) in the 119 soil samples. Of the 178 ASVs identified, 134 aligned with a virtual taxon in MaarjAM; 24 virtual taxa were represented. Taxon accumulation curves approached sampling saturation in all soil conditioning treatments and heat treatments (Figures S1 and S2). Each soil sample had a mean of 12 $\pm$ 1 AM fungal ASVs (range: 3–27), and each ASV was present in a mean of 8 samples $\pm$ 1 SE (range: 1–72). The ASVs encompassed eight AM fungal genera, with the most frequent in the dataset belonging to *Glomus* (order Glomerales), followed by *Archaeospora* (order Archaeosporales; Figure 2). Assessing the ten most frequent ASVs in each soil conditioning treatment, we saw that soils conditioned by *Chionochloa* were dominated by Glomeraceae, while soils conditioned by *Hieracium* were dominated by Glomeraceae and Paraglomeraceae (Table S3). The most frequent ASVs in soils conditioned by *Pilosella* encompassed Glomeraceae, Acaulosporaceae, and Archaosporaceae.

Soils conditioned by each plant species had a similar number of unique ASVs, and 37 ASVs were shared among all three soil conditioning treatments (Figure 3). However, soils conditioned by *Pilosella* and *Hieracium* shared more ASVs than either species did with *Chionochloa*. *Chionochloa*-conditioned soils had a number of ASVs in the order Diversisporales, but these were mainly *Acaulospora*. The two ASVs in *Diversispora* identified were only in hawkweed soils (ASVs 152 and 286), and only one of the seven ASVs in *Scutellospora* was present in *Chionochloa*-conditioned soils (and only in one sample: ASV 133). Within the order Archaeosporales, the hawkweeds had more *Archaeospora*, but *Ambispora* was more common in *Chionochloa*-conditioned soils; this genus was entirely absent from soils conditioned by *Pilosella*. Many ASVs were present across all heat treatments (Figures 2 and 3).

Statistical analyses supported that soil conditioning was a greater determinant of AM fungal community structure than heat. The first two axes of the principal coordinate analysis (PCoA) based on Jaccard's dissimilarity explained 17.7% of the variation in AM fungal communities (Figure 4). There was some clustering of AM fungal composition by soil conditioning but considerable overlap among samples from different heat treatments in ordination space. AM fungal community composition differed significantly by soil conditioning (pairwise permutational analysis of variance [PERMANOVA]: $F = 6.34$, $p = 0.001$) but not heat treatment ($F = 0.72$, $p = 0.990$) or the interaction of these two factors ($F = 0.58$, $p = 1$). Composition of AM fungal ASVs was statistically different among all three soil conditioning treatments (Table 1). Indicator ASVs for all three soil conditioning treatments belonged to the genera *Acaulospora*, *Archaeospora*, *Glomus*, and *Paraglomus* (Figure 5; Table S4). Only *Chionochloa*-conditioned soils had indicator ASVs from the genus *Ambispora*, while only hawkweed soils had indicator ASVs from the genus *Claroideoglomus* (although the identities of the significant ASVs of this genus differed for *Hieracium* and *Pilosella*).

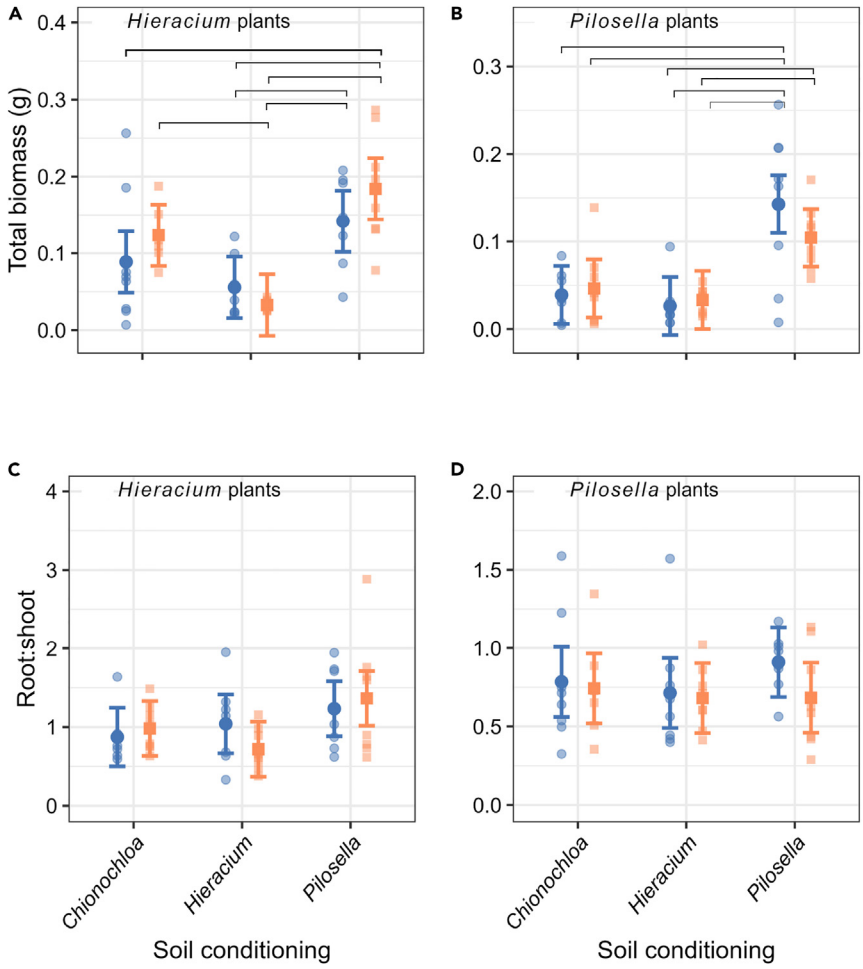

Figure 1. Dry total biomass and root-shoot ratios of *Hieracium* and *Pilosella* plants

Total biomass (A, B) and root-shoot ratios (C, D) of *Hieracium lepidulum* and *Pilosella officinarum* plants that were grown in soils conditioned by *Chionochloa macra*, *Hieracium lepidulum*, or *Pilosella officinarum* that were not heated (blue) or heated to 45°C (orange). Predicted means and upper and lower 95% confidence intervals (error bars) from linear models are shown (see also Table S1). Raw data are overlaid as semi-transparent points. Significant contrasts are shown based on Tukey's HSD *post hoc* contrasts ($p < 0.05$; see also Table S2). *Hieracium* plants: $n = 46$; *Pilosella* plants: $n = 48$.

