

Invasion Alters Plant and Mycorrhizal Communities in an Alpine Tussock Grassland

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Abstract

Plant invasions now impact even the world's most remote ecosystems, including alpine zones, altering key mutualisms that affect ecosystem functions. Plant-mycorrhizal associations can be sensitive to invasion, but previous studies have been limited in the types of mycorrhizas examined, favouring invaders that host arbuscular mycorrhizas or are non-mycorrhizal. Consequently, little is known about how invaders that host other types of mycorrhizas may affect community and ecosystem properties. We studied invasion by an ericoid mycorrhizal host plant (*Calluna vulgaris* L., heather) in alpine tussock grasslands in New Zealand. We investigate the effects of increasing *C. vulgaris* density on the plant and soil microbial community and on mycorrhization in the dominant native species (*Chionochloa rubra* Z., red tussock), an arbuscular mycorrhizal host. We show that variation in plant community composition among sites was driven by *C. vulgaris* density. Higher densities of *C. vulgaris* were associated with a reduced diameter of *C. rubra* plants and lower densities of neighbouring plant species, especially woody shrubs. Belowground, we show that higher densities of *C. vulgaris* were associated with lower rates of mycorrhization in native *C. rubra* and higher abundances of the fungal fatty acid methyl ester (FAME) biomarker but had little effect on the total microbial community biomass, suggesting that increased ericoid mycorrhizal fungal biomass increased the ratio of fungi relative to bacteria in high *C. vulgaris* density stands. By exploring invasion of a plant with an atypical mycorrhizal type, our study expands knowledge of the ecosystem consequences of biological invasions.

Introduction

Alpine ecosystems form isolated pockets of biodiversity covering ~ 5% of the terrestrial surface (Cavieres et al. 2014). Warming is leading to changes in plant community composition whereby low elevation species, particularly woody species, are colonising alpine areas, shrinking an already spatially restricted ecosystem (Gottfried et al. 2012; Halloy and Mark 2003; Lamprecht et al. 2018; Pauli et al. 2012; Steinbauer et al. 2018). Alpine ecosystems are characterised by cold temperatures and short growing seasons which limit plant growth and nutrient availability by slowing decomposition processes (Frazier and Brewington 2020; Pauchard et al. 2016). The stress-tolerant and often highly endemic alpine species adapted to these harsh abiotic conditions (Brooker 2006; Cavieres et al. 2014; Chapin and Shaver 1985) exhibit relatively little trait variation over environmental gradients (Rixen et al. 2022) and are over-represented among those species experiencing population declines and range contraction (Rumpf et al. 2018). By contrast, some woody species are expanding in grasslands and cold biomes in locations worldwide (Elmendorf et al. 2012; Mod and Luoto 2016; Myers-Smith et al. 2011; Stevens et al. 2017) and have become a particular threat to alpine grasslands (Brandt et al. 2013; Espunyes et al. 2019; Komac et al. 2011). As climatic warming continues to alleviate the harsh abiotic conditions in mountains, plant-plant interactions are likely to shift from facilitative to competitive (Brooker 2006; Grime 1977; Maestre et al. 2009).

Human-mediated plant introductions are a global phenomenon (Hulme 2009) and while only a few species become invasive, these species now affect even the most remote ecosystems, including alpine

zones (Alexander et al. 2016; Clavel et al. 2021). Most naturalised plant species function ecologically like resident native species, but those that become invasive can alter plant community composition and reduce local plant diversity and the diversity of higher trophic guilds (Reinhart and Callaway 2006). The direct negative effects of invaders on native plant species are well-characterised; competition for space and resources often leads to a reduced local abundance of natives (Bradley et al. 2019; Gioria and Osborne 2014). However, the indirect effects of invasive plants on natives, such as those affecting mutualistic interactions between species, like seed dispersal, pollination and mycorrhization, are less well understood (Grove et al. 2017; Lekberg et al. 2013; Traveset and Richardson 2014).

Mutualisms between plants and mycorrhizal fungi are ubiquitous positive interactions, which are obligate for many plant species (Smith and Read 2008). Mycorrhizas aid in plant establishment, nutrition, growth, defence, and survival and are particularly important in harsh environments such as alpine ecosystems (Cripps and Eddington 2005). Root colonisation by mycorrhizas directly affects plant nutrition and performance, while the extraradical mycorrhizal mycelium affects soil functioning, modifying the distribution of carbon (C) through the soil profile, affecting soil aggregation and the structure of the soil microbial community (Barceló et al. 2020). Invasive plants can disrupt the mycorrhizal symbioses of native plant species, contributing to their invasion success (Barto et al. 2011; Meinhardt and Gehring 2012; Wolfe et al. 2008), a phenomenon known as mycorrhizal interference (Grove et al. 2017). High invader densities can reduce mycorrhizal colonisation in native hosts, negatively affecting their survival and growth, and altering plant and fungal community composition and other ecosystem properties and functions (Grove et al. 2017; Meinhardt and Gehring 2012). However, previous studies have been limited in the types of mycorrhizas examined, favouring invaders that host arbuscular mycorrhizas or are non-mycorrhizal (Grove et al. 2017; Zubek et al. 2016), and relatively little is known of the ecosystem impacts of invaders that host other types of mycorrhizas.

Invaders of different mycorrhizal types are likely to have different impacts on ecosystem functions. Mycorrhizas play foundational roles in ecosystem C cycling (Bardgett and van der Putten 2014; Tedersoo et al. 2020), by influencing plant production and litter quality, being a key C source to other microbes, controlling soil organic matter turnover (Bardgett and van der Putten 2014; Hawkes et al. 2008) and ultimately the size of the soil C sink (Averill et al. 2014; Clemmensen et al. 2013; Orwin et al. 2011). Mycorrhizal types differ in their effects on ecosystem C cycling, with ectomycorrhiza (EcM) and ericoid mycorrhiza (ErM) fungi obtaining greater C per unit nitrogen than arbuscular mycorrhizal fungi (AMF) (Orwin et al. 2011; Phillips et al. 2013; Soudzilovskaia et al. 2015). Where plant invasions or woody plant expansions cause changes in the dominance of mycorrhizal types, we may expect them to have larger effects on ecosystem properties. Indeed, during plant invasions, mycorrhizal mismatch, whereby native and invasive species host different types of mycorrhizal fungi, is generally associated with significant ecosystem impacts (Grove et al. 2017), but this has been explored in only a few systems.

