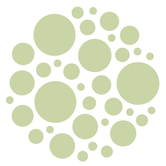

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RESEARCH

The mycorrhizal communities of *Lophomyrtus bullata* Burret (Myrtaceae) within three natural forest associations of New Zealand

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Abstract: The widespread endemic tree *Lophomyrtus bullata* (ramarama; Myrtaceae) is in serious decline. *Lophomyrtus bullata* is now considered threatened due to the ongoing spread of *Austropuccinia psidii*, a rust fungus causing myrtle rust disease. Mycorrhizal communities play an important role in the survival of plant species and have a potential role in disease resistance. Thus, we examined the fungal communities of *L. bullata*, with special emphasis on the arbuscular mycorrhizal fungi, together with vegetation and site characteristics in three forest associations in Northern New Zealand. Molecular analyses demonstrated a diverse fungal community, including representatives of nine families of arbuscular mycorrhizae. The family Archaeosporaceae was particularly abundant and diverse. Other fungal phyla (Ascomycota, Basidiomycota, and Zygomycota) were also found to associate with *L. bullata*. Mycorrhizal species composition across vegetation associations was similar but abundances differed. This is the first study to demonstrate the multiple fungal species associated with *L. bullata*, which may help in the remediation of this vulnerable plant.

Keywords: fungal endophytes, Glomeromycota, *Lophomyrtus bullata*, metabarcoding, zero-radius operational taxonomic unit

Introduction

Lophomyrtus bullata (ramarama) is one of two species in the endemic genus *Lophomyrtus* (Myrtaceae: Myrteae) in New Zealand (Burret 1941; Thornhill et al. 2015). *Lophomyrtus bullata* occurs throughout the North Island and the north-eastern corner of the South Island (absent from northwest Nelson) and south to the Wairau River, Marlborough (Cheeseman 1906; Allan et al. 1961; Dawson et al. 2011). *Lophomyrtus bullata* grows in shade or full sun, preferring open forest or forest margins (Salmon 1980), and is often a common mid-storey tree of lowland-podocarp forests; occasionally it grows on steep slopes and can form a large component of regenerating shrubland in wetter areas (de Lange 2020). In te reo Māori (Māori language) ramarama means "a gleam of light" most likely because of the crinkly, shiny leaves (Fig. 1). Traditionally ramarama leaves have been used by Māori to treat bruises and cuts (Riley 1994), presumably because of the cytotoxic and antimicrobial properties of a unique natural product, bullatenone (Woollard et al. 2008). Previously assessed as not threatened, this species was reclassified as Threatened, Nationally Critical because of the risk myrtle rust disease, caused by the exotic rust fungus *Austropuccinia psidii,* poses to *L. bullata* since it was first detected in New Zealand in 2017 (de Lange et al. 2018).

Over 90% of plant species have a mutually beneficial symbiotic association with mycorrhizal fungi (Bonfante & Genre 2010). These fungi have a major role in nutrient cycling (Kloepper 1994; Selosse & Roy 2009), providing the host plants with services such as improved nutrient status, growth, water absorption, and disease resistance, while the plant host is necessary for fungal growth and reproduction (Smith & Read 2008). Mycorrhizae are separated into endomycorrhizae, within which there are orchid, ericoid, and arbuscular mycorrhizae (AM), and ectomycorrhizae (ECM) (Bonfante & Genre 2010).

Members of New Zealand Myrtaceae such as *Metrosideros* Banks ex Gaertn. are known to support AM (Hafeel 2000), while others such as *Leptospermum scoparium* agg. J.R. Forst. & G. Forst. and *Kunzea* Rchb. (*Kunzea ericoides* (A. Rich) Joy Thomps. complex) are known to support both AM and ECM (Moyersoen & Fitter 1999; de Medina 2018). To date, we do not know which type of mycorrhizae associates with *Lophomyrtus bullata*. Members of the tribe Myrteae closely related to *Lophomyrtus,* such as *Rhodomyrtus* (DC.) Rchb. and *Psidium* L. are known to associate with AM (Koske et al. 1992) and in a phylogeny of Myrtaceae *Lophomyrtus* occurs

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Figure 1. Ramarama (*Lophomyrtus bullata*) in flower. December 31 2020 (M. Ford).

in a putative AM-only clade (Tedersoo & Brundrett 2017); we wanted to definitively demonstrate the mycorrhizal partners of the genus *Lophomyrtus*.

Our study is the first step in understanding the ecology of this often-overlooked plant species. Although the main aim of our study is to determine which species of fungi associate with *Lophomyrtus bullata* across three forest types in north-western Northland, our ultimate aim is the protection of this endemic species using its fungal associates.

Methods

Study sites

The study sites were in the Tutamoe Ecological District (Miller & Holland 2008) of the western far north of the North Island, New Zealand (35°70′0″ S, 173°5′0″ E). The three study sites, Six-Foot Track, The Domain, and Maunganui Bluff, were chosen to represent differences in elevation, forest associations, rainfall, soil, and distance to the coast (Table 1). Further, sites were chosen in areas of native vegetation with no known occurrences of myrtle rust disease (Campbell et al. 2020). The vegetation of all three sites have been modified (e.g. logging and fire) to some extent in the last 100 years (Ford 2021).

Vegetation analysis

In each study site, five mature *Lophomyrtus bullata* plants were randomly selected. The distance between the selected plants was three times the size of the canopy to minimise the overlap of the root system between plants. The location and elevation of all selected plants was recorded with a Garmin GPSMAP 65 (Garmin Ltd, Kansas).

The vegetation structure and floristic composition were recorded at each site, using the Atkinson system (Atkinson 1985) to characterise the composition of the dominant species in the canopy and describe the growth form (Appendix S1 in Supplementary Material). In addition, vascular plant species were recorded to assess the forest composition and diversity. The vegetation survey was done within one plot (30 m in radius, containing all five selected *L. bullata* plants) per site. A voucher specimen of *L. bullata* was collected from each site and deposited in the Auckland Museum Herbarium.

Soil sampling and analyses

One soil sample was collected 0.5 m from the stem, at random directions, of each selected plant. Leaf litter and the organic layer was removed before collecting a sample to 10 cm depth using a stainless-steel ring with a 3.5 cm radius. Samples were stored in plastic ziplock bags and transported in an insulated container with ice packs before being stored at 4°C.

Table 1. Characteristics of each location: Median annual average rainfall (Chappell 2014), median annual average temperature (Chappell, 2014), elevation (a.s.l.), underlying geology and soils (Fieldes 1968; Manaaki Whenua 2020).