We also observed that some *Acaulospora* and *Archaeospora* ASVs were indicators of hawkweed soils but not for *Chionochloa*-conditioned soils (Figure 5). In terms of AM fungal richness, soils conditioned by *Pilosella* tended to have the greatest richness (Figure 6). *Pilosella*-conditioned soils that were unheated had significantly higher richness than *Hieracium* soils across all heat treatments and *Chionochloa* soils heated to 60°C; these patterns were the same for *Pilosella*-conditioned soils that were heated to 60°C (Tables S5 and S6).

We found relationships between AM fungal communities in the field-collected soil and plant growth in the plant-soil feedback study. Total plant biomass was positively correlated with soil AM fungal community composition (PCoA axis 1 scores) for both *Hieracium* ($\rho = 0.34$, $p = 0.022$) and *Pilosella* plants ($\rho = 0.41$, $p = 0.005$; Figure 7). Similarly, there were positive correlations between total plant biomass and soil AM fungal richness for both *Hieracium* ($\rho = 0.32$, $p = 0.030$) and *Pilosella* plants ($\rho = 0.34$, $p = 0.020$).

DISCUSSION

Our study demonstrates that plant-soil feedback shapes both AM fungal community structure and the growth of two invasive hawkweed species; surprisingly, these were largely unaltered by heating to simulate fire. We infer that invasion by hawkweeds modifies soils and AM fungal communities and that this modification, particularly by *Pilosella*, promotes growth of these invasive plants. Both hawkweed species had greater biomass in soils conditioned by *Pilosella*, which correlated with AM fungal communities with greater richness and different composition, compared to soils conditioned by *Hieracium* (the other hawkweed) and *Chionochloa* (the dominant native grass). We observed evidence for a degree of convergence in AM fungal communities associated with invasion by hawkweeds. Firstly, soils conditioned by each of the three plant species differed in AM fungal community composition, but *Hieracium*- and *Pilosella*-conditioned soils shared a larger

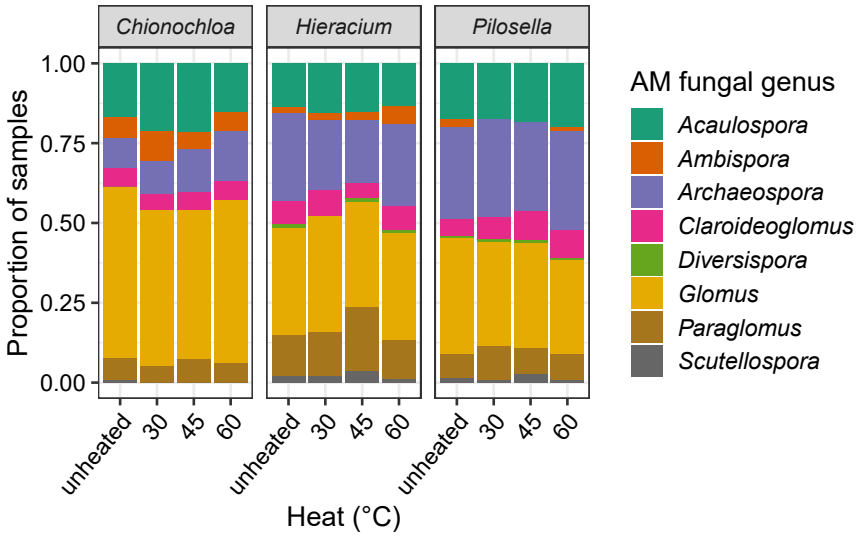

Figure 2. Representation of genera of AM fungi

Proportion of samples (frequency) in which genera of arbuscular mycorrhizal fungi were detected in soils conditioned by *Chionochloa macra*, *Hieracium lepidulum*, and *Pilosella officinarum* plants, subjected to different heat treatments. Data are from Illumina MiSeq of the SSU region. $n = 119$.

number of AM fungal ASVs than either species shared with *Chionochloa*. Secondly, while there were AM fungal genera that were significantly positively associated with all three plant species, there were distinct genera associated with *Chionochloa* that were not associated with either hawkweed species, and vice-versa. Together, our results suggest that hawkweed invasion alters AM fungal communities relative to the dominant native grass cover in this environment, but heat by fires could facilitate growth of *Hieracium* in these grasslands. Further, plant-soil feedback by *Pilosella* could facilitate invasion by *Hieracium*, suggesting soil-mediated invasional meltdown as a possible mechanism facilitating the spread of *Hieracium* across the landscape.

Our results suggest *Pilosella* may be an AM fungal generalist because its soils contained many AM fungal ASVs and had high richness. Being an AM fungal generalist is advantageous for invasive plants because their distribution is less limited by specific mutualist requirements[3,5]; however, there are examples of successful invaders with specific mutualist requirements, particularly woody species.[31] The greater richness of AM fungi in *Pilosella*-conditioned soils increases the probability that there are taxa that are beneficial for *Hieracium* growth, and this may have contributed to *Hieracium* growing better in *Pilosella*-conditioned soils in the growth chamber study compared to its own soil. Hawkweeds, which are closely related and are in the same family (Asteraceae), may benefit from the same AM fungal taxa compared to those in *Chionochloa* (Poaceae) soils, although a meta-analysis suggests that closely related plant species tend to have less similar AM fungal communities than plants that are more distantly related.[32] While AM fungal richness in our soil samples seems low (range: 3–27), it is comparable to results from sequencing AM fungi in roots in the same system (range: 3–30).[33] Our weak but significant relationships between hawkweed biomass and soil AM fungal richness and community composition indicate that AM fungi may play a part in growth of *Hieracium* and *Pilosella*, supporting previous research.[28,29,34–36] Conditioning of soil by *Pilosella* could have altered other attributes of the soil community or abiotic conditions that impacted growth of *Hieracium*. *Pilosella* alters soil pH and C:N[37] and may produce compounds that have allelopathic effects.[38] Our additional analyses found no differences in percent soil carbon among treatments (Figure S3; Tables S7 and S8). Soil pH also increases after fire and is a key determinant of soil fungal community structure.[39–41]