Calluna vulgaris (heather, Ericaceae) is a European shrub common to heathland, montane, and alpine ecosystems. *C. vulgaris* is invasive in New Zealand's Tongariro National Park (TNP), where it has become a common and often dominant member of the plant community over roughly a third (265/798 km²) of

the landscape (Effah et al. 2020; Giejsztowt 2019; Rogers and Leathwick 1996). Invasion by *C. vulgaris* is detrimental to the native red tussock, *Chionochloa rubra*, which forms the tussock-grassland ecosystems formerly typical non-forested areas of the park (Rogers and Leathwick 1996). High densities of *C. vulgaris* are associated with pollination limitation and suppressed seed masses in a native ericaceous shrub (Giejsztowt et al. 2020), altered herbivorous insect assemblages (Keesing 1995), and successional trajectories (Rogers and Leathwick 1996) in former *C. rubra* tussock grasslands, but we know little about concomitant effects on below-ground components of the ecosystem. *C. vulgaris* is an ErM generalist, allowing it to thrive in broad edaphic conditions and tolerate harsh environmental settings (Perotto et al. 2012). Mycorrhizal facilitation by native ErM species may contribute to the rapid spread of *C. vulgaris* within TNP, which now easily establishes in inter-tussock spaces (Chapman and Bannister 1990; Orlovich and Cairney 2004). Where *C. rubra* and *C. vulgaris* co-occur, the presence of the invasive species reduces the overall density of the native species and *C. vulgaris* is predicted to exhibit range expansion under warming (Giejsztowt 2019) potentially leading to greater competitive effects of *C. vulgaris* on *C. rubra* in the future (Brooker and Callaghan 1998; Callaway et al. 2002; Choler et al. 2001; Maestre et al. 2009).

This study focuses on the invasion of an alpine tussock grassland by the woody ErM host plant *C. vulgaris*. We investigate how a gradient of *C. vulgaris* density affects the surrounding plant community, mycorrhization in the dominant native host (*C. rubra*), and the soil microbial community. Our study took place in a comparably little-studied ecosystem; an alpine zone in the Southern hemisphere. We hypothesised (Hyp₁) that increasing *C. vulgaris* density leads to a shift in the composition of the plant community through the suppression of *C. rubra* and other native species. As a consequence of mycorrhizal mismatch between native *C. rubra* and invasive *C. vulgaris*, we hypothesised (Hyp₂) that higher densities of the invader would be associated with a reduction in AMF colonisation in the roots of *C. rubra*. Finally we hypothesised (Hyp₃) that mycorrhizal interference by invasive ErM *C. vulgaris* would lead to reduced AMF abundances in soils.

Methods

Study area

This study took place in Tongariro National Park (TNP), in the central North Island of New Zealand (39.19°S, 175.59°E), which contains the volcanos Ruapehu, Ngāuruhoe, and Tongariro. The disturbance history of TNP includes volcanic activity, fires and wind erosion, which contribute to a patchwork of forest and grassland ecosystems across the landscape (Keesing 1995). As New Zealand's first National Park and a UNESCO Cultural and Biological World Heritage Site, TNP is valued for its iconic open landscapes characterised by tussock grassland plant communities with high endemism (Halloy and Mark 2003). Prevalent among these are perennial tussock grasslands dominated by *Chionochloa* spp. (Poaceae) and interspersed with woody shrubs and perennial forbs, of which species belonging to the Ericaceae, Plantaginaceae, and Asteraceae are common. *Calluna vulgaris* (heather) was actively planted

in TNP in the early 20th century for aesthetic purposes and to create habitat for introduced game fowl. Generating an abundance of seed, *C. vulgaris* spread quickly along roadsides and in fire-disturbed areas. Colonising inter-tussock spaces, it invaded intact tussock grasslands displacing *Chionochloa rubra*, the dominant tussock-forming species, eventually becoming abundant over large areas (Rogers and Leathwick 1996). Climatic change is expected to increase the suitable habitat for *C. vulgaris* in TNP, leading to range expansion and increasing areas of high density *C. vulgaris* stands that exclude *C. rubra* (Giejsztowt 2019).

Experimental Design

Five study areas were selected over a ~ 40 km² of TNP in locations that varied in *C. vulgaris* density and were accessible by road or foot (see *Supplementary table 1*, *Supplementary Fig. 1*). Sites located in the southern end of the study area had sandier soils compared to those further north, which were higher in moisture and organic matter. Within each study area we selected three sites: one each with high, medium or low *C. vulgaris* density. The density of *C. vulgaris* was estimated visually as percent ground cover, with low density being $\leq 10\%$ cover, medium density between 11% – 60% cover, and high density $> 60\%$ cover. Within each of the 15 sites, 5 circular plots, with a diameter of 2 m were established around a focal *C. rubra* plant, resulting in a total of 75 plots. Each plot was a minimum of 5 metres distant from a neighbouring plot. Within each plot, we measured percent cover of all vascular plant species. The percent cover was estimated visually within 1% if $\leq 10\%$, within 5% if between 10% – 30%, and within 10% if $> 30\%$ cover. We calculated vascular plant species richness in all plots. Plant species were assigned to the structural classes: native ferns, native forbs, native grasses, native sedges and rushes, and pastoral, which included non-native grasses and forbs, all of which are considered weeds in this ecosystem. Plant species were categorised by mycorrhizal type: arbuscular mycorrhizal (AM), ericoid mycorrhizal (ErM), ectomycorrhizal (EcM), dual AM/EM, or non-mycorrhizal (NM), based on literature reports (see *Supplementary table 3*). The height of all focal *C. rubra* plants was measured from the soil surface to the tip of the tallest live leaves. The diameter of all focal *C. rubra* plants was measured as the horizontal distance between the tips of the longest live leaves, without repositioning leaves. Finally, two soil cores (6 cm diameter, 11 cm long) were collected at the base of the focal *C. rubra* plant for mycorrhizal and soil microbial measurements.

Root Length Colonisation

To measure AM colonisation in *C. rubra* we obtained *C. rubra* roots from both soil cores from each plot, cores were pooled by plot to ensure enough *C. rubra* roots were sampled in all plots. To separate plant roots, the soil cores were sieved to 4.0 mm and the sieved soil was reserved for soil nutrient and FAME analysis. Plant roots were then washed in tap water and *C. rubra* roots were collected while dead roots and those of non-target species were discarded. The roots of *C. rubra* were readily identified by their pale yellow to dark orange colour and elastic texture compared to the roots of co-occurring species. *C. rubra* roots were cut into ~ 15 mm sections and stored overnight in Formalin/alcohol/acetic acid/water (10%, 50%, 5%, 35%) fixative (FAA) (Sass 1958) in a refrigerator at 4°C. The roots were rinsed of FAA and transferred into a 10% KOH solution and cleared by autoclaving for 10 minutes at 121°C. Cleared roots

were then rinsed with several changes of water and transferred into a staining solution, consisting of equal volumes of 80% lactic acid, glycerine and distilled water with a chlorazol black E. stain for ~ 1.5 hours at 90°C (Brundrett et al. 1984). Stained *C. rubra* roots were stored in glycerine in a refrigerator at 4°C until measurements of percent root length colonisation were made.