Environmental parameter	Six- Foot Track (35°45′00″ S, 173°33′00″ E)	The Domain (35°30′00″ S, 173°28′00″ E)	Maunganui Bluff (5°39′00″ S, 173°39′0″ E)
Median annual average rainfall	1600–1800 mm	2000+ mm	1300–1400 mm
Median annual average temperature	12–13°C	11–12°C	14–15°C
Elevation (a.s.l.)	438 m	465 m	435 m
Underlying Geology	Tangihua Complex	Waipoua Basalt	Waipoua Basalt
Soils (Fieldes, 1968)	Brown granular loams and clays and associated soils	Brown granular loams and clays and associated soils	Brown granular loams and clays and associated soils
Soils (Manaaki Whenua 2020)	Orthic Recent	Perch-Gley Granular	Orthic Recent

Soil samples were prepared for analyses by air drying them at 35–40°C. Air dried samples were then crushed to pass through a 2 mm sieve. The volume to weight ratio was estimated using air-dried 2 mm sieved soil. Soil pH was measured in deionised water at a 1:2 soil:water slurry. Dumas high-temperature (1050°C) combustion was used to determine total carbon (TC) and total nitrogen (TN) (Blakemore et al. 1987). All soil analyses were carried out in an International Accreditation New Zealand (IANZ) laboratory.

Root sampling

Root samples were taken from each of the five selected mature plants of *L. bullata* at each site. Sampling was conducted over two weeks in Austral spring 2020. According to Stürmer and Bellei (1994), AM are active and producing spores during spring. Leaf litter and coarse organic matter were removed. Roots were then carefully tracked back to the trunk base of each selected *L. bullata* plant followed by the collection of fine roots (up to 3 mm). Root samples were stored in ziplock bags and transported in an insulated container with ice packs before being stored in a freezer (−20°C).

Root preparation and microscopy

Root samples were cleaned by washing twice with reverse osmosis (RO) water and brushing off soil with a soft toothbrush, followed by washing with 1% Tween® 20 (nonionic detergent), and a final rinse with RO water; care was taken to preserve the root structure although some fine roots may have been lost in the process of cleaning the roots. Samples were then frozen at −20°C. While preparing the root samples we detected that sample 6 and 7 (The Domain) contained *Dacrycarpus dacrydioides* (kahikatea) roots, a species which was commonly found intermixed with *L. bullata*. The *D. dacrydioides* root samples were excluded from further analyses.

Staining of root samples followed the protocol developed by Moukarzel et al. (2020) in which roots are covered with 75% ethanol overnight at room temperature and then autoclaved in 10% potassium hydroxide for 15 minutes. The roots were stained in 0.05% trypan blue. After staining, the roots were de-stained using lactoglycerol (1:1:1 lactic acid: glycerol: water) so they could be stored long-term at 4°C before microscopy work was undertaken.

Stained root samples were sectioned and mounted in water. The slides were studied using a Nikon Eclipse 80i compound microscope (Nikon Instruments Inc., Melville, New York) and any observed fungal structures were photographed using NIS elements imaging software 3.1.100 (2018). The percentage of mycorrhizal colonisation of *L. bullata* roots was determined using a gridline intersection (Brundrett et al. 1996); five roots from two samples per site were examined.

DNA extraction and Illumina sequencing

Approximately 0.3 g of powdered root material per sample was used for each extraction with the DNeasy Plant Mini kit (QIAGEN). The extracted DNA was sent to Genewiz, China (https://www.genewiz.com) for 18S ribosomal RNA (SSU small subunit) and ITS (internal transcribed spacer) amplicon sequencing on the Illumina MiSeq 2x250 platform. The 18S: v7–v8 region was amplified using primers CGWTAACGAACGAG and AICCATTCAATCGG designed by GENEWIZ (https://www.genewiz.com). The ITS2 region was amplified using GTGAATCATCGARTC and TCCTCCGCTTATTGAT designed by GENEWIZ (https://www.genewiz.com). Two gene regions were amplified to characterise the fungal community within *L. bullata*; the 18S region to target AMF (Öpik et al. 2010; Banos et al. 2018), and the ITS2 for the general fungal community (Hanif et al. 2019).

Bioinformatics and statistical analyses

All the steps for processing sequence reads into ZOTUs (zero-radius operational taxonomic units) were carried out using VSEARCH 2.14.2 (Rognes et al. 2016), except for trimming of adaptors and primers, which was done with Cutadapt 3.3 (Martin 2011). The forward (R1) and reverse (R2) strand sequences were merged, trimmed of adaptors and primers, and relabelled by sample. The trimmed sequences were then pooled together and filtered for errors and lengths (any sequences with maximum expected errors > 1.0, > 0 undetermined bases, and outside length ranges of 300 to 415 for 18S, or 190 to 415 for ITS, were discarded). The filtered sequences were dereplicated, denoised into ZOTUs, and filtered for chimeras. The 18S ZOTUs were identified by BLAST with the MaarjAM database (https://maarjam.botany.ut.ee/) which contains Glomeromycota sequences. The ITS data were identified using the RDP naïve Bayesian classifier with the fungal ITS database option (derived from the UNITE fungal ITS database) (Wang et al. 2007). Zero-radius operational taxonomic unit abundances were inferred by mapping the trimmed sequences against the ZOTU sequences at a 97% identity threshold.

Molecular data (18S and ITS) were analysed using R (Version 4.0.3.) using the packages vegan (Oksanen et al.

2013), ggplot2 (Wickham 2016), and dplyr (Wickham et al. 2021). Both datasets provided ZOTU richness values per sample which were compared between forest types. The 18S data were filtered to exclude ZOTUs with BLAST bitscores of less than 300, while the ITS data were filtered to exclude ZOTUs with RDP identification confidence scores of less than 0.5 at taxonomic class level, to exclude ZOTUs with unreliable identifications. For analyses of richness differences between forest associations, the data sets were filtered again to exclude families represented by fewer than 30 ZOTUs. Means and standard errors of ZOTU richness per family were calculated per sample and plotted as bar graphs to display the richness trends of mycorrhizal families across the three forest associations. ANOVA tests were carried out to test for significant differences in the richness of mycorrhizal families between the forest associations. P-values were adjusted for multiple comparisons according to the Benjamini & Hochberg false discovery rate (Benjamini & Hochberg 1995) method. Multivariate community structure of 18S and ITS molecular data sets were analysed by calculating Bray-Curtis distances between samples. To investigate the relationships between fungal community composition and soil characteristics, principal coordinate analyses (PCoA) were carried out on the ZOTU abundance tables (Table 3). The first three axes of variation were extracted and used in a mixed model with soil data as independent variables and site as a random variable, using the R package lmerTest (Kuznetsova et al. 2017).

Results

Plant community composition and soil characteristics

Canopy, understorey, and groundcover plant species composition differed between the three sites (Table 2; Appendix S2). Six-Foot Track was dominated by *Pterophylla sylvicola* (tōwai) and *Kunzea robusta* (kānuka) in the canopy (Appendix S2). The Domain was dominated by *Dacrycarpus dacrydioides* (kahikatea); large *Metrosideros robusta* (northern rātā) trees were seen in The Domain but were not dominant. The canopy of Maunganui Bluff was dominated by *Knightia excelsa* (rewarewa) and *Beilschmiedia tarairi* (taraire). The highest number of vascular species was found at The Domain (*n* =

Table 2. Plant community composition at Six-Foot track, The Domain, and Manganui Bluff. Following Atkinson (1985), the three leading species (based on the range of % cover) in each category are listed.

