Diversity and Distributions, (Diversity Distrib.) (2015) 1–11

BIODIVERSITY RESEARCH

Testing the co-invasion hypothesis: ectomycorrhizal fungal communities on *Alnus glutinosa* and *Salix fragilis* in New Zealand

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ABSTRACT

Aim It has been proposed that co-invasion with ectomycorrhizal (EM) fungi is a common mechanism by which non-indigenous trees overcome symbiont limitation, yet virtually all prior evidence has come from a single plant family, the Pinaceae. We tested the co-invasion hypothesis by examining the EM fungal communities associated with a specialized host, *Alnus glutinosa* (black alder), and a generalist host, *Salix fragilis* (crack willow), in New Zealand, where both trees are invasive. We aimed to find out if these two hosts, which often co-occur on invaded riverbanks, (1) were forming novel EM fungal associations in New Zealand and (2) had potential to facilitate each other through shared EM fungi.

Location New Zealand.

Methods We collected root tip samples from both host plants at riparian sites on the North Island and South Island and used DNA sequence-based identification to characterize EM fungal communities.

Results Both trees relied upon exotic EM fungi from their indigenous ranges and did not associate with any known endemic New Zealand EM fungi. *Alnus* had highly similar communities on both islands, while the *Salix* communities were distinct. All EM fungi on South Island *Alnus* were also present on South Island *Salix*, while North Island *Salix* did not substantially share EM fungal associates with *Alnus*.

Main conclusions Overall, our study indicates that plant hosts with specialized and more generalist EM fungal communities can both successfully invade new habitats with non-indigenous EM fungi. While there may be some potential for facilitation between these two EM plants hosts via shared non-indigenous fungi, this outcome was context specific. Our findings suggest that the specificity of fungal mutualists is not a major barrier to the spread of invasive plants and cannot be taken as evidence an introduced plant will not become invasive.

Keywords
Biological invasions, facilitation, fungi, host specificity, invasive species, mycorrhizal inoculum.

INTRODUCTION

Invasion by non-indigenous species can significantly change the structure and function of ecological communities (Davis, 2009). Although the success of non-indigenous organisms in new areas can be difficult to predict, species interactions are often important (Shea & Chesson, 2002; Ricciardi *et al.*, 2013). In particular, 'enemy release' – in which an non-indigenous invader escapes specialized pathogens and competitors when it leaves its indigenous range – is frequently cited as a major contributor to non-indigenous invasion success (Mitchell *et al.*, 2006; Reinhart & Callaway, 2006; Diez *et al.*, 2010). Fewer studies, at least until recently, have considered that non-indigenous species will likely be missing

DOI: 10.1111/ddi.12304
http://wileyonlinelibrary.com/journal/ddi

A Journal of Conservation Biogeography
Diversity and Distributions

more than their enemies: specialized mutualists may also be absent in the new environment (Richardson *et al.*, 2000; Pringle *et al.*, 2009; Nuñez & Dickie, 2014). Despite this lag in our understanding of the role of mutualistic interactions in invasion, there is growing evidence that the absence of mutualists can be just as important as the absence of enemies in determining the success of non-indigenous establishment and spread (Richardson *et al.*, 2000; Thiet & Boerner, 2007; Nuñez *et al.*, 2009; Rundel *et al.*, 2014).

For obligate mutualisms, non-indigenous species can adopt several strategies to successfully establish partnerships in new areas. Dickie *et al.* (2010) presented a conceptual framework involving three possibilities: a non-indigenous organism can (1) co-invade with mutualists from its native range, (2) associate with indigenous but globally distributed (a.k.a. cosmopolitan) mutualists, or (3) acquire novel associates in the new environment, either with indigenous species or other exotic, yet novel, symbionts (co-xenic novel associations; Nuñez & Dickie, 2014). Evidence supporting all of three possibilities has been found, with the invasion scenario depending on the biology of the mutualism (Nuñez & Dickie, 2014). For example, invasions by non-indigenous plants requiring arbuscular mycorrhizal fungi or nitrogen-fixing bacteria, which generally show low host specificity, appear to involve either cosmopolitan or novel associations (Lafay & Burdon, 2006; Parker *et al.*, 2006; Moora *et al.*, 2011). In contrast, invasions by exotic plants requiring ectomycorrhizal (EM) fungi or specialized pollinators, which often have greater host specificity, tend to be dominated by co-invasion and cosmopolitan associations (Ramirez & Montero, 1988; Díez, 2005; Tedersoo *et al.*, 2007; Nuñez *et al.*, 2009; Dickie *et al.*, 2010; Hynson *et al.*, 2013).