For each of the 75 samples, 5 root segments were randomly selected and placed on a microscope slide for observation under a compound microscope. Fungal colonisation was recorded at 6 locations along each root segment, resulting in 30 locations quantified for each individual plant sampled. We observed AMF hyphae, arbuscules, coils, vesicles, and dark septate endophyte (DSE) structures using a magnified intersection method (McGonigle et al. 1990). Intersections of fungal structures along the 10 marks of the microscope reticle were recorded at a magnification of 400X. The reticle was positioned parallel to the length of the root segment, along the root cortical cells. At each of the 30 locations per individual AMF root colonisation was scored out of 10, with 0 = no structures, and 10 = fully colonized by AMF hyphae, arbuscules, and vesicles. The sum of these scores was divided by 30 as a measure percent root length colonised. Each location of quantification was at least an entire field of view distance from the previous site.

Soil nutrient analysis

For soil nutrient analyses, soil samples were pooled by site (n = 15), oven dried and ground with a ball-mill (Anton Parr BM500, Graz, Austria) to < 2mm. Total carbon, total nitrogen and Olsen P analyses were conducted at Hill laboratories, New Zealand. Olsen P was gained through Olsen extraction followed by Molybdenum Blue colorimetry. Total carbon and total nitrogen were determined by Near Infrared Spectroscopy (NIR) and calibration based on Total Carbon or Total Nitrogen by Dumas combustion.

Phospholipid and neutral lipid fatty acid analysis

Sieved soil samples were pooled by plot, resulting in 75 samples for lipid analyses. Soil samples were frozen to -20 °C and lyophilised (LABCONCO). For each sample, between 0.45–0.55 g of dry soil was weighed directly into 10 ml screw-cap glass tubes. Lipids were extracted from the soil using a modified high-throughput Bligh & Dyer method (White 1979) described by Lewé and co-authors (2021). The resulting FAME samples were run on a Shimadzu Gas Chromatograph and Mass Spectrometer, which separated the individual FAMES on a Restek RXi5-Sil (30 m x 0.25 mm x 0.25 µm) capillary column. The mass spectrometer had an injection temperature of 260°C and was run in spitless mode with helium as a carrier gas. GC-MS solutions version 4.44 (Shimadzu) software was used to run the instruments and for the qualitative and quantitative analysis of the chromatograms produced. To correct for sample loss during extraction, the concentration of each lipid was determined as $peakarea \times \left(\frac{0.266667}{IS_{area}} \right) \times (1/RF)$. Where IS_{area} is the peak area of the internal standard 19:0 and RF is a unique response factor calculated for each FAME based on a calibration curve for its commercially available standard (see Lewé et al. 2021). This value was then multiplied by the volume of solvent (75 µL of hexane) and divided by soil sample dry weight, resulting in FAME lipid concentrations expressed as nmol g⁻¹ DW soil. Seven PLFA

biomarkers (10Me16:0, 16:0, 16:1ω5, 16:1ω7c, 18:0, 18:1ω9c, delta19:0) were resolved to quantify bacterial biomass and the PLFA biomarker 18:2ω6 was used to quantify fungi (Table 1). Their sum was taken as a measure of total microbial biomass. We calculated the proportional abundance of fungi in soil samples as $\frac{18:2\omega6}{\text{total microbial biomass}} \times 100\%$. The phospholipid 16:1ω5 is present in both AMF and bacteria (Butler et al. 2003). Therefore, we characterised the NLFA fraction of total lipid extracts and used the NLFA16:1ω5 as a biomarker for AMF. As neutral lipids are the primary storage compounds in AMF, 16:1ω5 is an appropriate indicator of AMF lipid biomass (Bååth 2003).

Table 1
FAME biomarkers characterised as an indicator of soil microbial biomass in this study

Microbial group	FAME biomarker	Reference
<i>Arbuscular mycorrhizal fungi</i>	16:1ω5	(Olsson et al. 1995)
<i>Fungi</i>	18:2ω6	(Ahlgren et al. 1992; Zelles 1997)
<i>Microbial Biomass</i>	10Me16:0, 16:0, 16:1ω5, 16:1ω7c, 18:0, 18:1ω9c, 18:2ω6, delta19:0	(Francisco et al. 2016; Vestal and White 1989; Olsson et al. 1995; Zelles 1997; Ahlgren et al. 1992; Parker et al. 1982; Wilkinson and Ratledge 1988; Francisco et al. 2016)

Statistical Methods

To explore how increasing densities of *C. vulgaris* effected plant community composition, we used non-metric multidimensional scaling (NMDS). The NMDS ordination was performed on a rectangular matrix of the percent cover of all plant species in the 75 plots. We used Indicator Species Analysis (ISA) to gain insight to which species were significantly associated with the *C. vulgaris* cover categories (high, medium or low density). Likewise, we explored spatial variation in plant occurrence by ISA of 3 categorical factors: zone (northern, central, southern), Area (A to E); and site (1 to 15). Indicator values were calculated with the method of Dufrêne and Legendre (1997). Thirty-six vascular plant species were identified in this study, so we would expect 1.8 species to be significant indicators at alpha = 0.05, we therefore interpret only categorical predictors with > 2 significant indicator species. NMS and ISA analyses were conducted in PCOrd version 7.08 (Wild Blueberry Media, Corvallis, USA).

Other statistical analyses were conducted in R v 4.3.1 (R Core Team 2023). A stacked bar graph was used to visualise the effect of *C. vulgaris* density categories (high, medium and low) on the composition of the subordinate plant community by structural class, excluding the two focal species, *C. vulgaris* and *C. rubra*. To account for differences in total vegetative cover among plots, we used two-proportions z-tests to compare the proportional abundances of plant structural classes in the three *C. vulgaris* density categories.

We applied linear mixed effect models to assess the effect of *C. vulgaris* density on *C. rubra* morphology (height, diameter), AMF root colonisation, and soil nutrients (Olsen P, Total C, Total N and C:N) using the

lme4 package and *lmer* function in R. Each model included *C. vulgaris* density as a fixed effect and study area (A-E) as a random effect. In the root colonisation of *C. rubra* model we also included soil nutrients (Olsen P, Total C, Total N) as co-variables, and used the *step* function for backwards elimination of terms to determine the best model of root colonisation. AMF root colonisation data were log transformed to improve their compliance with the assumption of normality for the linear model. Likewise, we used linear mixed effect models to assess the effect of *C. vulgaris* density on lipid biomarkers for AMF, total microbial biomass (the sum of 8 PLFAs) and the proportional abundance of fungi in soil samples. Each model included *C. vulgaris* density as a fixed effect and geographic region (southern, central, northern) as a random effect. We included region rather than study area as a random effect in these models because including study area yielded singular model fits, indicating that study site explained no variation in the response variables. We also explored geographic variation in soil nutrients using simple linear models of Olsen P, Total C, Total N and C:N as a function of geographic region of plots (southern, central, northern). All models were fit by REML and comparisons calculated using type III sums of squares with Satterthwaite's method. Finally, because the NLFA biomarker for AMF biomass was detected in relatively few soil samples, we applied logistic regression to determine if its likelihood of detection was effected by *C. vulgaris* density. We modelled the detection of 16:1ω5 as a function of *C. vulgaris* density using a generalized linear model (*glm* function) with a binominal distribution using the *glm.predict* package in R.