The different AM fungal communities and positive plant-soil feedback observed in *Pilosella*-conditioned soils suggest that these attributes may reinforce growth and local abundance of this invasive species once it colonizes a new area. Although both hawkweed species are highly invasive in these grasslands, *Pilosella* is often in much greater relative abundance (percent cover) at fine spatial scales than *Hieracium* (0.025 m^2).[42] *Pilosella* plants tend to invest more resources into clonal growth (rhizomes) when nutrient availability is high and competes best when inoculated with AM fungi,[43] so associating with effective soil mutualists that enhance plant nutrients could facilitate localized abundance. It is therefore advantageous for clonal species, such as *Pilosella*, to experience positive plant-soil feedback. Conversely, the negative plant-soil feedback observed in plants in *Hieracium*-conditioned soils, combined with its inability to reproduce clonally, may explain why it has lower local abundance than *Pilosella* in the field.[42] The negative feedback experienced by *Hieracium* could be due to associating with ineffective AM fungi,[44] or an accumulation of negative soil biota, such as pathogens or pests. These negative interactions could drive *Hieracium* to colonize new areas, which is consistent with its expanding distribution on the landscape.[42] The ability of *Hieracium* to grow better in heated soils conditioned by *Chionochloa* suggests that fire could facilitate further invasion of this plant. Fires have been shown to reduce soil pathogens and increase growth of invasive species[45] and even reverse the direction of plant-soil feedback.[7]

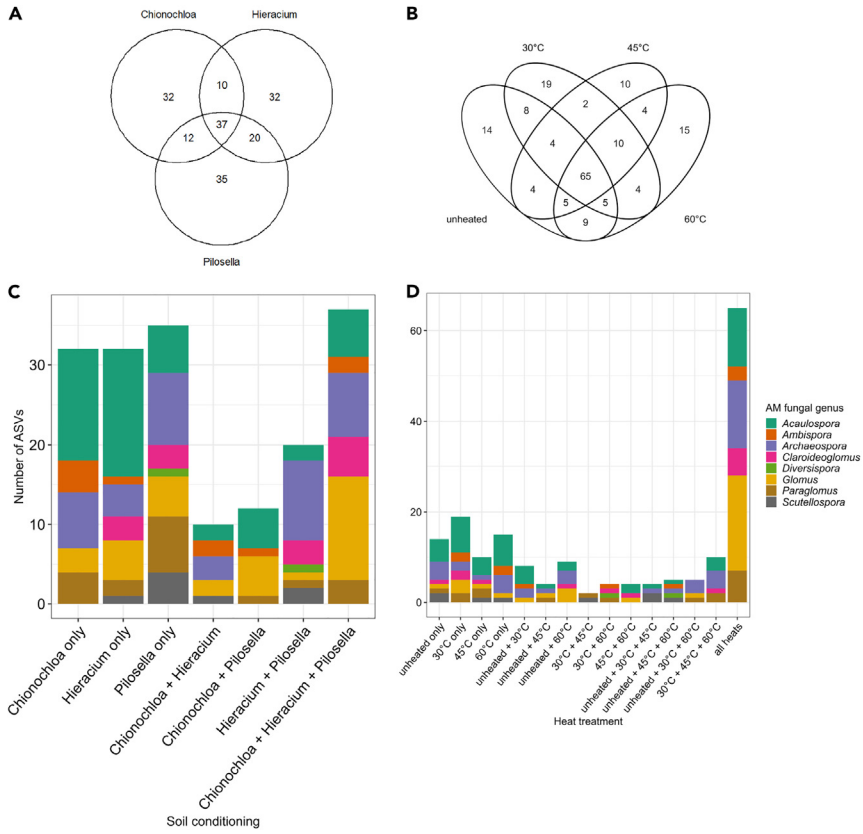

Figure 3. Unique and shared AM fungal ASVs in soil conditioning and heat treatments and by genus

Venn diagrams showing the number of arbuscular mycorrhizal fungal amplicon sequence variants (ASVs) unique to, or shared among, (A) soil conditioning treatments (*Chionochloa macra*, *Hieracium lepidulum*, and *Pilosella officinarum*) and (B) heat treatments (unheated, 30°C, 45°C, 60°C), and stacked bar graphs showing the number of ASVs in each genus unique to, or shared among, (C) soil conditioning and (D) heat treatments. Data are from Illumina MiSeq of the SSU region. $n = 119$.

While soil AM fungal communities among the three plant species differed, *Pilosella*-conditioned soils were particularly distinct; richness was greater and more ASVs were indicators. Soils conditioned by the two hawkweeds had more similar indicator AM fungal ASVs than either one had with *Chionochloa*-conditioned soils. For example, ASVs in the genus *Claroideoglomus* were indicators of soils conditioned by both *Hieracium* (one ASV) and *Pilosella* (three ASVs), but not *Chionochloa*. In contrast, ASVs in the genus *Ambispora* were indicators of soils conditioned by *Chionochloa* (2 ASVs) but not the hawkweeds. Dominant ASVs in soils also differed under the different species. *Hieracium* soils were dominated by ASVs in two families (Glomeraceae and Paraglomeraceae), but the native *Chionochloa* soils were dominated by ASVs in only one family (Glomeraceae). The most frequent ASVs in *Pilosella* soils encompassed three families (Glomeraceae, Acaulosporaceae, and Archaeosporaceae), further supporting that this species is an AM fungal generalist. Previous work in Aotearoa New Zealand's grasslands showed that AM fungal communities in roots of exotic species differed from those in roots of native species, with natives dominated by Acaulosporaceae ASVs.[33] Although we did not observe this pattern in the soils of our three plant species, only one of the 30 species in the study by Ramana et al.[33] overlapped with ours (*Pilosella*) and results may not be directly comparable because at least some AM fungal groups show preference for either soil or roots.[46] Although plant-soil feedback studies often use a "conditioning" phase in the glasshouse, our multi-decadal knowledge of the site where soils were collected and the biology of the plant species,[26,42,47] particularly in terms of establishment times and sizes and the patchy distribution of vegetation at the site, provides confidence that the soil conditioning effect observed is due to plant-soil feedback and *in situ* soil conditioning.