Differences in plant mutualistic specificity have been widely suggested to have a significant effect on plant invasions, with more specific, obligately associated mutualists being less likely to succeed than those with the flexibility to associate with cosmopolitan or novel partners (Richardson *et al.*, 2000; Schwartz *et al.*, 2006; Pringle *et al.*, 2009). If true, this could suggest that symbiont specificity could be used as a trait to focus allocation of resources for biosecurity or invasive species removal. Ectomycorrhizal plants and fungi provide an excellent system in which to examine the relationship between mutualistic specificity and invasion outcomes because they exhibit a broad gradient of specificity by both partners (Molina *et al.*, 1992). Virtually all prior work on invasive EM plants has, however, focused on a single plant family, the Pinaceae (Nuñez *et al.*, 2009; Dickie *et al.*, 2010; Salgado Salomón *et al.*, 2011; Hynson *et al.*, 2013), with one study of naturalized *Eucalyptus* being the only exception (Díez, 2005) (all plant nomenclature follows the International Plant Names Index, www.ipni.org, accessed 26 August 2014). This raises an important question of whether results regarding patterns of symbiont association based on a single plant family can be generalized to other invasive ectomycorrhizal trees. While a range of fungal symbionts have been observed on exotic Pinaceae, the closely related, Pinaceae-specific fungal genera *Suillus* and *Rhizopogon* are frequently dominant players both in invasions and in early successional establishment within the indigenous range (Terwilliger & Pastor, 1999; Ashkannejhad & Horton, 2006; Collier & Bidartondo, 2009) (except where noted, all fungal nomenclature follows the NZfungi database, nzfungi2.landcareresearch.co.nz, accessed 26 August 2014). The dominance of Pinaceae-specific *Suillus* and *Rhizopogon* suggests a strong possibility that Pinaceae results may not be widely generalizable but rather are specific to this one plant family (Dickie & Moyerson, 2008).

To more thoroughly assess the co-invasion pattern, we examined the invasion of two EM angiosperms, *Alnus glutinosa* and *Salix fragilis*, in New Zealand. *Salix fragilis* was introduced in 1880 and considered a problematic invasive within 15 years of introduction (Webb *et al.*, 1988). It is presently widespread along waterways on both the North and South Islands. *Alnus glutinosa* was introduced in 1914 and has spread less widely than *S. fragilis*, forming extensive stands on the North Island and with more isolated naturalization and invasion elsewhere. Importantly, *A. glutinosa* and *S. fragilis* have different degrees of specificity for their EM fungal partners. *Salix* species generally associate with EM fungi that have cosmopolitan distributions and broad host ranges (Nara & Hogetsu, 2004; Nara, 2006; Tedersoo *et al.*, 2010, 2013; Ryberg *et al.*, 2011), while *Alnus* species are limited to a set of host-specific EM fungal mutualists (Molina, 1981; Tedersoo *et al.*, 2009; Bogar & Kennedy, 2013; Roy *et al.*, 2013). Given these differences in specificity, we predicted that *S. fragilis* would expand its range into New Zealand by associating with both cosmopolitan and indigenous New Zealand fungi, while *A. glutinosa* would likely maintain its small pool of exotic, specialized EM fungal associates as it invades. Despite the specificity of *A. glutinosa* for its particular EM fungi, based on recent studies suggesting that neighbouring EM hosts can influence *Alnus*-associated EM fungal assemblages (Bogar & Kennedy, 2013), we also hypothesized that *A. glutinosa* could share EM fungi with *S. fragilis* in the mixed species stands that we examined.