Results

Effect of *C. vulgaris* on the plant community

Plant community composition varied strongly in response to *C. vulgaris* density in our study. Two NMS ordination axes described 78.5% of the variation in plant community composition (Fig. 1A). *C. vulgaris* density was highly correlated ($r^2 = 0.820$, $\tau = 0.739$) to Axis 1, which accounted for 63.1% of the variation in plant community composition among plots. Across all study plots the average density of *C. vulgaris* was 35%; varying from < 0.1%, 40–65% in the low, medium, and high *C. vulgaris* percent cover categories, respectively (see supplementary table 2). Plant communities were quite heterogeneous among low *C. vulgaris* sites but converged to a homogenous community dominated by *C. vulgaris* in medium and high-density categories. As the density of the invader increased, native shrubs declined most dramatically. Native shrub cover was significantly negatively correlated ($r^2 = 0.360$, $\tau = -0.400$) to Axis 1. Further, morphology of the focal species *C. rubra* changed over the *C. vulgaris* density gradient. Plant diameter of *C. rubra* was significantly negatively affected by increasing density of invasive *C. vulgaris*, with a 0.16 cm decrease in diameter for every 1% increase in *C. vulgaris* density ($F(1) = 6.44$, $p = 0.013$, Supplementary Fig. 2). However, we found no effect of *C. vulgaris* density on the height or percent cover *C. rubra* in study plots. Taken together, these results provide strong support for our first hypothesis that increasing *C. vulgaris* density drives a shift in the composition of the plant community.

In addition to the very strong gradient in plant community composition associated with increasing percent cover of *C. vulgaris*, we observed a significant gradient in grassiness across plots. NMS Axes 2,

which accounted for an additional 15.4% of the variance in the vegetative composition of plots, was correlated with the percent cover of native grasses ($r^2 = 0.304$, $\tau = 0.331$) as well as to the percent cover of the focal *C. rubra* tussocks ($r^2 = 0.183$, $\tau = 0.283$). Indicator species analysis of geographic zones (northern, central, southern), study area (A to E), and sites (1 to 15) indicated very little spatial autocorrelation in plant species composition occurred that was unaccounted for by our sampling design (Fig. 1A, *supplementary tables 4,6*).

The subordinate plant community, which excluded *C. vulgaris* and *C. rubra*, also responded strongly to *C. vulgaris* cover categories. Total subordinate plant cover was reduced nearly 7-fold from an average of 78.6% cover in the low to only 11.5% cover in the high *C. vulgaris* cover categories, a change that was highly significant ($F(2) = 10.08$ p-value < 0.001, Fig. 1B). The subordinate plant community was predominantly composed of native shrubs, which hosted AM (9 species), ErM (7 species) and dual AM/EcM (1 species) mycorrhizas or were non-mycorrhizal (2 species, see *supplementary table 3*). Native shrub cover was reduced by a factor of 6 between low and high *C. vulgaris* cover categories, and the proportional reduction in shrub cover was significant between each of the *C. vulgaris* cover categories (High < Low: $z = 28.13$, p-value < 0.001; High < Medium: $z = 6.39$, p-value < 0.001; Medium < Low: $z = 28.87$, p-value < 0.001). While they comprised a relatively small proportion of the plant community, other structural classes of plants also responded to increasing *C. vulgaris* density. Non-*C. rubra* grass cover declined significantly High < Low: $z = 5.52$, p-value < 0.001; High < Medium: $z = 5.57$, p-value < 0.001), while the native forbs and the native sedges and rushes categories were completely absent in the high *C. vulgaris* cover category. Likewise, native ferns were absent in all but the lowest *C. vulgaris* cover category. The strong and significant declines in native grasses and AM shrubs among the subordinate plant community provides further support for our first hypothesis that higher densities of *C. vulgaris* density suppress *C. rubra* and other AMF hosts, leading to a shift in the composition of the plant community.

Despite the very strong effect of *C. vulgaris* on plant community structure, total plant species richness did not vary significantly over the *C. vulgaris* density gradient; with 24 plant species present in both the high and medium density sites and 26 plant species in low-density sites. Excluding *C. vulgaris*, the plant communities of all plots were dominated by native alpine species, which comprised 31 of those 36 recorded. Only four other non-native species occurred (*see supplementary Table 3*) all of which are associated with pastoral agriculture, a dominant land-use adjacent to TNP. Mean percent cover of the four non-native pastoral species was less than 1% across all plots. Indicator species analysis suggested a gradient of weediness across the study region from Southern to Northern sites, with pasture species comprising three of the six significant indicator species for the Northern part the study region (*supplementary Table 4*). Also notable were the 11 species that were significantly associated with the density categories of *C. vulgaris* (*supplementary Table 5*). Seven of these indicator species were significantly associated with the low *C. vulgaris* cover category, of which five were native shrubs, and one a native fern. *Dracophyllum subulatum*, a common and abundant native shrub that is endemic to the frost flat region of the Eastern (lee) side of TNP, declined 6-fold from an average cover of 30.8% in low *C.*

vulgaris sites only 4.8% in high density sites, while the native shrubs *Veronica tetragona*, *Olearia nummulariifolia*, *Carmichaelia nana* and the native fern *Gleichenia dicarpa* were absent from all but the low *C. vulgaris* cover sites. *Hypochaeria radiacata*, the only strongly wind-dispersed non-native species in the community, was also significantly associated with the high-density *C. vulgaris* cover class. *H. radiacata*, was common in all study areas occurring in 44% (33 of 75) of plots, but never abundant, increasing from an average of 0.6% cover to 2.4% cover from the low to high density categories of *C. vulgaris*. By contrast, the medium and high-density categories were associated with only two indicator species each; of which two were non-native (*C. vulgaris* – high; *Lolium perenne* -medium) and one was a native species which is also wind-dispersed and disturbance-adapted and more typically associated with wetlands and forest margins *Austroderia toetoe* (high). These results provide further support for our hypothesis 1 that increasing *C. vulgaris* density causes a shift in the composition of this alpine plant community.

We hypothesised (hyp2) that higher densities of *C. vulgaris* would result in lower AM root colonisation in native *C. rubra*. We found strong support for this hypothesis in the significant negative relationship between *C. vulgaris* density and fungal colonisation in *C. rubra* roots ($F(1) = 94.01$, $p < 0.001$; Fig. 2, Supplementary Table 7). Our final model for AM root colonisation in *C. rubra* did not include any soil nutrient, indicating a strong effect of *C. vulgaris* in suppressing mycorrhization in *C. rubra* regardless of soil nutrient content. Across all samples we observed a 3% decrease in AMF colonisation for every 10% increase in *C. vulgaris* density.