Vegetation	Six–Foot Track	The Domain	Maunganui Bluff
Canopy	*Pterophylla sylvicola* (Sol. ex A. Cunn) Pillo et H.C. Hopkins, *Kunzea robusta* de Lange et Toelken	***Dacrycarpus dacrydiodes* (A. Rich.) de Laub.), *Pterophylla sylvicola***	*Knightia excelsa** R. Br. *Beilschmiedia tarairi** (A. Cunn.) Benth. et Hook. f. ex Kirk
Understorey	*Lophomyrtus bullata*	[*Dicksonia squarrosa* (G. Forst.) Swartz -*Lophomyrtus bullata*]	[*Myrsine australis* (A. Rich.) Allan*, *Geniostoma ligustrifolium A. Cunn* var. *ligustrifolium**, *Rhopalostylis sapida* H. Wendl. et Drude]
Groundcover	*Schoenus maschalinus* Roem. et Schult.	*Selaginella kraussiana* (Kunze) A. Braun	*Asplenium lamprophyllum* Carse
Epiphytes		*Asplenium* L. spp., *Astelia* Banks & Sol. ex R. Br. spp. and *Hymenophyllum* Sm. spp.	
Lianas		*Ripogonum scandens* J.R. Forst. et G. Forst., *Freycinetia banksii* A. Cunn.	

spp. = multiple species
*denotes plants of same height tier, bolded species means more than 50% cover, underlined species means 20–49%, and square brackets means 10–19% cover.

Table 3. Recognised generic diversity of Glomeromycota families (Wijayawardene et al. 2020), numbers of 18S ZOTUs recovered, and numbers of unique sequence names among ZOTU-matched MaarjAM database sequences.

Family	Generic diversity of families	ZOTUs	Unique Sequence Names
Acaulosporaceae Gerdemann & Trappe	1	68	6
Ambisporaceae C. Walker, Vestberg & Schüßler	1	487	3
Archaeosporaceae	3	2433	9
Diversisporaceae C.Walker & A.Schüssler	7	11	3
Entrophosporaceae Ames & Schneider	3	105	5
Gigasporaceae J.B. Morton & Benny	1	610	9
Glomeraceae	17	576	37
Pacisporaceae Oehl & E. Sieverd.	1	4	1
Paraglomeraceae J.B. Morton & Redecker	2	189	5
Total	**36**	**4483**	**78**

137) followed by Six-Foot Track (n = 112) and Maunganui Bluff (n = 80) (Appendix S2).

Soil pH at Maunganui Bluff ranged from 5.5 to 6.5 whereas pH at both Six-Foot Track and The Domain was less than 5.5 (Fig. 2). Low volume weight (< 0.5 g ml^{-1}) in two of the Six-Foot Track samples suggest a larger proportion of organic matter compared to Manganui Bluff and The Domain, which partly explains the high total carbon concentration (up to 18%) measured in some of the Six-Foot Track samples (Fig. 2). High variation in carbon and nitrogen concentration and C:N ratios were found at each site (Fig. 2).

Mycorrhizal fungal colonisation

Root staining provided evidence of AM colonisation, such as hyphal coils, arbuscules, and vesicles (Figs. 3a–c). This confirmed that *Lophomyrtus bullata* in all three forest associations supports AM relationships. Root samples were not associated with any AM spores. Using the gridline intersection method demonstrated that 68.5% of root length was colonised by mycorrhizal fungi across the samples examined; this percentage is most likely much higher as some sampled roots were too dark to see the mycorrhizal structures. Dark septate endophytes (usually Ascomycota) were also observed in *L. bullata* roots alongside AM (Fig. 3c). We did not observe any ECM fungal structures such as a mantle or a hartig net.

Molecular analyses

Among the 18S dataset, seven families from the phylum Glomeromycota were represented by over 30 ZOTUs (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Gigasporaceae, Glomaraceae, and Paraglomeraceae), with 68 to 2433 ZOTUs per family (Table 3); Diversisporaceae and Pacisporaceae were also amplified but with fewer than 30 ZOTUs. Similar trends were observed across the forest associations with the family Archaeosporaceae J.B. Morton & Redecker (2001) being the most dominant for all three forest associations (Fig. 4). Archaeosporaceae was approximately four times as abundant than the next most abundant AMF family, Gigasporaceae, in terms of ASV richness and relative sequence abundance (Fig. 4). Although there were 2433 ZOTUs in Archaeosporaceae, each of these matched one of only 20 different database sequences, with only nine different names, compared with c. 1110 18S sequences and 14 different names from this family in the MaarjAM database (Öpik et al. 2010). One sequence (*Archaeospora* sp.; HM159462) was a match for 2351 ZOTUs with a mean