METHODS

Field sampling

In November 2012, we collected soil samples from two sites in New Zealand, one on the South Island and one on the North Island. The South Island site was located along the Waiau River (42°39′14.56″S, 173°1′49.69″E) where most trees were associated with lenses of fine sand in a cobble braided river bed. Sampling varied from individual clumps of trees to more contiguous forest along the edge of the river bed, but entire areas showed clear indications of frequent inundation. The plant community at the site was dominated by *S. fragilis*, *A. glutinosa*, *Lupinus arboreus* and *Cytisus scoparius*, with scattered individuals of *Ulex europeaus*, *Populus nigra* and *Populus alba*. No indigenous woody plant species were

observed. We collected samples at arbitrary intervals along the river, sampling from east to west for roughly 600 m and sampling evenly in the outer river bed and the inner channel in order to capture any variation in species composition along the water inundation gradient. Samples were collected alternately from beneath *A. glutinosa* and *S. fragilis* individuals, all of which were separated by at least 5 m (total 40 South Island samples) in an effort to limit spatial autocorrelation among samples (Lilleskov *et al.*, 2004). Soil samples were located less than a metre from the main trunk of the target tree and were all roughly 15 cm wide, long and deep, excavated with a narrow-blade shovel.

The North Island site was located along the Waikato River in Hamilton, NZ (37°45′24.66″S, 175°15′50.75″E). The sampling area consisted of a narrow strip (5–10 m width) of vegetation beside the river, which was dominated by *A. glutinosa* and *S. fragilis*. Other vegetation present included *Ligustrum sinense*, *Solanum pseudocapsicum*, *Coprosma robusta* (indigenous), *Melicytus ramiflorus* (indigenous) and *Populus nigra*. We employed the same sampling scheme as on the South Island along 500 m of the riverbank (total 40 North Island samples). Neither of the study sites has recently supported any native EM host vegetation.

Soil samples were refrigerated for up to 10 days between collection and processing. All roots collected were washed gently under cold running water and viewed under a dissecting microscope. For each soil sample, the first non-senescent root tip with a visually apparent fungal mantle on a randomly selected root fragment was collected, and the root fragment discarded; this was repeated until 8 EM root tips were collected. In samples that did not contain eight distinct, EM root fragments, we resampled the available EM root fragments at random to obtain 8 EM tips. EM status of root tips was confirmed using a compound microscope to check for mantle development. Samples containing no healthy EM root tips were discarded (final $N = 38$ samples South Island, $N = 39$ samples North Island).

Molecular analyses

We extracted total root tip DNA from each EM root tip collected using the Sigma-Aldrich REDExtract-N-Amp kit (Sigma-Aldrich, St. Louis, MO, USA) as described in Kennedy *et al.* (2012) after Avis *et al.* (2003). Polymerase chain reactions (PCR) were performed and optimized as in Bogar & Kennedy (2013). Briefly, we used ITS1F and ITS4 primers to amplify the EM fungal DNA (Gardes & Bruns, 1993), with multiple-banded reactions further assessed using reverse primers ITS4A and ITS4B in two separate reactions (Gardes & Bruns, 1993; Larena *et al.*, 1999). All single-banded PCR products were cleaned using EXOSAP-IT (Affymetrix, Santa Clara, CA) and sent to the University of Arizona Genetics Sample for Sanger sequencing in the forward direction with ITS1F.

To identify the host plant associated with a given EM root tip, we used the *trn*C and *trn*D primer pair developed by Taberlet *et al.* (1991) to amplify the chloroplast *trn*L region. We found that incubating the *trn*C–*trn*D PCR product with enzyme RsaI produced restriction patterns that allowed us to reliably distinguish between *A. glutinosa*, *S. fragilis* and other common plants at both sites when visualized on an agarose gel (see Fig. S1 for an example of *A. glutinosa* and *S. fragilis* restriction patterns). The reliability of this assay was confirmed using RsaI to digest *trn*C–*trn*D amplicons from positively identified leaf samples from all observed plants at each site.