Effect of *C. vulgaris* on the soil microbial community

To test hypothesis 3, that mycorrhizal mismatch would cause higher densities of *C. vulgaris* to be associated with reduced AMF abundances in soil, we characterised soil microbial phospho- and neutral lipids as a proxy for the biomass of different soil microbial groups. Across all plots, total microbial biomass was low, with a mean value of $123.05 \text{ nmol g}^{-1} \text{ DW soil}$, and was unaffected by *C. vulgaris* density ($F(1) = 1.22$, $p = 0.27$, Fig. 3, Supplementary table 8). However, the PLFA biomarker for fungi 18:2 ω 6 increased significantly over the *C. vulgaris* gradient ($F(1) = 4.28$, $P = 0.042$, Fig. 3, Supplementary table 9), with an increase of $0.89 \text{ nmol g}^{-1} \text{ DW soil}$ for every 10% increase in *C. vulgaris* density. The NLFA biomarker for AMF was detected in less than a third of the samples (22 of 75), and its likelihood of detection was not significantly affected by the density of *C. vulgaris* ($z = -1.509$, $p = 0.131$, Supplementary table 10, Supplementary Fig. 3). Therefore, we did not find support for our third hypothesis that mycorrhizal mismatch would cause higher densities of *C. vulgaris* to reduce soil AMF abundances.

Soil nutrients

Soils at our study sites had low C, N and P contents and C:N ratios that varied from 25–33, suggesting infertile soils with low labile carbon availability. *C. vulgaris* density had no effect on the nutrient content of soils. However, we found significant geographic variation in soil N and P. Geographic region had a

significant effect on the soil total percent nitrogen ($F(2) = 9.57$, $p = 0.0033$) and Olsen P ($f(2) = 6.23$, $p = 0.014$, *Supplementary table 11*). Total percent nitrogen more than doubled from 0.13–0.29% from Southern to Northern sites while Olsen P increased by 50% over this geographic gradient. While total percent carbon simultaneously increased by 58%, this change was not statistically significant due to the high variation in soil carbon content among samples. Likewise, we found no significant geographic variation in soil C:N (Table 2).

Table 2

Mean ($\pm$ standard deviation) percent carbon, percent nitrogen, C:N, Olsen P and microbial biomass of soils samples by geographic region (northern, central and southern) of the study sites.

Region	Total C (%)	Total N (%)	C:N	Olsen P (mg/L)	Microbial biomass (nmol g ⁻¹ DW soil)
North	7.20 $\pm$ 1.1	0.29 $\pm$ 0.02	25	5.00 $\pm$ 0	146.94 $\pm$ 45.6
Central	5.78 $\pm$ 2.3	0.18 $\pm$ 0.05	32	4.00 $\pm$ 1	125.41 $\pm$ 47.3
South	4.23 $\pm$ 2.3	0.13 $\pm$ 0.05	33	2.57 $\pm$ 0.8	110.84 $\pm$ 74.7

Discussion

We studied the effects of increasing *C. vulgaris* density on the plant and soil microbial community and on mycorrhization in the dominant native species, *C. rubra*, an arbuscular mycorrhizal host of alpine tussock grasslands in Aotearoa New Zealand. We found that *C. vulgaris* was the major driver of variation in plant community composition, with higher densities of *C. vulgaris* associated with reduced cover of subordinate plant species, especially shrubs, as well as altered morphology of *C. rubra* tussocks. Belowground, we found that higher densities of *C. vulgaris* were associated with lower rates of AMF mycorrhization in native *C. rubra* and significantly higher abundances of soil fungi, which include ErM, but had little effect on the biomass of AMF or on total soil microbial biomass. In what follows, we discuss these results in detail and consider the potential for mycorrhizal mismatch to be contributing to invasion by ErM *C. vulgaris* which is converting these *C. rubra* tussock grasslands into shrublands.

Our study plots were centred on *C. rubra* individuals to facilitate its root sampling and to ensure that it was present in every plot, because field observations suggested that *C. rubra* became much less abundant on the landscape in high density *C. vulgaris* stands. Our measurements of plant morphology for *C. rubra* revealed a significant decline in plant diameter, despite high variability across the across the *C. vulgaris* density gradient. However, we found no significant effect on plant height or the overall cover of *C. rubra* across this gradient. This suggests that *C. rubra* responds to increasing competition from *C. vulgaris* by reducing its lateral spread and exhibiting a more upright stature, maintaining its maximal height to persist in the densest *C. vulgaris* thickets. While physical space is not a consumable resource, it is important for gaining access to light, water, and nutrients, all of which can determine the success of

the plant (Gioria and Osborne 2014). In a classical study of uninvaded grasslands of the closely related *Chinochloa rigida*, tussock height and diameter vary synchronously across elevational gradients (Mark 1969). The decoupling of plant height and diameter of *C. rubra* tussocks across the *C. vulgaris* density gradient in our study suggests that *C. rubra* is altering its morphology to avoid competitive exclusion by *C. vulgaris* as affected through light limitation.

Perhaps the strongest evidence in support of our first hypothesis -that increasing *C. vulgaris* density leads to a shift in the composition of the plant community through the suppression of native species- is the dramatic seven-fold decline in total subordinate plant cover of plots over the *C. vulgaris* density gradient. While plant species richness (alpha diversity) was unaffected by invader density, our NMS ordination revealed a reduction in species turnover (beta diversity), among plots along the *C. vulgaris* density gradient, this finding is parsimonious with the homogenisation of alpine plant communities associated with the upward shift of invasive plant species as climate warms in the Swiss Alps (Jurasinski and Kreyling 2007). Importantly, the reduction of plant beta diversity and subordinate species cover was accompanied by the total loss of two functional groups of native plants from our study plots: ferns and sedges/rushes. This finding suggests a reduction in the functional diversity of the plant community with higher densities of the invader, a finding that agrees with that of a recent review on this subject (Renault et al. 2022).