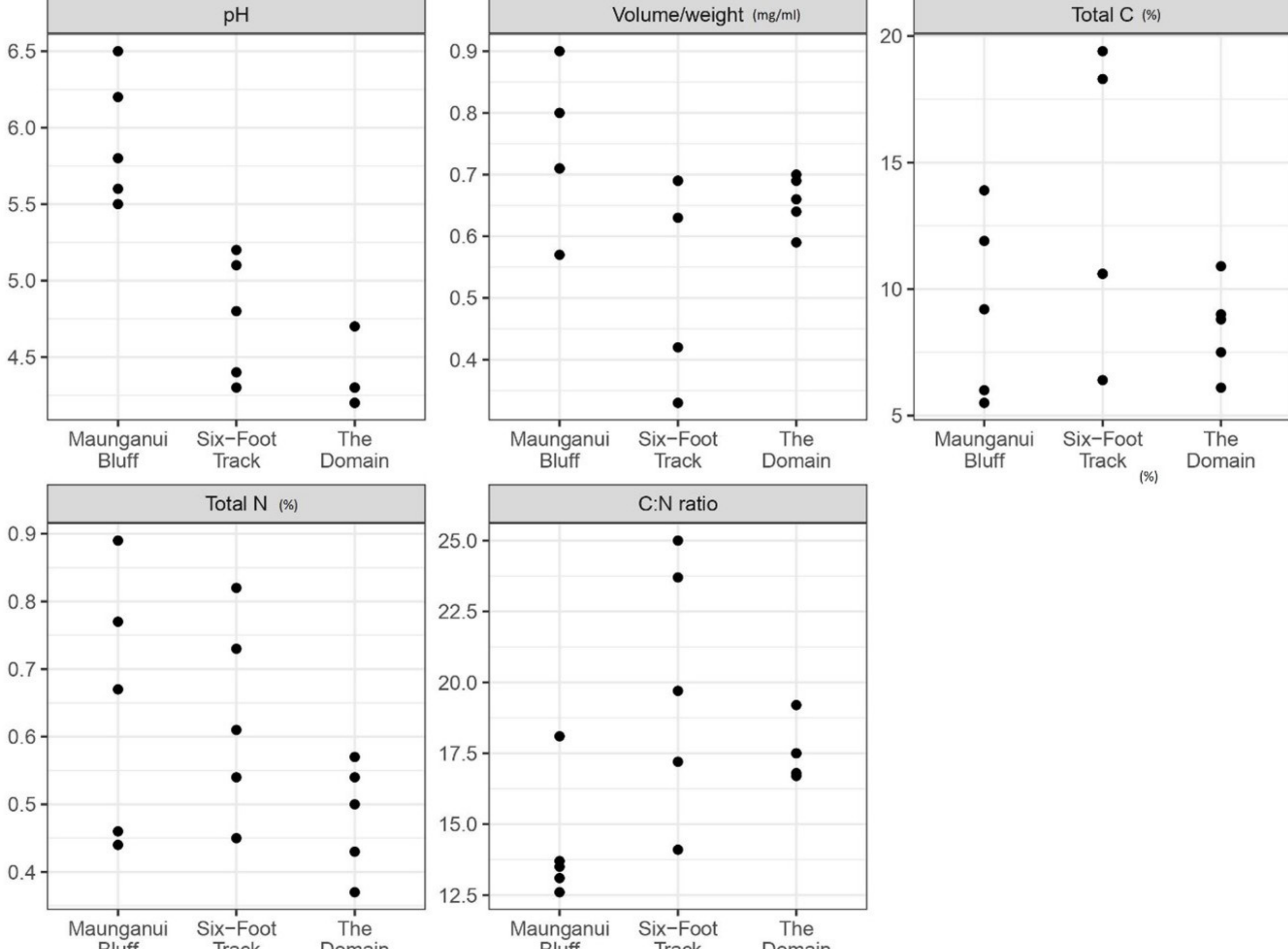

Figure 2. Soil characteristics (pH, volume weight, total carbon and total nitrogen concentration, C:N ratio) at Manganui Bluff, Six-Foot Track, and The Domain.

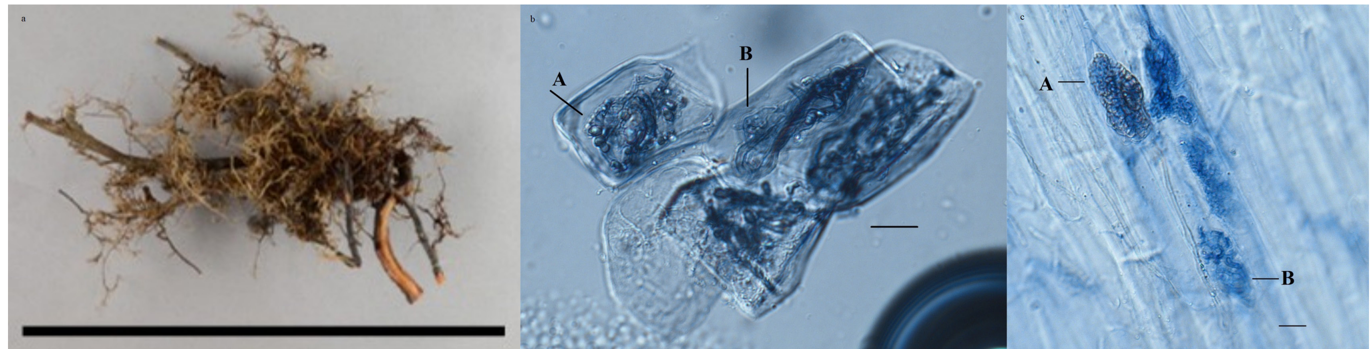

Figure 3. (a) Roots of *L. bullata* after cleaning Sample 9 from The Domain. Scale bar 15 cm. September 22 2021 (M. Ford), (b) AM fungi within the root cells of *L. bullata* from Sample 1, Six-Foot Track. A is an arbuscule and B is a fungal coil. The AM fungal coils appear dark blue due to the trypan stain. Magnification 400×, scale bar 10 μm, (c) AM fungal structures and a dark septate fungal endophyte within the root cells of *L. bullata*, The Domain. A is a dark septate endophyte and B is an arbuscule. Fungal structures appear dark blue due to the trypan stain. Magnification 400×, scale bar 20 μm.

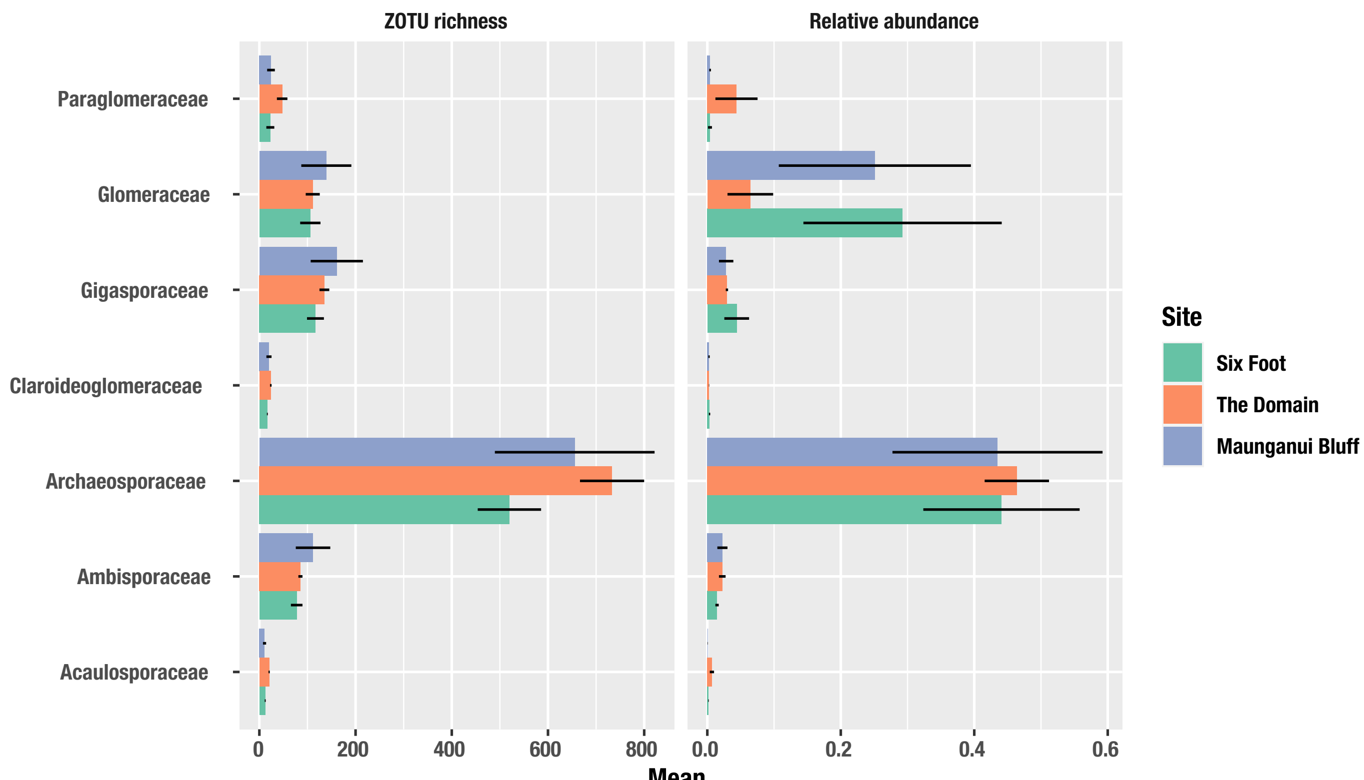

Figure 4. Mean and SE of ZOTU (ASV) richness (left) and relative sequence abundance (right) in Glomeromycota families detected at different sites by metabarcoding analyses of 18S genes.

pairwise identity of 94%; this sequence represents 96.6% of the recovered ZOTUs. Based on ANOVA tests, no significant differences in mycorrhizal richness were detected between sites for any Glomeromycota families in the 18S dataset.