EM fungal taxon designation

Sequence chromatograms were processed using SEQUENCHER v4.10 (Gene Codes, Ann Arbor, Michigan, USA). To facilitate accurate identification of EM fungal taxa, we excluded reads of 450 or fewer base pairs from the data set. Sequences were assembled at ≥97% sequence similarity. We identified EM fungal taxa as in Walker *et al.* (2014), naming them to species when they matched a database entry with at least 97% identity over 450 bp or more. All of the best database matches to our taxa were collected in Europe (the native range of the two plant hosts) or North America; none came from New Zealand or nearby (see Table S1 in Supporting Information). Although operational taxonomic units based upon 97% identical ITS sequences do not correspond to biological species in all fungal groups (Nilsson *et al.*, 2008), they are a proxy used broadly in ecological studies, particularly of ectomycorrhizal fungi (Peay *et al.*, 2008; Kennedy *et al.*, 2012; Tedersoo *et al.*, 2012). As such, the taxon richness encountered here should be comparable to the findings of other sequence-based studies of environmental ectomycorrhizas, but caution should be used when interpreting these findings in the context of any particular species. Representative sequences for each taxon are available in NCBI's GenBank (accession numbers KM522805–KM522830).

Statistical analyses

We calculated EM fungal taxon accumulation curves and estimated minimum richness in ESTIMATES (Colwell, 2013), using the abundance-based bias-corrected Chao 1 estimator. Differences in EM fungal community structure between hosts and sites were assessed using weighted UniFrac distances in the phyloseq package in R (McMurdie & Holmes, 2013). UniFrac distances account for both compositional differences and phylogenetic distances between communities, unlike traditional dissimilarity matrices such as Bray–Curtis, which only address the former. We visualized differences in community structure by running Non-metric Multidimensional Scaling (NMDS) analyses in the vegan package in R (metaMDS function) (Oksanen *et al.*, 2013). To test the influence of host plant and site on fungal community structure, we used a permutational multivariate analysis of variance (PERMANOVA; adonis function). We also examined differences in sample-to-sample heterogeneity (dispersion) between the

EM communities associated with each host and site by performing a permutational dispersion analysis using the betadisper function. This allowed us to determine whether differences detected in the PERMANOVA were due to differences in community composition (i.e. centroids in multivariate space), or to differences in the sample-to-sample heterogeneity associated with each host and site (i.e. multivariate dispersion within groups) (Azeria *et al.*, 2011). Each of the four possible combinations of host and site was used as a group for the dispersion analysis (*A. glutinosa* on the North Island, *A. glutinosa* on the South Island, *S. fragilis* on the North Island and *S. fragilis* on the South Island), with statistical differences across host and sites determined using a permutation test (permutest function, vegan package) and an analysis of variance (ANOVA; anova function), with the Tukey 'Honest Significant Difference' test (TukeyHSD function) to assess the significance of pairwise differences in dispersion.

To assess patterns of host specificity, we used the CLAssification Method program (CLAM) implemented in R (Chazdon *et al.*, 2011). This program uses a multinomial statistical approach to classify taxa as specialists or generalists based on relative abundance data in binary habitats, which in our study was the two host species sampled. The algorithm assesses whether the relative abundance of a species is significantly greater in one habitat or the other by performing a one-sided statistical test, with the significance threshold adjusted for multiple comparisons. Although this method cannot distinguish between specialization on a host and specialization on other factors that covary with host (Zobel & Öpik, 2014), our sampling scheme minimizes environmental covariance between the two hosts of interest and should allow detection of host preference in EM fungi. One advantage of this method over others is that it has greater power to assign habitat designations to lower abundance taxa (Chazdon *et al.*, 2011). As EM fungal communities are typically dominated by taxa with low abundance (Horton & Bruns, 2001), this method was attractive to more accurately determine patterns of specificity. In addition, for the handful of multihost EM fungal taxa observed (see results below), this classification method allowed us to statistically assess whether they should be considered host specialists or generalists. Importantly, however, this method only allows for inference within the data set used and cannot be used to infer specialization in other locations.