We were surprised that soil heating did not significantly impact AM fungal communities because they have repeatedly been shown to decline in abundance and richness after fire.[6] There is generally poor understanding of temperatures required to kill microbes.[48] The heat treatments we used of low temperatures and short periods reflect the only knowledge we are aware of regarding soil temperatures during fires in New Zealand's tussock grasslands, where soil temperatures did not exceed 59°C and cooled rapidly.[49] Temperatures used in our study are comparable to those observed during fires in tussock grasslands in other regions, where temperatures at 2 cm depth in soils seldom exceed 65°C and return to ambient temperature within just a few minutes[23,50–52]; this is similar to grassland fires as a whole.[24,53] In support of our results from the growth chamber, a study in a comparable tussock grassland site in Otago, New Zealand, found no effect

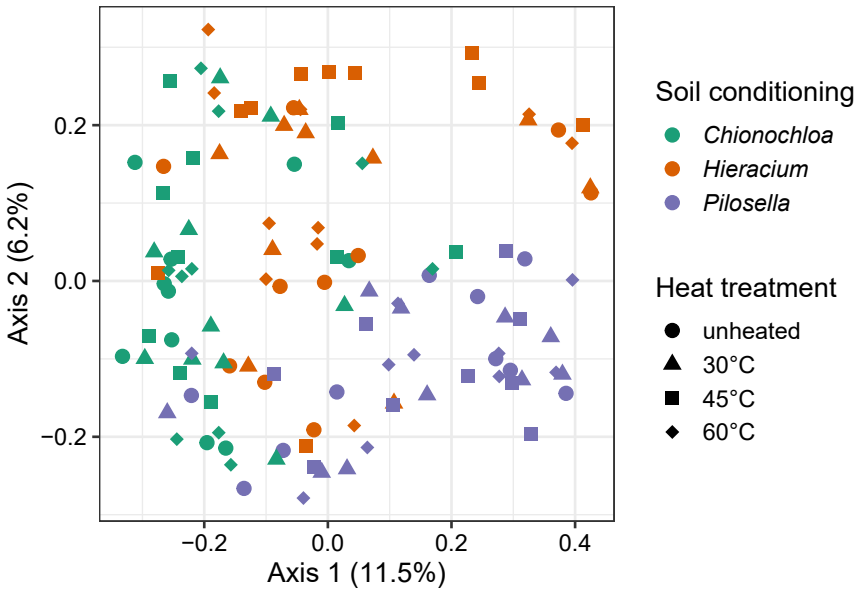

Figure 4. PCoA of AM fungi

Sample scores from principal co-ordinates analysis of Jaccard dissimilarities of arbuscular mycorrhizal fungal amplicon sequence variants in samples. Color of points shows the soil conditioning treatment (*Chionochloa macra*, *Hieracium lepidulum*, and *Pilosella officinarum*), and shapes of points show the heat treatment (unheated, 30°C, 45°C, 60°C). $n = 119$.

of wildfire on soil fungal composition (MiSeq, ITS1 region).[54] In Pedley's[54] study, the soil temperature during the fire was not measured and sampling occurred nine weeks after fire, so it is possible that soil fungal communities had recovered between the wildfire and sampling time. In South Australian tussock grasslands, soil fungal community composition shifted in response to increased fire frequencies, with the authors suggesting that changes in plant communities underlay this pattern, particularly for AM fungi that closely associate with plants.[25]

Our study contributes valuable information to the scarce knowledge on potential impacts of fires on plant-soil feedback, a disturbance that is thought to have been infrequent in our study region until ~750 years ago.[55,56] Our experiments suggest that moderate heat from wildfires in tussock grasslands could modify soils in a way that facilitates invasion by *Hieracium* into *Chionochloa*-dominated areas, but *Pilosella* can invade regardless of fire. Both *Pilosella* and *Hieracium* have traits that facilitate persistence through fires because they have low flammability,[57] and studies after recent wildfires in multiple tussock grassland sites strongly suggest that hawkweeds had survived with intact root systems.[58] This persistence re-enforces plant-soil feedback through fire disturbance. We speculate that plant-soil feedback and low flammability may account for invasion of hawkweeds in tussock grasslands in the past and may facilitate further invasion in the future.

Overall, our study demonstrates the importance of plant-soil feedback and AM fungal communities when attempting to predict outcomes of the combined crises of changing biodiversity and climate. Soil conditioning was consistently more important than heat, and the lack of significant effects of soil heating on AM fungal communities was surprising. Both hawkweed species had greater biomass in soils conditioned by *Pilosella*, and these soils also had greater AM fungal richness compared to soils conditioned by *Chionochloa* or *Hieracium*. Together, our results suggest that plant-soil feedback and AM fungi influence hawkweed growth and that *Pilosella* is an AM fungal generalist. Fire impacts on soils will likely not impact invasion by *Pilosella*, but they could facilitate further invasion of *Hieracium* into native tussock grasslands. Further, modification of soils and AM fungal communities by *Pilosella* may facilitate invasion by *Hieracium* in this environment, suggesting soil-mediated invasional meltdown.

Limitations of the study

Our experiments were conducted in the lab and growth chamber, so they may not fully reflect phenomena that occur in the field. Future experiments should measure the temperatures reached in the soils during the heating treatments, or the duration of maximum temperatures. Heating represents only one aspect of fires, and it is likely indirect factors, such as smoke and changes in other edaphic factors, could impact plant-soil feedback and mycorrhizal fungi. It is possible that our results reflect physical disturbance due to disruptive sampling to collect soils, but we minimized this by putting samples on ice immediately at the time of collection. Pooling soil samples within species from the one site was done due to space constraints but may have reduced intraspecific variation in responses in our experiments.[59,60] It is possible that our PCRs amplified relic or extracellular DNA from dead AM fungi. We chose primers that resulted in good taxonomic resolution of AM fungi, but

Table 1. Pairwise PERMANOVA of AM fungal ASVs among soil conditioning treatments

Soil conditioning comparison	df	SS	*F*	$p_{adjusted}$
Chionochloa - Hieracium	1	2.08	5.75	0.003
Chionochloa - Pilosella	1	2.87	8.33	0.003
Hieracium - Pilosella	1	2.05	5.62	0.003

Results for pairwise permutational analysis of variance (PERMANOVA) comparing variation in composition of arbuscular mycorrhizal fungal amplicon sequence variants (Jaccard dissimilarities) among soil conditioning treatments. df, degrees of freedom; SS, sums of squares. *n* = 119.

it is possible that other primers would have picked up more or different taxa. The sequencing-based approach of the study means that plant growth cannot be attributed to any particular abiotic or biotic effect, such as AM fungal taxon or ASV, and absolute abundances of AM fungi was not measured (e.g., by estimating colonization of roots).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to Dr. Nicola Day (nicola.day@vuw.ac.nz).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- DNA sequence data have been deposited to NCBI under BioProject PRJNA1086500.
- Plant biomass and ASV data have been deposited in Mendeley dataset https://data.mendeley.com/datasets/ty3z4tmpgm/1.
- All original code is available in this paper's supplemental information. Any additional information required to reanalyze the data reported in this paper is available from the corresponding author upon request.