The ferns and sedges/rushes functional categories consist of species associated with the wettest microhabitats of the red-tussock ecosystem, commonly abundant on the Rangipo desert only in wet depressions (Mark 2021). The exclusion of these species from the highest density *C. vulgaris* plots indicates that high invader densities are associated with drier soils. Since we placed all study plots at flat locations, this may suggest that high density *C. vulgaris* thickets alter site moisture locally, potentially drying soils through increased vegetative evapotranspiration. Two mechanisms could potentially contribute to this change; firstly, *C. rubra* is more conservative in its water use, especially at lower soil moisture, than *C. vulgaris* leading to measured transpiration rates in *Chinochloa* tussock grasslands that are less than half that reported for *C. vulgaris* shrublands (Espie 1999, Campbell and Murray 1990, Miranda 1982). Secondly, higher leaf areas are likely to be achieved in densely invaded areas, creating more surface area for evapotranspiration. Total vegetative cover is greatest in areas of the highest *C. vulgaris* density in this ecosystem (Giejsztowt 2019), and woody shrub invasion increases evapotranspiration in the Swiss Alps (van den Bergh et al. 2018) and across grassland ecosystems of the American South-West (Shen et al. 2022). Unfortunately, we did not measure soil moisture and therefore we cannot exclude the alternative explanation that the highest density *C. vulgaris* stands simply did not occur in the wettest microsites in our study. However, *C. vulgaris* has broad edaphic and hydrologic tolerances and it commonly occurs in moist to wet habitats throughout its native range (Rayner 1911, 1913), which are comparatively much wetter than the Rangipo desert ecosystem of TNP (Giejsztowt 2019), so it seems unlikely that excessive soil moisture limited *C. vulgaris* density in our study. Therefore our results suggest that one possible mechanism through which increasing *C. vulgaris* density leads to a shift in the composition of the plant community is by excluding the most drought

sensitive native species by promoting drier soils, although this conclusion requires further experimental verification.

The mycorrhizal type of an invader can be a major determinant of its ecological impacts (Pringle et al. 2009, Grove et al. 2017). Invaders often reduce mycorrhization of natives species (Busby et al. 2013, Zubek et al. 2016, Řezáčová et al. 2021) and these effects tend to be large where mycorrhizal mismatch occurs (Wolfe et al. 2008, Meinhardt and Gehring 2012). Supporting our second hypothesis, we show that higher densities of *C. vulgaris*, an ErM invader, reduced AMF colonisation in the native grass *C. rubra*. AMF use their expansive hyphal networks to forage for growth-limiting nutrients, which they exchange with their hosts for C, increasing soil nutrient retention in low nutrient ecosystems (Cavagnaro et al. 2015). The reduction in AMF colonisation we observed is therefore likely to have had negative effects on the mineral nutrition and biomass production of *C. rubra* in this low-fertility alpine tussock grassland, contributing to its smaller size and lower cover in the high *C. vulgaris* stands. High densities of *C. vulgaris* also displaced AMF hosts, which may have reduced AMF dispersal to *C. rubra* via root-to-root contact, the primary mechanism of AMF infection (Read et al. 1976, Powell 1979). Indeed, mycorrhizal host displacement by an invader is associated with reductions in mycorrhizal colonisation in remaining hosts in other systems (Tanner and Gange 2013, Zubek et al. 2016).

The strong negative relationships we show between *C. vulgaris* density and percent *C. rubra* root length colonised by AMF and *C. rubra* diameter point to a high mycorrhizal dependence of *C. rubra*. Theoretical work to explore the mechanism of mycorrhizal disruption by invaders found that the strength of mycorrhizal disruption depends on both the population size of the invasive plant and the strength of the mycorrhizal mutualism in the native plant species, with weak and strong native mutualisms more susceptible to invasion while intermediate native mutualisms were more resistant. They predicted strongest declines in mycorrhization of native plants where densities of the invader and mycorrhizal dependence of the native species were both high (McCary et al. 2019). That study considered AMF colonisation in a native species affected by a non-mycorrhizal invader. We are aware of no previous empirical or theoretical studies of mutualism interference by an ErM invader. However, a review by Grove et al (2017) found that non-mycorrhizal invaders reduced root colonisation in both AM or EcM hosts while AM invaders reduced or had no effect on root mycorrhizal colonization of EcM plants. Our study aligns and extends these results, demonstrating a reduction in root colonisation in an AM host due to mycorrhizal disruption by and ErM invader.

We found no support for our third hypothesis that higher densities of *C. vulgaris* would be associated with reduced soil AMF abundances. This suggests that mycorrhizal interference by invasive *C. vulgaris* was insufficient to reduce AMF biomass in soils, despite that it drove strong and significant declines in AM-host species cover and intraradical AMF structures in *C. rubra* across the *C. vulgaris* density gradient. As obligate biotrophs, AMF allocate greater biomass to plant roots than soils, especially when considered by volume or mass of substrate (Hiiesalu et al. 2014) and consequently, AMF are more difficult to detect in soil relative to plant roots (Ngosong et al. 2012). Moreover, even in highly AMF dominated systems, AMF typically account for only a small proportion of total soil microbial biomass

(Hiiesalu et al. 2014). In our study system, average microbial biomass across all sites was low and the AMF biomarker was detected in less than a third (22/75) of samples, limiting our ability to detect effects of increasing *C. vulgaris* density on AMF biomass. However, the non-significant but negative relationship between the likelihood of NLFA 16:1 ω 5 detection and *C. vulgaris* density revealed by our glm may indicate a trend toward less AMF biomass in soils of heavily invaded areas. While previous studies do not generally examine AMF biomass in both roots and soils as we have done, more studies appear to report reduced AMF colonisation in native species than reduced AMF biomass in soils with invasion (Bunn et al. 2015). Regardless, the strong decline in intraradical AMF structures in *C. rubra* together with no significant concomitant decline in NLFA 16:1 ω 5 in soils across the *C. vulgaris* density gradient suggests that the primary mechanism of mycorrhizal interference of *C. vulgaris* on *C. rubra* occurs through reduced root-to-root spread of AMF to *C. rubra*, rather than by reduced germination of soil AMF spores.

Studying invasion by an ErM host, we found no change in total microbial biomass but a marginally significant increase in fungal biomass over the *C. vulgaris* density gradient. These results suggest an increase in the ratio of fungi to bacteria in soils with *C. vulgaris* invasion, which agrees with the effect of *C. vulgaris* on soil microbes in a mesocosm study (Witt & Setälä 2010). In the absence of EcM species, 18:2 ω 6 is a sensitive indicator of changes in ErM biomass in soils (Wallander et al. 2013). We recorded no EcM plant species in plots, and while the dual AM-EcM shrub *Leptospermum scoparium* was common, it was unlikely to have hosted EcM fungi at our sites given the absence of other EcM hosts (Moyersoen & Fitter 1999, Cooper 1976). Therefore, the increase of PLFA 18:2 ω 6 over the *C. vulgaris* density gradient is likely to reflect increasing ErM biomass associated with the invader in our study. Similarly, invasion by rose into coastal dunes increased the biomass of its mycorrhizal symbiont in soils (Stefanowicz 2019). Mycorrhization in *C. vulgaris* is negatively related to soil nutrient availability (Caporn et al. 1995) and positively related to plant aboveground biomass, but not plant tissue nutrient content when grown in low nutrient soils (Strandberg & Johansson 1999). Soils in the study region are nutrient poor. Therefore, our findings suggest that *C. vulgaris* uses ErM to compete successfully with native plants for growth-limiting soil nutrients, which it allocates to the growth of aboveground biomass, effectively competing with native species for space and light, as evidenced by the altered morphology of *C. rubra* over the invasion gradient. Larger *C. vulgaris* shrubs with higher leaf area are able to shuttle more C belowground to their ErM symbionts, which respond with increased biomass in invaded soils. Presumably this greater ErM biomass further enhances the mineral nutrition of *C. vulgaris*, generating a positive feedback on the invasion cycle.