Assessing fungal geographic origins

To determine whether the EM fungal partners of these invasive plants are indigenous to New Zealand, invading from Europe or indigenous to both New Zealand and Europe (i.e. cosmopolitan, following Dickie *et al.*, 2010), we explored two sources of information. First, we used the NZfungi database maintained by New Zealand's Landcare Research program (nzfungi2.landcareresearch.co.nz, accessed 29 July 2014) to determine the status of each fungal species in New Zealand (exotic, indigenous, endemic or not in the database). Second, we noted the provenance of the best NCBI or UNITE match for each taxon's ITS sequence. When both NZfungi and NCBI or UNITE suggested that a given species was exotic to New Zealand, we accepted that label. Fungi identified at the genus level or above were not classified, with the exception of *Alnicola* (syn. *Naucoria:* Moreau *et al.*, 2006) and *Otidea* species. We know of no well-supported records of indigenous *Alnicola* in New Zealand (Segedin & Pennycook, 2001), and no species in the genus *Otidea* is listed as indigenous to New Zealand on NZfungi. Accordingly, we considered both genera to be exotic to New Zealand.

Phylogenetic analyses

To further investigate whether *A. glutinosa* and *S. fragilis* were co-invading with EM fungi from their indigenous ranges, or associating with indigenous New Zealand fungi, we conducted a phylogenetic analysis of the family Thelephoraceae, which was the best represented fungal family in our study. Our alignment included sequences of the ITS1–5.8S–ITS2 region from three sources: (1) a representative of each of the *A. glutinosa* and *S. fragilis*-associated Thelephoraceae species in our data set, (2) a representative sample of Thelephoraceae associated with *Alnus* and *Salix* EM hosts in Europe (Tedersoo *et al.*, 2009; Hrynkiewicz *et al.*, 2012; Roy *et al.*, 2013) and (3) several sequences of Thelephoraceae taxa obtained from indigenous New Zealand EM tree hosts in the Nothofagaceae (i.e. genera *Fuscospora* and *Lophozonia*: Heenan & Smissen, 2013) (Dickie *et al.*, 2010, Johnston & Dickie unpublished, Johnston & Park unpublished, Orlovitch *et al.* unpublished). Details and accession numbers for sequences used in the alignment are available in Table S2.

Sequences were aligned using the MUSCLE algorithm, with sequences trimmed at starts and ends following alignment in MEGA 6.06 (Tamura *et al.*, 2013) to ensure that the same portion of the ITS was included for all taxa. The same program was used to select an appropriate model of evolution for the data set (Kimura 2-parameter with a gamma distribution). The alignment of 76 taxa and 590 characters was then analysed under the selected model in MRBAYES 3.2 using default parameters for 1,450,000 generations (until the standard deviation of split frequencies dropped below 0.01) (Ronquist *et al.*, 2012) and in PHYML 3.0 using the aLRT SH-like fast likelihood-based optimization method (Guindon *et al.*, 2010). For the Bayesian analysis, chain convergence was assessed in TRACER 1.6 (Rambaut *et al.*, 2014) and the first 10% of generations were discarded as burn-in.

We also constructed a phylogenetic tree from the EM fungal LSU sequences associated with *A. glutinosa* and *S. fragilis* in New Zealand for use in the UniFrac analysis. For this analysis, the LSU sequences were aligned, trimmed and tested for an appropriate model as described above. The alignment was the analysed by maximum likelihood using the selected

model (Tamura-Nei) in MEGA 5.2.2 under default parameters (Tamura *et al.*, 2011).

RESULTS

Molecular plant identification of EM root tips revealed that many soil samples contained root tips from both of our target host species, *A. glutinosa* and *S. fragilis,* despite our individual host sampling strategy. Because of the intentional host bias in our sampling strategy, we excluded non-target-host EM root tips from all final analyses (i.e. only molecularly confirmed *S. fragilis* EM tips were included in samples taken from directly beneath *S. fragilis* trees and vice versa for *A. glutinosa*). Applying these criteria, we retained a total of 294 identified root tips (from the 616 originally collected) across 74 soil samples: 159 from the North Island (91 tips in 20 samples under *A. glutinosa*, 68 tips in 17 samples under *S. fragilis*) and 135 from the South Island (77 tips in 18 samples under *A. glutinosa*, 58 tips in 19 samples under *S. fragilis*).