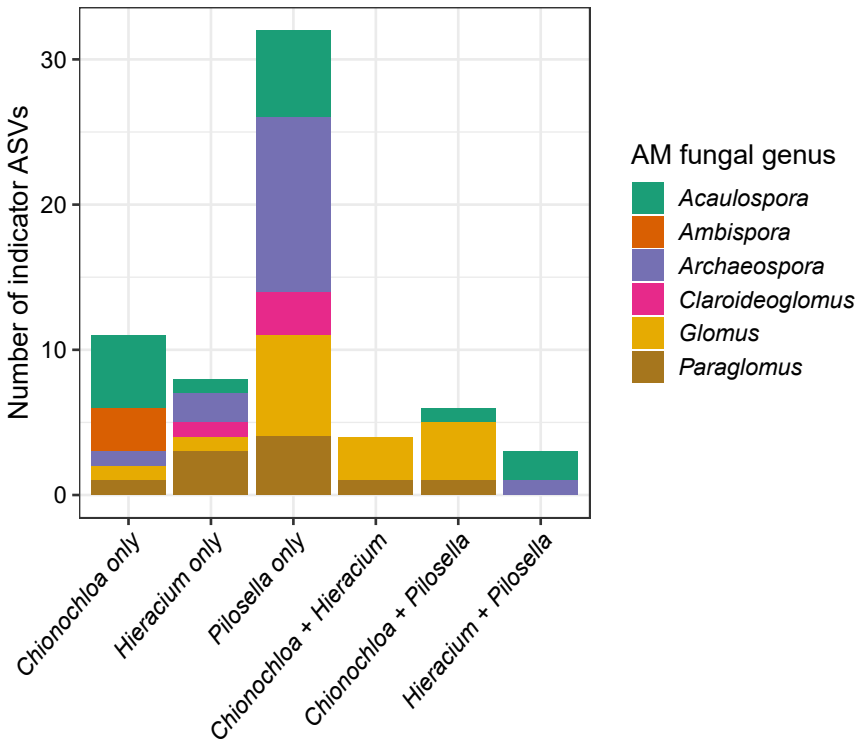

Figure 5. Significant indicator AM fungal ASVs according to soil conditioning

Summary of indicator species analysis, showing the number of arbuscular mycorrhizal fungal amplicon sequence variants (ASVs) that were significant indicators of each soil conditioning treatment or combination of treatments and their genus (see also Table S4). *n* = 119.

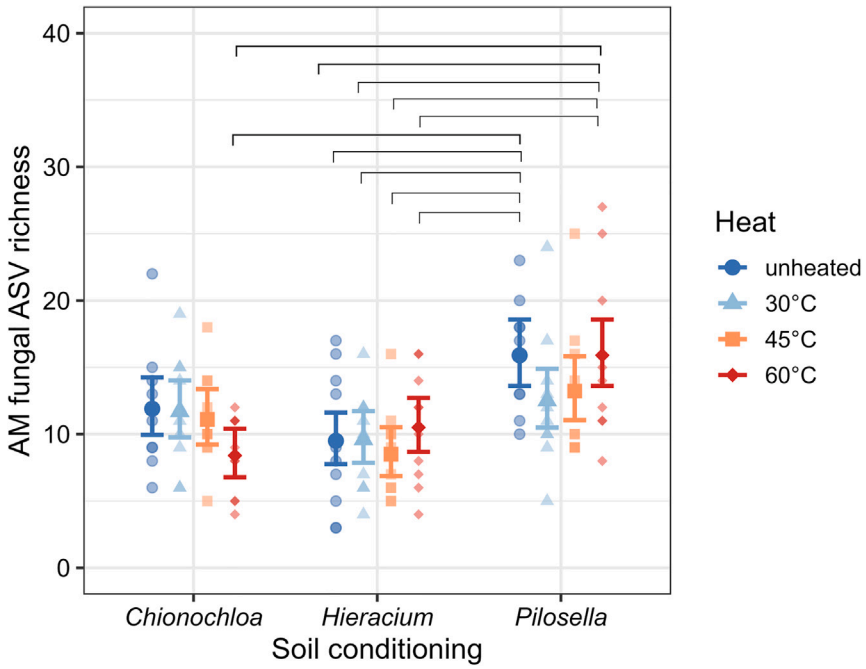

Figure 6. AM fungal richness by soil conditioning and heat treatments

Arbuscular mycorrhizal (AM) fungal richness of amplicon sequence variants (ASVs) in samples taken from three soil conditioning treatments (*Chionochloa macra*, *Hieracium lepidulum*, and *Pilosella officinarum*) and subjected to heat treatments (unheated, 30°C, 45°C, 60°C). Predicted means and upper and lower 95% confidence intervals (error bars) from a generalized linear model with Poisson errors are shown (Table S5). Raw data are overlaid as semi-transparent points. Significant contrasts are shown based on Tukey's HSD *post hoc* contrasts ($p < 0.05$; see also Table S6). *Nn*= 119.

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AUTHOR CONTRIBUTIONS

Conceptualization, N.J.D. with K.E.M., C.M.H., and H.L.B.; methodology, K.E.M. and N.J.D.; investigation, K.E.M.; formal analysis, K.E.M., N.J.D., and M.T.; software, K.E.M., N.J.D., and M.T.; resources, N.J.D., H.L.B., and C.M.H.; data curation, K.E.M. and N.J.D.; writing – original draft, K.E.M.; writing – review and editing, N.J.D., K.E.M., H.L.B., M.T., and K.E.D.; visualizzation, K.E.M. and N.J.D.; supervision, N.J.D., H.L.B., and C.M.H.; project administration, K.E.M. and N.J.D.; funding acquisition, K.E.M., N.J.D., and H.L.B.