In the ITS dataset, Ascomycota had the highest richness (1361 ZOTUs), with many ZOTUs identified as Archaeorhizomycetaceae Rosling and T. James, Herpotrichiellaceae Petr. and Hyaloscyphaceae Nannf, followed by Basidiomycota (561 ZOTUs), while 60 ZOTUs were detected from Glomeromycota. Only one ZOTU (zotu1051; Appendix S3) matched an ectomycorrhizal species, *Scleroderma xanthochroum* (SH1525914.08FU), with a sequence similarity of > 0.96%. Twelve fungal families were represented by over 30 ITS ZOTUs, with varying richness trends between sites (Fig. 5). The mean richness of Hyaloscyphaceae (but no other families in the ITS dataset) differed significantly between sites ($F_2 = 30.4$, $p.adj = 0.00118$), with mean values of 16.2 ASVs in samples from The Domain, compared to 2.25 and 5.25 in Maunganui Bluff and Six-Foot Track samples, respectively. The first three PCoA axes of variance represented 28.7% of ITS community variance and 43.9% of 18S community variance. For the ITS dataset, there were no significant effects or correlations between the first three PCoA axes and soil variables. For the 18S dataset, the PCoA axis 2 was significantly negatively correlated with total C and C:N ratio (F13 = −2.55 and −2.28, p = 0.0242 and

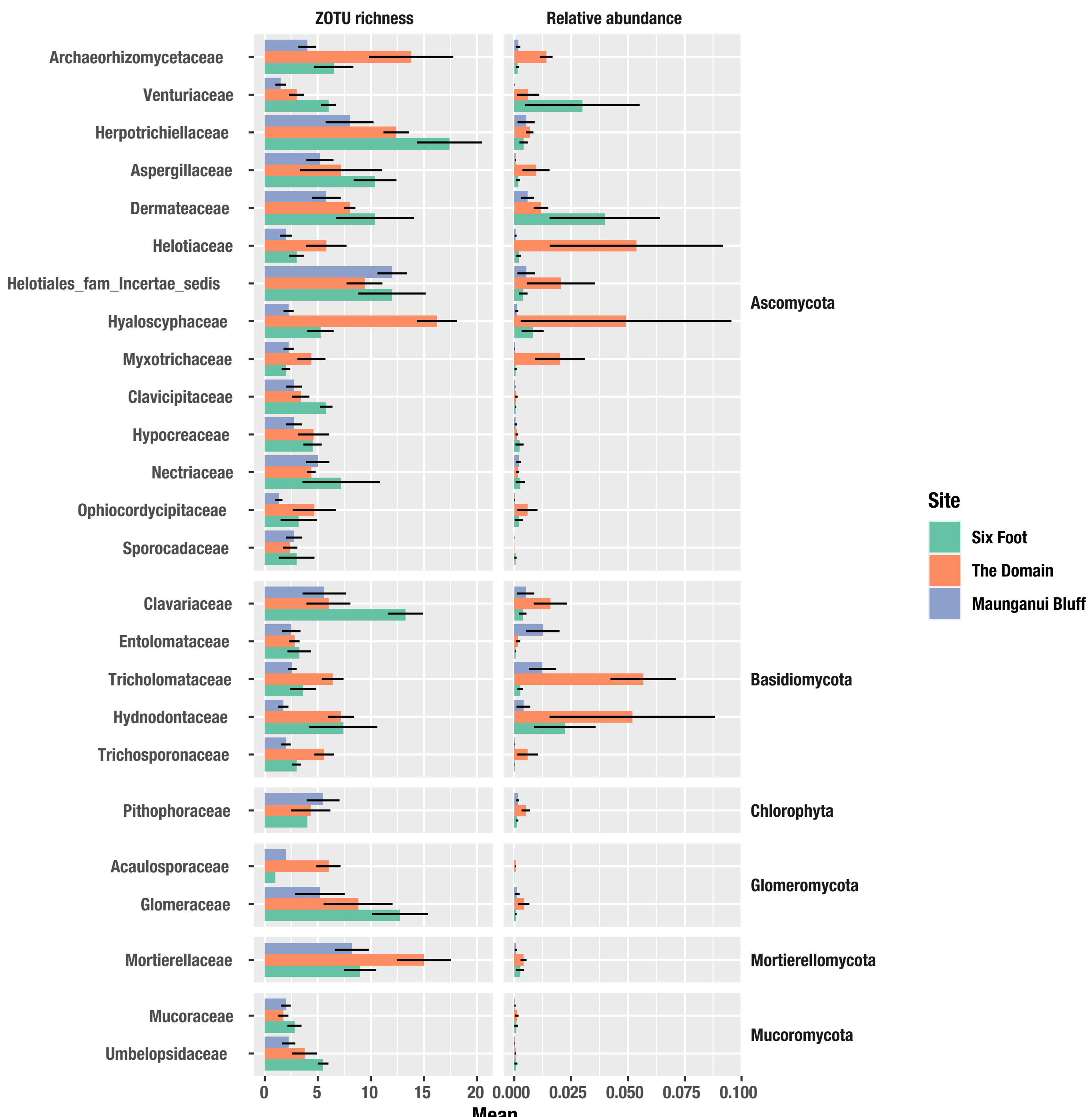

Figure 5. Mean and SE of ZOTU/ASV richness (left) and relative sequence abundance (right) of fungal families grouped by phylum detected at different sites by metabarcoding analyses of the ITS locus.

0.0403, respectively), and axis 3 was significantly positively correlated with total N (F11.8 = 2.29, p = 0.0414).

Discussion

Our study confirms the prior hypothesis (Tedersoo & Brundrett 2017) that *Lophomyrtus bullata* are colonised by arbuscular mycorrhizae (AM); additionally, we did not find any evidence of ectomycorrhizal fungi (ECM) on *L. bullata* roots. Although plants generally support only one type of mycorrhizae, such as AM or ECM (Moyersoen & Fitter 1999), there are Myrtaceae and other plant families that support both groups (Chilvers et al. 1987). The occurrence of both AM and ECM fungi associated with some Myrtaceae species such as *Eucalyptus* (Chilvers et al. 1987) has been explained by their ecology. In plant species able to support both AM and ECM, AM fungi are more dominant in the early stages of plant establishment (i.e. the seedling stage) but are quickly outcompeted by ECM as the roots of the plant branch and spread (Chilvers et al.