We detected a total of 26 unique EM fungal taxa, nine associated with *A. glutinosa* (five EM fungal taxa on the South Island and seven on the North Island) and 25 with *S. fragilis* (19 taxa on the South Island, 13 on the North Island) (Fig. 1). Visual inspection of taxon accumulation curves for EM fungal communities associated with each host and site indicated that we did not sample any of the four communities to saturation; this was especially true of the *S. fragilis*-associated EM fungal communities (Fig. S2). Estimated EM fungal taxon richness for *A. glutinosa* ranged from 6 ± 2 (Chao 1 mean $\pm$ standard deviation, South Island) to 8 ± 3 (North Island), while *S. fragilis* was estimated to associate with 33 ± 13 EM fungal taxa on the South Island and 14 ± 1 EM fungal taxa on the North Island.

The EM fungal community associated with *A. glutinosa* at both sites was dominated by *Alnicola escharoides*, *Tomentella testaceogilva* and *Thelephora alnii*, while the EM fungal community associated with *S. fragilis* was more even, and the dominant taxon varied between the North and South Islands (Fig. 1). There was overlap in the taxa present on both hosts (8 of the 25 taxa), but the abundance of the shared EM fungal taxa was highly asymmetrical on the two hosts. The CLAM analysis showed that many of these shared EM fungal taxa significantly preferred one EM host species host over the other – *Thelephora alnii*, *Tomentella testaceogilva* and *Alnicola escharoides* specialized on *A. glutinosa*, while *Clavulina cinerea*, Pezizales sp. 1 and Pezizaceae sp. 1 specialized on *S. fragilis.* Most of the EM fungal taxa, however, were not adequately abundant to determine host specialization class (Fig. 1).

Host species, site and their interaction were all significant predictors of EM fungal community structure (PERMANOVA, host: $F_1 = 22.72$, $R^2 = 0.220$, $P < 0.001$, site: $F_1 = 5.73$, $R^2 = 0.056$, $P < 0.01$, host:site: $F_1 = 4.79$, $R^2 = 0.046$, $P < 0.05$). In addition, EM fungal sample-to-sample heterogeneity (dispersion) differed significantly between the host/site pairings ($P < 0.001$ in both the permutation test and ANOVA). Tukey HSD tests showed that EM fungal dispersion was significantly lower in *A. glutinosa*-associated EM fungal communities than in those associated with *S. fragilis*, but that dispersion did not differ significantly between islands. These findings were consistent with the NMDS results, which showed tight clustering of *Alnus*-associated EM fungal samples, regardless of geography, while *Salix*-associated samples were more loosely clustered and strongly influenced by sampling site (Fig. 2).