DECLARATION OF INTERESTS

H.L.B. is a guest editor of this special issue Plant-Microbe-Soil Interactions. He was not involved in any editorial decisions related to this article.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- METHOD DETAILS
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  - Simulated fire and AM fungal communities
- QUANTIFICATION AND STATISTICAL ANALYSES

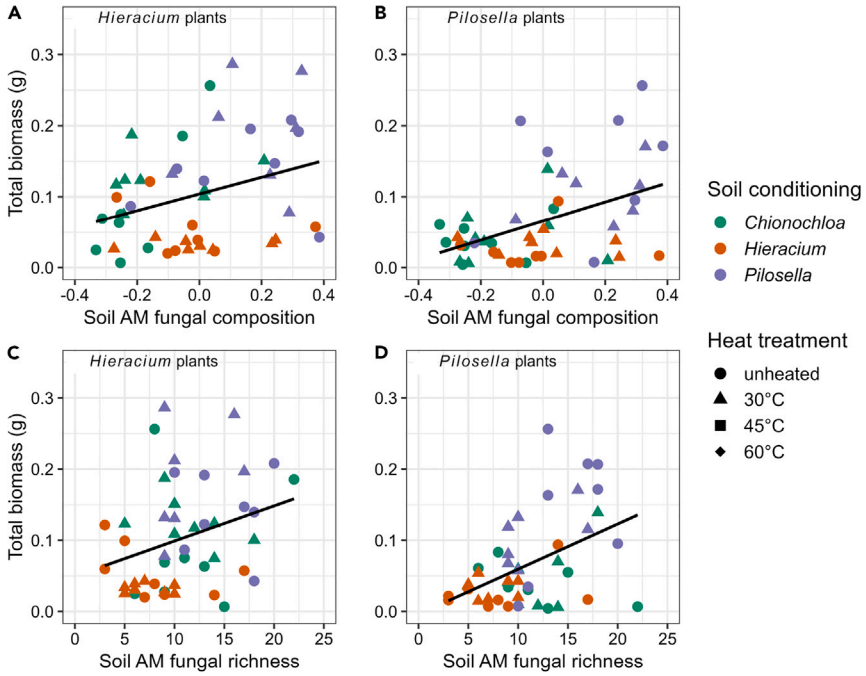

Figure 7. Relationships between *Hieracium* and *Pilosella* plant biomass and AM fungal composition and richness

Scatterplot of relationships between total plant biomass and (A) soil arbuscular mycorrhizal (AM) fungal composition (axis 1 of principal co-ordinates analysis; see also Figures 4 and 6) of *Hieracium* plants and (B) *Pilosella* plants, and total plant biomass and soil AM fungal richness for (C) *Hieracium* plants and (D) *Pilosella* plants. Color of points shows the soil conditioning treatment (*Chionochloa macra*, *Hieracium lepidulum*, and *Pilosella officinarum*), and shapes of points show the heat treatment (unheated, 30°C, 45°C, 60°C). Correlation lines are shown in black. $n = 119$.

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Soil conditioned by *Chionochloa macra*, *Hieracium lepidulum*, and *Pilosella officinarum*	Porters Pass, Canterbury, New Zealand (43°16′12.0″S 171°37′51.6″E)	N/A
Critical commercial assays		
DNeasy PowerSoil pro kit	Qiagen	Cat. No. 47016
KAPA HIFI HotStart Readymix	Roche	Cat. No. KK2602
Beckman Coulter AMPure XP	Beckman Coulter	A63881
Metagenomic Sequencing Library Preparation	Illumina	Part # 15044223 Rev. B
Qubit dsDNA Quantification Assay Kit	Invitrogen	Q32851
Deposited data		
Raw DNA sequences	This study	NCBI BioProject PRJNA1086500
Plant biomass data and processed DNA sequences	This study	Mendeley dataset https://data.mendeley.com/datasets/ty3z4tmpgm/1
Experimental models: Organisms/strains		
Hieracium lepidulum (Stenstr.) Omang	Seeds collected from Porters Pass, Canterbury, New Zealand (43°16′12.0″S 171°37′51.6″E); Lammerlaw Ranges, Otago, New Zealand (45°44′06.0″S 169°51′38.9″E)	N/A
Pilosella officinarum Vaill.	Seeds collected from Porters Pass, Canterbury, New Zealand (43°16′12.0″S 171°37′51.6″E); Lammerlaw Ranges, Otago, New Zealand (45°44′06.0″S 169°51′38.9″E)	N/A
Oligonucleotides		
Forward primer WANDA: 5′-CAG CCG CGG TAA TTC CAG CT-3′	Integrated DNA Technologies	Dumbrell et al.[61]
Reverse primer AML2: 5′- GAA CCC AAA CAC TTT GGT TTC C-3′	Integrated DNA Technologies	Lee et al.[62]
Software and algorithms		
R code	This study	Supplementary file
R software v. 4.3.3	R Core Team[63]	https://cran.r-project.org
MaarjAM database (6 May 2019)	Öpik et al.[30]	https://maarjam.ut.ee/
QIIME 2 2021.8	Bolyen et al.[64]	https://qiime2.org/
cutadapt	Martin et al.[65]	https://cutadapt.readthedocs.io/en/stable/
DADA2	Callahan et al.[66]	https://benjjneb.github.io/dada2/
q2-RESCRIPt	Robeson et al.[67]	https://library.qiime2.org/plugins/rescript/27/
q2-feature-classifier classify-consensus-blast	Camacho et al.[68]	https://github.com/qiime2/q2-feature-classifier/blob/dev/q2_feature_classifier/_blast.py

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
tidyverse	Wickham et al.[69]	https://cran.r-project.org/web/packages/tidyverse/index.html
egg	Auguie[70]	https://cran.r-project.org/web/packages/egg/index.html
ggvenn	Yan[71]	https://cran.r-project.org/web/packages/ggvenn/index.html
ggeffects	Lüdecke[72]	https://cran.r-project.org/web/packages/ggeffects/index.html
emmeans	Lenth[73]	https://cran.r-project.org/web/packages/emmeans/index.html
vegan	Oksanen et al.[74]	https://cran.r-project.org/web/packages/vegan/index.html
pairwiseAdonis	Martinez Arbizu[75]	https://github.com/pmartinezarbizu/pairwiseAdonis
indicspecies	De Cáceres and Legendre[76]	https://cran.r-project.org/web/packages/indicspecies/index.html

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study system and plant species

Fire is thought to have been infrequent in Aotearoa New Zealand prior to human arrival ~750 years ago.[56] The eastern part of the South Island is at particular risk of increased fire activity in coming decades,[77] most of which is covered in indigenous grasslands dominated by perennial tussocks (bunchgrasses) in the genus *Chionochloa* Zotov (Poaceae).[78] Despite the increased risk of fire in tussock grasslands, the only study to our knowledge that has measured soil heat did not register temperatures at or above 59 °C at depths of 2.5 and 5 cm,[49] depths where AM fungi would be present. AM fungi are generally present in *Chionochloa rigida*,[27,79] and likely associate with other grasses in this genus.