1987; Lodge & Wentworth 1990). *Kunzea* and *Leptospermum* may characterise early successional vegetation stages in New Zealand forests; however, they appear to be more closely affiliated with ECM when establishing in areas dominated by ECM tree species (e.g. members of the Nothofagaceae) and with AM in areas dominated by AM tree species (e.g. *Podocarpus* spp.) (Moyersoen & Fitter 1999; Medina 2018). Many dominant tree species in New Zealand such as *Agathis australis* and Podocarpaceae Endl. support AM relationships (Russell et al. 2002; Dickie & Holdaway 2011; Padamsee et al. 2016). Although we do not know much about fungal associations of forest understorey plants, it was not unexpected that AM fungi associate with *L. bullata*, especially in areas that were initially dominated by *Agathis australis* that is solely associated with AM fungi (Ogden et al. 1992).

Lophomyrtus bullata supported four orders (Diversisporales, Glomerales, Archaeosporales, and Paraglomerales) within Glomeromycota and nine of 16 families using the classification system outlined in Wijayawardene et al. (2020). Surprisingly, Glomeromycota diversity in *L. bullata* was higher than *A. australis* (Padamsee et al. 2016) and New Zealand Podocarpaceae (Russell et al. 2002). The high abundance (four times as abundant) of Archaeosporaceae in *L. bullata* as compared with other Glomeromycota families is also noteworthy. In studies of other NZ tree species, Archaeosporaceae occurred in low numbers or was absent in Podocarpaceae (Russell et al. 2002), *A. australis* (Padamsee et al. 2016), and *Leptospermum scoparium* (de Medina 2018). The Archaeosporaceae ZOTUs matched approximately 2% of diversity in the MaarjAM database. Additionally, the mean percent identity of matches for those 2433 ZOTUs to the 20 MaarjAM sequences is 93.8%, (minimum 85%; maximum 99%), which implies that although a lower number of species were recovered in the analyses, the ZOTUs may represent Archaeosporaceae species and/or sub-species yet to be recognised. In fact *Archaeospora* sp. (HM159462), that was a close match for 96.6% (2351 ZOTUs) of the total recovered 18S ZOTUs, was initially found in a study examining AMF associated with a fly ash pond in India (Giridhar Babu & Sudhakara Reddy 2011). This species has not been formally described and we do not know how many of the 2351 ZOTUs represent this undescribed species as there is only 94% pairwise sequence identity match on average.

It may also be expected that one traditionally defined species may have more than one ZOTU, and that an OTU may represent more than one species. ZOTUs were used in this study as they enable resolution of closely related strains with potentially different phenotypes that would otherwise be lumped into 97% similar OTUs (Porter & Hajibabaei 2018). The family Archaeosporaceae had far fewer ZOTUs (three) in the ITS data, which may be due to the sequencing platform (de Medina 2018). Another possibility for the lack of Archaeosporaceae ZOTUs is that the ITS primers may be biased against amplifying sequences from this family (Tedersoo & Lindahl 2016).

Not surprisingly, the ITS dataset was dominated by Ascomycota. However, the ZOTU richness of Archaeorhizomycetales, including a representative of the uncultured sister lineage of Archaeorhizomycetes GS31, was interesting as we do not know much about its ecology other than that they are commonly recovered in environmental sequencing studies (Kalsoom Khan et al. 2020). Although the ITS dataset (zotu1051; Appendix S3) indicated the presence of one species of ectomycorrhizal fungus, this was most likely a symbiont of *Kunzea robusta* rather than *L. bullata*. *Scleroderma xanthochroum* is known to be ectomycorrhizal and associates with *Kunzea* (McKenzie et al. 2006), which forms the canopy where this ZOTU was recovered (Six-Foot Track). Apart from the strictly arbuscular mycorrhizal family Glomeraceae, the ITS data demonstrate the presence of Helotiales which have ericoid mycorrhizal members but are also known to be saprotrophs (Rice & Currah 2006; Chambers et al. 2008). The ITS data demonstrate the presence of *Phaeohelotium* Kanouse an aggregate genus that has both mycorrhizal and saprotrophic species (Baral et al. 2013); however, it is difficult to confirm if these organisms are in symbiosis with *L. bullata* or are living as saprotrophs in the environment. Ascomycota were observed microscopically and with ITS data. Dark septate endophytes (Ascomycota; Faeth & Hammon 1997; Larran et al. 2007) were observed in *L. bullata* roots; they have also been found to be associated with *A. australis* (Padamsee et al. 2016) and *Spinifex sericeus* (Poaceae) (Johansen et al. 2016). Further research in different areas throughout the range of *L. bullata* will be needed to fully characterise and understand the role of its root endophytes.

Carbon, nitrogen, and C:N ratio influenced the composition of 18S communities, but not ITS communities. This difference might be due to the different communities detected by the targeted gene regions. The three sites differed in soil characteristics and fungal communities resulting in a lack of replication. In this study, the highest abundance of Glomeraceae was found at the site with the lowest soil pH (The Domain, pH c. 4.5). Our finding contrasts with previous studies reporting that Glomeraceae prefer alkaline and neutral substrates (Bainard et al. 2015). Since we found that Glomeraceae abundance associated with low soil pH, it may be possible that other soil properties (e.g. nitrogen and/or phosphorus content; He et al. 2003) and plant species identity (Lekberg & Waller 2016) may be driving mycorrhizal community composition in these sites.

Arbuscular mycorrhizal communities have been shown to differ in composition between broadly defined habitats (tropical forests, temperate forests, grasslands, etc.) and the number of AM taxa on a host can differ between habitats (Öpik et al. 2006). We found that the AM ZOTUs across the three forest associations were similar even though there are some differences in plant community composition across the three sites (Fig. 4). The occurrences and abundances of ZOTU data suggest that individual trees may have the same fungal species composition but not the same species abundances. Further sampling of AM associated with *L. bullata* in other habitats and sites may confirm if this trend is observed elsewhere and enable further insights on the influence of *L. bullata*.

Our findings indicate that *L. bullata* supports a diverse range of AM fungi, especially Archaeosporaceae diversity, which has not been seen at this level in association with any other New Zealand plant species. With the threat of myrtle rust, these AM communities may also be under threat as *L. bullata* experience dieback and potential death. In 2021 myrtle rust disease was found on some of the sampled plants, which underscores the urgency to understand the ecology of this threatened species.

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Additional Information and Declarations

Author contributions: MF, MP, LS and PJdL conceptualised and designed the study. MF conducted the field and lab work and wrote the original manuscript; MP, LS and PJdL reviewed and edited the work. AD helped with the molecular analysis and presentation of data.

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References

Allan HH 1961. Flora of New Zealand. Wellington, RE Owen. 330 p.

Atkinson IA 1985. Derivation of vegetation mapping units for an ecological survey of Tongariro National Park, North Island, New Zealand. New Zealand Journal of Botany 23: 361–378.