We have shown that invasion by ErM *C. vulgaris* into tussock grasslands formerly dominated by AM *C. rubra* is associated with reduced mycorrhization in the native host, which appears to have high mycorrhizal dependency. High densities of the invader were associated with increased ErM biomass in soils, which likely enhanced competition by *C. vulgaris* for growth-limiting nutrients in these poorly fertile soils, enabling it to increase aboveground biomass, effectively competing with *C. rubra* and other native species for space and light. Dense *C. vulgaris* thickets were associated with reduced plant beta and functional diversity, partly due to the decline or absence of species associated with wet microhabitats,

which may reflect reduced soil moisture due to higher vegetative evapotranspiration by *C. vulgaris*. Reduced cover of native AM hosts appears to be more important than reduced soil AMF inoculum availability in affecting mycorrhizal interference by *C. vulgaris* and its symbionts on *C. rubra*. Taken together, our data clearly point to a role for mycorrhizal mismatch to be contributing to invasion by an ErM host into a AM dominant system, effectively converting these *C. rubra* tussock grasslands into shrublands with reduced cover and functional diversity of native plant species.

Declarations

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Code availability: This publication does not use novel code, the statistical code and necessary data files used in this manuscript are available at <https://github.com/deslippeju/Moyle-DeslippeTNP>

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Figures

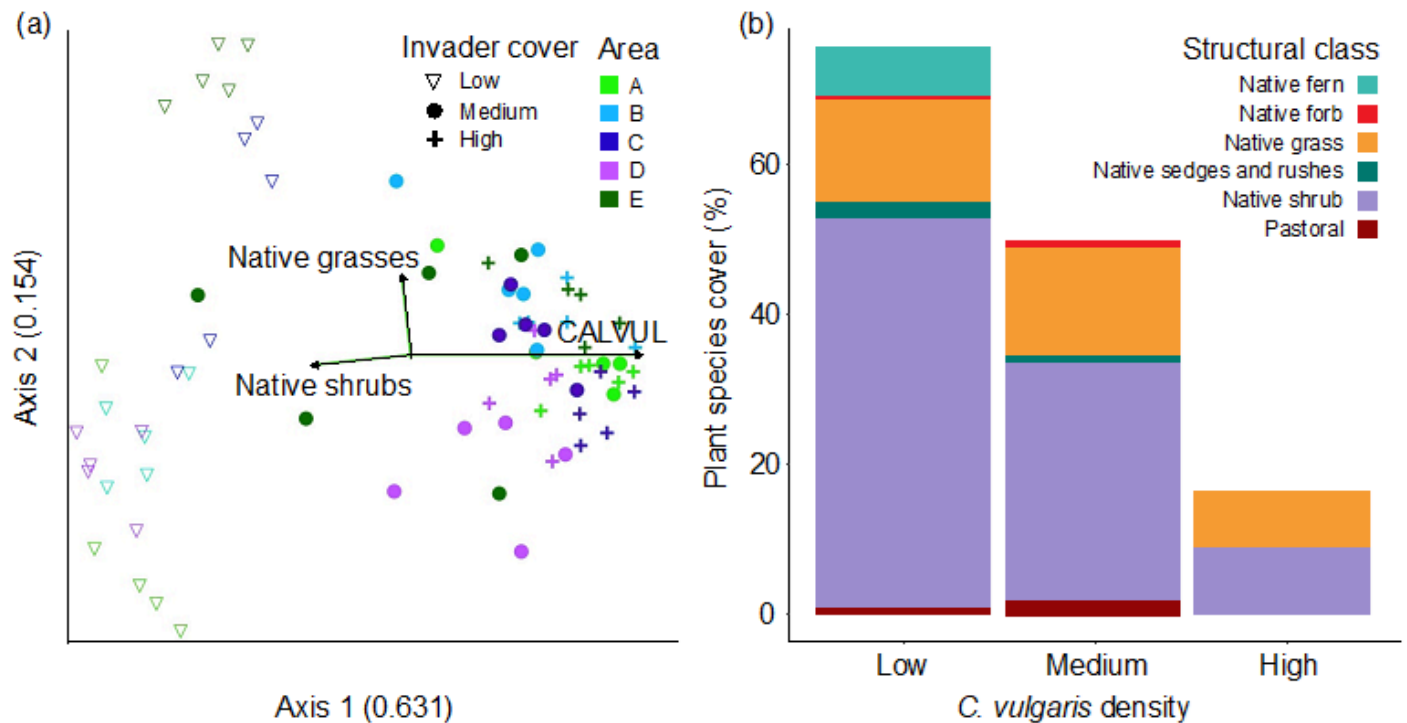


Figure 1

Response of the plant community to changes in *C. vulgaris* cover. a) NMS ordination of the variation in plant community composition in 75 study plots. Symbols represent *C. vulgaris* cover categories of each site; low $\leq 10\%$ cover, medium density between 11% - 60% cover, and high density $> 60\%$ cover. Colours represent the geographic area of study plots from Southern sites (Area A) to Northern sites (Area E). b) Average total percent cover of subordinate plant species (all species excluding *C. vulgaris* and *C. rubra*) by structural class in the three *C. vulgaris* cover categories.