Chionochloa spp. are commonly observed locally coexisting with invasive hawkweeds (Asteraceae) at fine spatial scales (e.g., 0.25 m^2).[26] Hawkweeds are invasive in many countries outside their native European range[47] and invasion by two of these species, *Pilosella officinarum* Vaill. and *Hieracium lepidulum* (Stenstr.) Omang, has been a concern for managers of both pastoral and conservation land in Aotearoa New Zealand since at least the 1950s.[80] Both of these species of hawkweeds have continued to expand locally and colonise new sites at decadal timescales since at least the 1960s.[42,80,81] *Pilosella* can reproduce from stolons and from seed, but *Hieracium* relies entirely on seed to grow and invade new sites.[47] *Pilosella* is highly dependent on AM fungal colonisation to grow and compete,[29,36,43,82] but while *Hieracium* grows better with AM fungi, its competitive ability is unaffected.[28,34,35] The two hawkweed species may therefore interact and respond differently to soil biota, including AM fungi, with *Pilosella* growth potentially more at risk from impacts of fire-mediated changes in soil biota than *Hieracium*.

Site description

Soil for this study was collected to assess impacts of simulated fire on arbuscular mycorrhizal fungal communities via DNA sequencing and a plant-soil feedback experiment. We collected soil for all experiments from one site at Porters Pass (43°16′12.0″S 171°37′51.6″E), Canterbury, Aotearoa New Zealand. The site was at 1160 m above sea level, had a slope of 28°, and an east-facing aspect. Climate averages from 1980 to 2010 showed precipitation was 1055 mm per year, mean winter temperature was 2.2°C, and summer temperature was 12.1°C.[83] Abiotic soil parameters at the site were characterized by sampling five locations at the site, pooling, and sending for physico-chemical analysis (Hills Laboratories, New Zealand): Soil had an average pH of 5.5, Olsen phosphorus concentration of 16 mg/L, organic matter content of 6.6%, a C:N ratio of 12.9, and a cation exchange capacity of 12 me/100 g. These samples were collected separately from those used in experiments (below). This site forms part of a network of long-term monitoring transects,[26,42] and the vegetation in the site over the previous 40 years was tall tussock grassland with native shrubs.

Soil and seed collection

In February 2020, we collected soil samples directly underneath ten individuals each of the three focal plant species to assess impacts of simulated fire on arbuscular mycorrhizal fungal communities via DNA sequencing and a plant-soil feedback experiment: *Chionochloa macra*, *Hieracium lepidulum*, and *Pilosella officinarum*. All three species are perennial and had been present at this site for at least 40 years[26] and were patchily distributed among bare soil at the site. We therefore assumed plant-soil feedbacks and soil conditioning had occurred naturally in the environment. All plants were within ~100 m of each other and were at least 5 m apart. The top 5 cm of soil under each plant was collected with gardening knives that were disinfected between samples; each sample weighed approx. 200 g. Samples were kept in separate plastic zipper storage bags, stored on ice in an insulated box in the field, and transported to Auckland University of Technology. We collected seeds of *Hieracium* and *Pilosella* from at least 30 individuals per species, which were pooled from plants at Porters Ski field and the Lammerlaw Ranges, Otago. We were unable to obtain seeds from *Chionochloa* because this species produces seeds by masting and it was not seeding in 2020.

METHOD DETAILS

Plant-soil feedbacks and simulated fire

A plant growth experiment was established to assess the role of plant-soil feedbacks and simulated heat from fire on growth of *Hieracium* and *Pilosella*. The experimental design was fully factorial with two treatments: soil conditioning and heat. Soil conditioning had three

levels: *Chionochloa*, *Hieracium*, and *Pilosella*. Heat treatment had two levels: Unheated and 45°C. There were eight individual plants per conditioning-heat combination per species (i.e., replicates or pots) resulting in 48 experimental units for each plant species.

Seeds of *Hieracium* and *Pilosella* were surface disinfected in 1% bleach for 5 min and rinsed with Millipore water, with pilot studies proving this resulted in no fungal growth and had no impact on seed germination after 14 days.[84] Seeds were pre-germinated for 2 weeks prior to transplanting into the main experiment. Seeds were placed in trays of autoclaved mix of sand and pumice in a 1:1 ratio, watered with Millipore water twice weekly, and grown underneath 55 W Starlite 6400K twin fluorescent tubes for a 16/8 h day/night photoperiod at 21°C.

All potting equipment was soaked in diluted bleach (1%) for a minimum of 20 min, rinsed, and air dried. The workspace was scrubbed and disinfected with Trigene 1:20 between preparation and potting of each soil inoculum. Soils collected from Porters Pass in February 2020 within each conditioning treatment (*Chionochloa*, *Hieracium*, *Pilosella*) were pooled and sieved (5 mm). From these three pooled soils (one from each species), subsamples were taken for later DNA extraction for analysis of AM fungal communities (below), using a spoon to take the subsample into separate plastic zipper storage bags. The spoon was disinfected with Trigene between subsampling. Half of the remaining larger sieved soil sample was then placed in aluminum trays at a 5 mm depth and covered with tin foil. Soils for the heated treatment were subjected to 45°C in an oven for 10 min and unheated soil was left at room temperature. All soil was rested at room temperature overnight before potting. At the time of potting for the experiment, soil of each conditioning-heat combination was mixed with autoclaved pumice and sand in a 2:1:1 ratio (i.e., 2 soil: 1 pumice: 1 sand) and added to 300 mL plastic pots on saucers into which one seedling was transplanted per pot. Plants were watered with Millipore water twice weekly and grown underneath 55 W Starlite 6400K twin fluorescent tube for a 16/8 h day/night photo period at 21°C. Pots were randomised in the growth chamber and were re-randomised four times during the experiment. After 12 weeks, seedlings were destructively harvested, and the roots and shoots separated. Shoots were dried in separate paper bags at 65°C for 48 h before weighing to obtain biomass. Roots were cleaned by washing with tap water and patting dry with paper towels, then dried and weighed in the same manner as the shoots.