Bainard LD, Dai M, Gomez EF, Torres–Arias Y, Bainard JD, Sheng M, Eilers W, Hamel C 2015. Arbuscular mycorrhizal fungal communities are influenced by agricultural land use and not soil type among the Chernozem great groups of the Canadian Prairies. Plant and Soil 387: 351–362.

Banos S, Lentendu G, Kopf A, Wubet T, Glöckner F, Reich M 2018. A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. BMC Microbiology 18: 190.

Baral HO, Galán, R, Platas G, Tena R 2013. *Phaeohelotium undulatum* comb. nov. and Phaeoh. *succineoguttulatum* sp. nov., two segregates of the *Discinella terrestris* aggregate found under *Eucalyptus* in Spain: taxonomy, molecular biology, ecology and distribution. Mycosystema 32: 386–428.

Benjamini Y, Hochberg Y 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological) 57: 289–300.

Blakemore LC 1987. Methods for chemical analysis of soils. NZ Soil Bureau Scientific Report 80. Lower Hutt, Department of Scientific and Industrial Research. 103 p.

Bonfante P, Genre A 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nature Communications 1(4): 48.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N 1996. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Canberra, Australia. 374 p.

Burret M 1941. Myrtaceen-Studien. Notizblatt des Botanischen Gartens und Museums zu Berlin–Dahlem 15: 479–550.

Campbell R, Teasdale S, Bradshaw P 2020. Aotearoa myrtle rust surveillance library. A Plant & Food Research report prepared for: Ministry for the Environment. PFR SPTS No. 19875. 39 p.

Chambers SM, Curlevski NJ, Cairney JW 2008. Ericoid mycorrhizal fungi are common root inhabitants of non–Ericaceae plants in a south-eastern Australian sclerophyll forest. FEMS Microbiology Ecology 65: 263–270.

Cheeseman TF 1906. Manual of the New Zealand flora. Wellington, J. Mackay, Government Printer. 1163 p.

Chilvers GA, Lapeyrie FF, Horan DP 1987. Ectomycorrhizal vs endomycorrhizal fungi within the same root system. New Phytologist 107: 441–448.

Dawson J, Lucas R, Connor J, Brownsey PJ 2011. New Zealand's native trees. Nelson, Craig Potton. 318 p.

de Lange PJ 2020. *Lophomyrtus bullata Fact Sheet*. New Zealand Plant Conservation Network. https://www.nzpcn.org.nz/flora/species/lophomyrtus–bullata/ (Accessed 28 July 2021)

de Lange PJ, Rolfe JR, Barkla J, Courtney SP, Champion PD, Perrie LR, Beadel SM, Ford KA, Breitwiser I, Schonberger I, Hindmarsh–Walls R, Heenan PB, Ladley K 2018. Conservation status of New Zealand indigenous vascular plants, 2017. New Zealand threat classification series 22. Wellington, Department of Conservation. 82 p.

de Medina JB 2018. Companion biota associated with *Leptospermum scoparium* (mānuka; Myrtaceae). Unpublished PhD thesis, Massey University, Manawatū, New Zealand.

Dickie IA, Holdaway RJ 2011. Podocarp roots, mycorrhizas, and nodules. Smithsonian Contributions to Botany 95: 175–187.

Faeth SH, Hammon KE 1997. Fungal endophytes in oak trees: long-term patterns of abundance and associations with leafminers. Ecology 78: 810–819.

Ford M 2021 The mycorrhizal communities of *Lophomyrtus bullata* Burret (Myrtaceae) within three natural forest associations of New Zealand. Unpublished MSc thesis, University of Auckland, Auckland, New Zealand.

Giridhar Babu A, Sudhakara Reddy M 2011. Diversity of arbuscular mycorrhizal fungi associated with plants growing in fly ash pond and their potential role in ecological restoration. Current Microbiology 63: 273–280.

Guo D, Xia M, Wei X, Chang W, Liu Y, Wang Z 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. New Phytologist 180: 673–683.

Hafeel KM 2000. Arbuscular mycorrhizal associations of *Metrosideros excelsa* and *M. robusta* (Myrtaceae). Unpublished PhD thesis, Victoria University of Wellington, Wellington, New Zealand.

Hanif MA, Guo Z, Moniruzzaman M, He D, Yu Q, Rao X, Liu S, Tan X, Shen W. 2019. Plant taxonomic diversity better explains soil fungal and bacterial diversity than functional