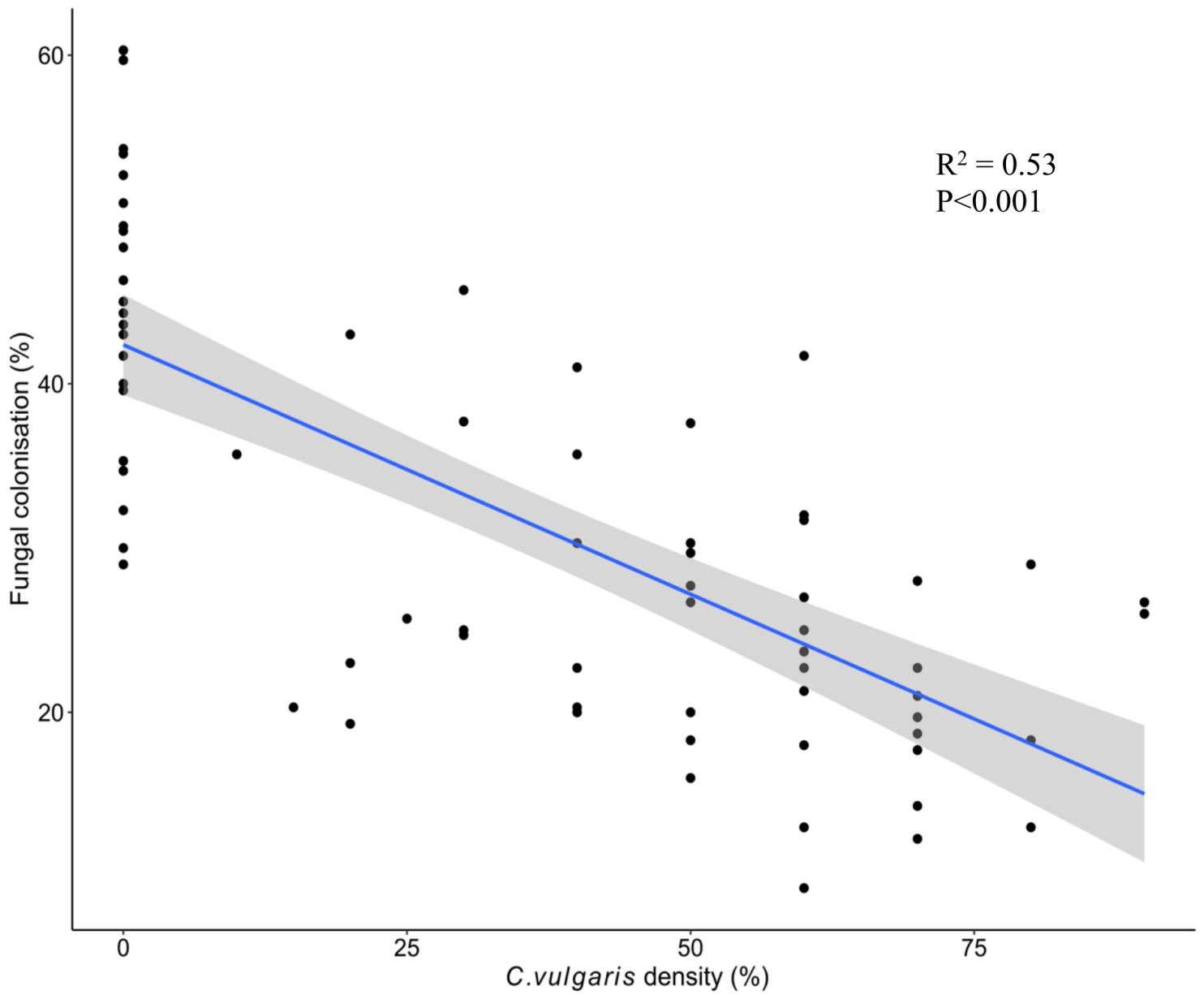


Figure 2

The negative relationship between *C. vulgaris* density (%) and the percent root length of *C. rubra* colonised by AMF, fitted with a linear trend line and 95% confidence interval.

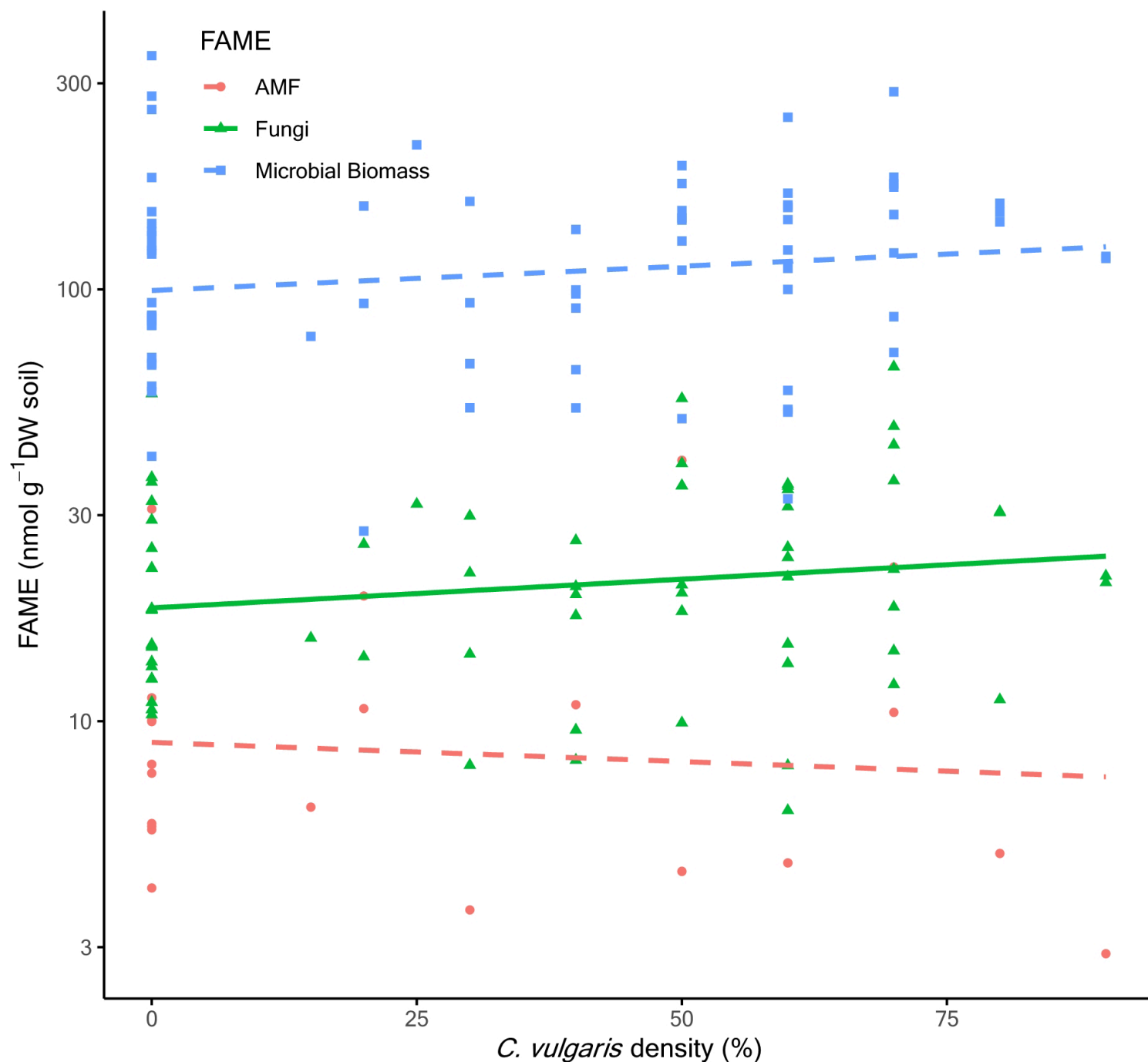


Figure 3

Lipid biomarkers for soil microbial groups in response to a gradient of *C. vulgaris* density: AMF biomass is indicated by the concentration of NLFA 16:1 ω 5 in soil; fungi are indicated by the concentration of PLFA 18:2 ω 6 in soil and total microbial biomass is indicated by the sum of 8 PLFAs in soil. Data are fitted with linear trend lines and 95% confidence intervals, solid lines indicate significant linear relationships. Note log scale transformation of y-axis. See supplementary materials for detailed model results.

Supplementary Files

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