Simulated fire and AM fungal communities

We used four heat treatments to assess the impacts of simulated heat from fire on community structure of AM fungi in soils associated with each plant species (*Chionochloa, Hieracium, Pilosella*): Unheated, 30°C, 45°C, or 60°C. A subsample of each sieved soil sample that had been processed from the field (above) was placed into an autoclaved 6 cm diameter aluminum dish measuring 2 cm deep and was covered with autoclaved aluminum foil. Samples were then heated in an oven for 10 min, cooled at room temperature for 30 min, then stored on ice overnight before DNA extraction. Samples for the unheated treatment were stored at room temperature for 10 min then left on ice overnight. There were ten biological replicates per soil conditioning-heat combination, for a total of 120 samples (3 soil conditioning treatments × 4 heat treatments × 10 replicates).

DNA was extracted from 250 mg of soil using the Qiagen DNeasy PowerSoil Pro kit following the manufacturer's instructions. Prior to PCR, all DNA extracts were quantified using a NanoVue spectrophotometer (GE Healthcare) and standardised to 5 ng/μL by diluting with nuclease-free water. Glomeromycota DNA was amplified from the DNA extracts using the small ribosomal subunit (18S) rRNA primers WANDA[61] and AML2.[62] These primers were modified to include Illumina flow cell adaptor sequences. Each reaction consisted of 12.5 μL of Kapa HIFI HotStart Readymix, 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. PCR conditions consisted of an initial denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR products were cleaned using AMPure XP beads. Illumina MiSeq libraries were prepared and sequenced as per the manufacturer's protocol. DNA in PCR products was quantified using Qubit. Index adaptors for Illumina MiSeq sequencing were added via a second PCR step. Each reaction had 12.5 μL of Kapa 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 min, then 8 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s, with a final extension at 72°C for 5 min. Following PCR, libraries on each plate were pooled into one tube. These were cleaned twice using AMPure XP beads, then quantified using Qubit. The sequence quality and length were assessed by running samples on a bioanalyzer (Agilent, Santa Clara, CA, USA). Paired-end sequencing (2 × 300 bp) was carried out using an Illumina MiSeq machine at the Auckland University of Technology sequencing facility.

Bioinformatics analyses were carried out in QIIME 2.[64] We analyzed forward reads only because this yielded a higher number of AM fungal reads than merged paired-end reads (248,522 vs. 193,198, respectively). Using q2-cutadapt trim-paired,[65] demultiplexed forward reads were trimmed to remove the forward and reverse primer sequences. Then, we used DADA2 in the plugin q2-dada2[66] for denoising, dereplication, and chimera-filtering. To assign taxonomy to the amplicon sequence variants (ASVs) obtained in this step, we used the MaarjAM reference database (version: 6 May 2019).[30] The database was subjected to a series of cleaning steps to use it with our amplicon sequence data.[85] Using cutadapt,[65] we first trimmed the forward primer and excluded reads that did not match its sequence or were shorter than 300 bp. Then, we trimmed reads at the reverse primer but kept those that did not have it (or those longer than 600 bp), since several reads in the database included our whole target sequence except the reverse primer. Using q2-RESCRIPt,[67] the remaining reads were dereplicated retaining those with unique taxonomies (mode 'uniq') and quality-filtered by removing those with more than seven degenerate bases and/or homopolymers of 10 or more bp. The clean database comprised a total of 31,034 reads and 387 virtual taxa (VT). Taxonomic classification was carried out using BLAST+ local alignment with q2-feature-classifier classify-consensus-blast[68] at 97% sequence similarity and 95% query alignment coverage, according to previous studies.[86–88] Following this step, non-AM fungal reads (i.e., not belonging to Glomeromycota) were excluded. One sample (*Pilosella* soil heated to 45°C) did not yield any Glomeromycota sequences and was excluded (N = 119 samples).

QUANTIFICATION AND STATISTICAL ANALYSES

Statistical analyses were performed in R 4.3.1[63] with packages 'tidyverse',[69] 'egg',[70] 'ggvenn',[71] and additional packages specified below. R code is provided (Supplementary Material). Significance for all analyses was set at $\alpha = 0.05$. Linear models were run in base R to assess the impacts of soil conditioning and heat treatment on plant growth of the two hawkweed species (Experiment 1, $N = 96$ pots). Separate models were run for each species for two plant variables: plant biomass and root-shoot ratio. An interaction term was specified between soil conditioning and heat treatment. Two replicates were removed from the root:shoot model for *Hieracium* due to high leverage impacting model fit, although they did not impact statistical significance. Predicted values and confidence intervals were computed using the 'ggeffects' package.[72] 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.[73]

When evaluating if soil conditioning and heat impacted AM fungal community structure (Experiment 2, $N = 119$), we used presence-absence data from the sequencing output due to the low abundance of AM fungi in our samples and in soils in general.[89] We recognise this means that ASVs that are in low abundance are treated equally as those in high abundance, but DADA2 removes singletons[66] and we consider using presence-absence data as a better way to investigate our dataset given the known problems with rarefying high throughput sequencing data.[90,91]

To evaluate if soil conditioning and heat impacted AM fungal community composition, we performed a principal coordinate analysis (PCoA) using Jaccard dissimilarities because it represented the data best and showed the greatest spread of points in two dimensions; the results were qualitatively the same using abundance-based measures, such as Bray-Curtis, and a presence-absence dissimilarity measure that corrects for variation in alpha diversity, Raup-Crick. We tested for effects of soil conditioning and heat on AM fungal community composition by conducting a permutational multivariate analysis of variance (PERMANOVA) in 'vegan'.[74] A pairwise PERMANOVA was used to assess differences in composition among soil conditioning treatments using the 'pairwiseAdonis' package.[75] We determined significant indicator ASVs of each soil conditioning treatment using the 'indicspecies' package.[76] A generalised linear model with Poisson-distributed errors was constructed to test if soil conditioning and heat impacted AM fungal species richness (number of ASVs). Soil conditioning and heat treatment were predictors, and AM fungal ASV richness was analyzed as count data. An interaction term was specified between soil conditioning and heat. *Post hoc* pairwise comparisons, predicted values and confidence intervals were computed using the same packages indicated for plant biomass analyses.

We correlated plant biomass data from the plant-soil feedback study with AM fungal community structure from the soils. For each plant species, we undertook Spearman rank correlation tests in base R between total plant biomass and scores from the first PCoA axis of soil AM fungal communities, which was considered a measure of soil AM fungal community composition. We repeated this correlation between total plant biomass and soil AM fungal richness.