diversity in restored forest ecosystems. Plants 8: 479.
He XH, Critchley C, Bledsoe C 2003. Nitrogen transfer within and between plants through common mycorrhizal networks (CMNs). Critical Reviews in Plant Sciences 22: 531–567.
Johansen RB, Johnston P, Mieczkowski P, Perry GL, Robeson MS, Burns BR, Vilgalys R 2016. A native and an invasive dune grass share similar, patchily distributed, root–associated fungal communities. Fungal Ecology 23: 141–155.
Kalsoom Khan F, Kluting K, Tångrot J, Urbina H, Ammunet T, Eshghi Sahraei S, Rydén M, Ryberg M, Rosling A 2020. Naming the untouchable – environmental sequences and niche partitioning as taxonomical evidence in fungi. IMA Fungus 11: 23.
Kloepper JW 1994. Plant growth–promoting rhizobacteria (other systems). Azospirillum/plant associations 187: 137–166.
Koske RE, Gemma JN, Flynn T 1992. Mycorrhizae in Hawaiian angiosperms: a survey with implications for the origin of the native flora. American Journal of Botany 79: 853–862.
Larran S, Perelló A, Simón MR, Moreno V 2007. The endophytic fungi from wheat (*Triticum aestivum* L.). World Journal of Microbiology and Biotechnology 23: 565–572.
Kuznetsova A, Brockhoff PB, Christensen RHB 2017. lmerTest Package: Tests in linear mixed effects models. Journal of Statistical Software 82: 1–26.
Lekberg Y, Waller LP 2016. What drives differences in arbuscular mycorrhizal fungal communities among plant species? Fungal Ecology 24: 135–138.
Lodge DJ, Wentworth TR 1990. Negative associations among VA–mycorrhizal fungi and some ectomycorrhizal fungi inhabiting the same root system. Oikos 57: 347–356.
Manaaki Whenua 2020: New Zealand soil classification. Soil Map Viewer. https://soils–maps.landcareresearch.co.nz/. (Accessed 28 July 2021)
Martin M 2011. Cutadapt removes adapter sequences from high–throughput sequencing reads. EMBnet Journal 17: 10–12.
McKenzie EHC, Johnston PR, Buchanan PK 2006. Checklist of fungi on teatree (*Kunzea* and *Leptospermum* species) in New Zealand. New Zealand Journal of Botany 44: 293–335.
Miller NC, Holland W 2008. Natural areas of Tutamoe Ecological District: reconnaissance survey report for the Protected Natural Areas Programme. Whangarei, Department of Conservation. 242 p.
Morton JB, Redecker D 2001. Two new families of Glomales, Archaeosporaceae and Paraglomaceae, with two new genera *Archaeospora* and *Paraglomus*, based on concordant molecular and morphological characters. Mycologia 93: 181–195.
Moukarzel R, Ridgway HJ, Guerin–Laguette A, Jones EE 2020. An improved clearing and staining protocol for evaluation of arbuscular mycorrhizal colonisation in darkly pigmented woody roots. New Zealand Plant Protection 73: 33–39.
Moyersoen B, Fitter AH 1999. Presence of arbuscular mycorrhizas in typically ectomycorrhizal host species from Cameroon and New Zealand. Mycorrhiza 8: 247–253.
Ogden J, Wilson A, Hendy C, Newnham RM, Hogg AG 1992. The late Quaternary history of kauri (*Agathis australis*) in New Zealand and its climatic significance. Journal of Biogeography 19: 611–622.
Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MH, Wagner H, Oksanen MJ 2013. Package 'vegan'. Community ecology package, version, 2, 1–295.
Öpik M, Moora M, Liira J, Zobel M 2006. Composition of root–colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. Journal of Ecology 94: 778–790.
Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytologist 188: 223–241.
Padamsee M, Johansen RB, Stuckey SA, Williams SE, Hooker J E, Burns BR, Bellgard SE 2016. The arbuscular mycorrhizal fungi colonising roots and root nodules of New Zealand kauri *Agathis australis*. Fungal Biology 120: 807–817.
Porter TM, Hajibabaei M 2018. Scaling up: A guide to high-throughput genomic approaches for biodiversity analysis. Molecular Ecology 27: 313–338.
Rice AV, Currah RS 2006. *Oidiodendron maius*: Saprobe in sphagnum peat, mutualist in ericaceous roots? In: Schulz BJE, Boyle CJC, Sieber TN eds. Microbial root endophytes. Soil Biology 9. Berlin, Springer. Pp. 227–246.
Riley M 1994. Māori healing and herbal: New Zealand ethnobotanical sourcebook. Wellington, Viking Sevenseas NZ. 528 p.
Rognes T, Flouri T, Nichols B, Quince C, Mahé F 2016. VSEARCH: a versatile open-source tool for metagenomics. PeerJ 4: e2584.
Russell AJ, Bidartondo MI, Butterfield BG 2002. The root nodules of the Podocarpaceae harbour arbuscular mycorrhizal fungi. New Phytologist 156: 283–295.
Salmon JT 1980. The native trees of New Zealand. Wellington, Craig Potton Publishing. 384 p.
Selosse MA, Roy M 2009. Green plants that feed on fungi: facts and questions about mixotrophy. Trends in Plant Science 14: 64–70.
Smith SE, Read DJ 2008. The symbionts forming arbuscular mycorrhizas. Mycorrhizal Symbiosis 2: 13–41.
Stürmer SL, Bellei MM 1994. Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. Canadian Journal of Botany 72: 359–363.
Tedersoo L, Lindahl B. 2016. Fungal identification biases in microbiome projects. Environmental Microbiology Reports 8: 774–779.
Thornhill AH, Ho SY, Külheim C, Crisp MD 2015. Interpreting the modern distribution of Myrtaceae using a dated molecular phylogeny. Molecular Phylogenetics and Evolution, 93: 29–43.
Wang Q, Garrity GM, Tiedje, JM, Cole JR 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73: 5261–5267.
Wickham H 2016. ggplot2: Elegant graphics for data analysis. 2nd edn. New York, Springer–Verlag. 260 p.
Wickham H, François R, Henry L, Müller K 2021. dplyr: A grammar of data manipulation. R package version 1.0.6.
Wijayawardene NN, Hyde KD, Al-Ani LK, Tedersoo L, Haelewaters D, Rajeshkumar KC, Zhao RL, Aptroot A, Leontyev D, Saxena RK, Tokarev YS, Dai DQ, Letcher PM, Stephenson SL, Ertz D, Lumbsch HT, Kukwa M, Issi IV, Madrid H, Phillips AJL, Selbmann L, Pfliegler WP, Horváth E, Bensch K, Kirk PM, Kolaříková K, Raja HA,

Radek R, Papp V, Dima B, Ma J, Malosso E, Takamatsu S, Rambold G, Gannibal PB, Triebel D, Gautam AK, Avasthi S, Suetrong S, Timdal E, Fryar SC, Delgado G, Réblová M, Doilom M, Dolatabadi S, Pawłowska JZ, Humber RA, Kodsueb R, Sánchez-Castro I, Goto BT, Silva DKA, de Souza FA, Oehl F, da Silva GA, Silva IR, Błaszkowski J, Jobim K, Maia LC, Barbosa FR, Fiuza PO, Divakar PK, Shenoy BD, Castañeda-Ruiz RF, Somrithipol S, Lateef AA, Karunarathna SC, Tibpromma S, Mortimer PE, Wanasinghe DN, Phookamsak R, Xu J, Wang Y, Tian F, Alvarado P, Li DW, Kušan I, Matočec N, Mešić A, Tkalčec Z, Maharachchikumbura SSN, Papizadeh M, Heredia G, Wartchow F, Bakhshi M, Boehm E, Youssef N, Hustad VP, Lawrey JD, Santiago ALCMA, Bezerra JDP, Souza-Motta CM, Firmino AL, Tian Q, Houbraken J, Hongsanan S, Tanaka K, Dissanayake AJ, Monteiro JS, Grossart HP, Suija A, Weerakoon G, Etayo J, Tsurykau A, Vázquez V, Mungai P, Damm U, Li QR, Zhang H, Boonmee S, Lu YZ, Becerra AG, Kendrick B, Brearley FQ, Motiejūnaitė J, Sharma B, Khare R, Gaikwad S, Wijesundara DSA, Tang LZ, He MQ, Flakus A, Rodriguez-Flakus P, Zhurbenko MP, McKenzie EHC, Stadler M, Bhat DJ, Liu JK, Raza M, Jeewon R, Nassonova ES, Prieto M, Jayalal RGU, Erdoğdu M, Yurkov A, Schnittler M, Shchepin ON, Novozhilov YK, Silva-Filho AGS, Gentekaki E, Liu P, Cavender JC, Kang Y, Mohammad S, Zhang LF, Xu RF, Li YM, Dayarathne MC, Ekanayaka AH, Wen TC, Deng CY, Pereira OL, Navathe S, Hawksworth DL, Fan XL, Dissanayake LS, Kuhnert E, Grossart HP, Thines M 2020. Outline of Fungi and fungus-like taxa. Mycosphere 1: 1060–1456.

Woollard JMR, Perry NB, Weavers RT, van Klink JW 2008. Bullatenone, 1, 3–dione and sesquiterpene chemotypes of *Lophomyrtus* species. Phytochemistry 69*:* 1313–1318.

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Supplementary material

Additional supporting information may be found in the supplementary material file for this article:

Appendix S1. Field sheet for recording the vegetation structure at each site.

Appendix S2. List of vascular plant species by site.

Appendix S3. ZOTU ITS data of species with a sequence similarity > 0.97.

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