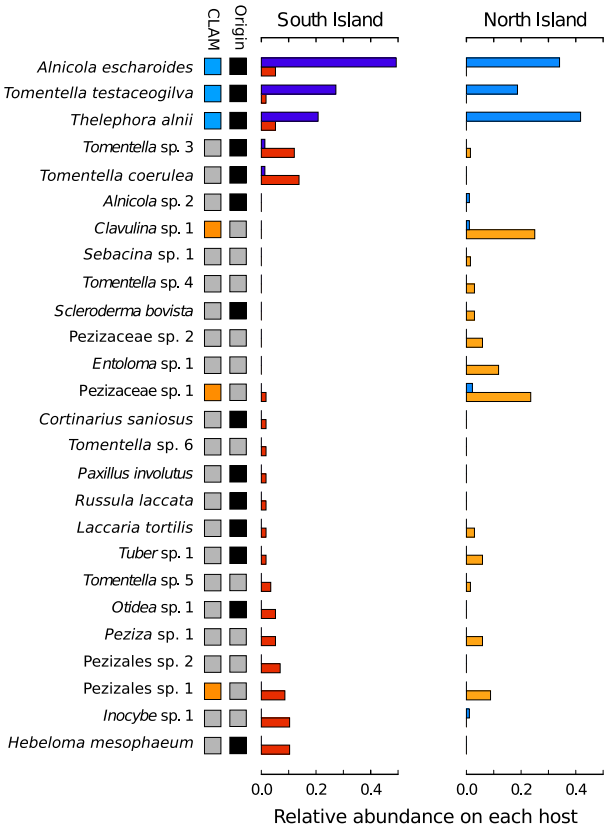

Figure 1 Relative abundances of ectomycorrhizal (EM) fungi associated with *Alnus glutinosa* (L.) Gaertner and *Salix fragilis* L. at two sites in New Zealand. Warm colours (yellow, red) indicate the relative abundances of EM fungal taxa on *S. fragilis* roots, while cool colours (blue, purple) indicate their relative abundances on *A. glutinosa* roots. Coloured boxes in the 'CLAM' column indicate the host preference category assigned to each taxon by a Classification Method analysis. Colours denote the same host affinities as in the bar chart, while grey boxes indicate fungal taxa that were insufficiently abundant to classify. The boxes in the 'Origin' column indicate the likely origin of each fungal taxon: black boxes indicate exotic fungi (typically indigenous to Europe) and grey boxes denote fungi that we were unable to classify. No fungi in this data set were found to be indigenous to New Zealand.

Although only 12 of 26 EM fungal taxa in this data set were identifiable to a species level, all 12 were exotic taxa (Table S1), with no EM fungus in the data set known to be endemic to New Zealand. Furthermore, no EM fungus in the data set returned a best match to a New Zealand ITS

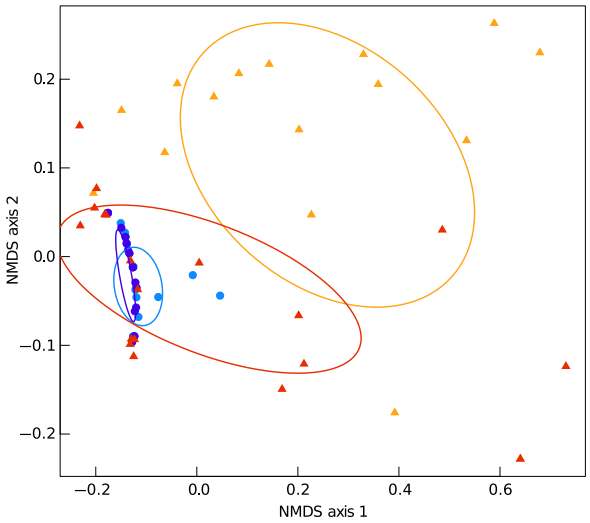

Figure 2 Non-metric Multidimensional Scaling (NMDS) analysis of UniFrac distances among the sampled ectomycorrhizal (EM) fungal communities. *Alnus glutinosa*-associated EM fungal communities from both the North and South Islands of New Zealand cluster tightly together, while the *Salix fragilis*-associated communities are more broadly dispersed and segregated by island. Triangles indicate *Salix*-associated samples, while circles indicate those associated with *Alnus*. Yellow represents *S. fragilis* on the North Island, red is *S. fragilis* on the South Island, light blue is *A. glutinosa* on the North Island, and violet is *A. glutinosa* on the South Island.

sequence; all of the best database results were from Europe and North America. The Bayesian and maximum likelihood (ML) trees constructed from Thelephoraceae ITS sequences had mostly concordant and well-supported topologies among their more recent divergences, although both trees were poorly resolved at deeper nodes (Figs. 3 and S3). Both topologies revealed *A. glutinosa* and *S. fragilis*-associated New Zealand Thelephoraceae taxa that clustered tightly with European taxa. Although the inclusion of many European taxa in the alignment means that New Zealand-derived sequences could be sister to European taxa by chance, several New Zealand taxa were separated by very short branch lengths from European ones, suggesting a European origin for these New Zealand collections. Specifically, the phylogeny allowed us to identify two additional taxa from this study as probable European invaders to New Zealand, bringing the number of exotic taxa in our data set to 14. The indigenous Nothofagaceae-associated New Zealand Thelephoraceae, in contrast to the *A. glutinosa* and *S. fragilis*-associated taxa from this study, generally formed well-supported monophyletic clades that contained no European representatives.

DISCUSSION

This study provides the first description of EM fungal associates of either *Salix* or *Alnus* as invasive species, and only the second study on EM fungal communities on any invasive non-Pinaceae (Díez, 2005; Nuñez & Dickie, 2014). It has been previously shown that *A. glutinosa* associates with a much more specific EM fungal community than *S. fragilis* in the indigenous European range of both plants (Tedersoo *et al.*, 2009, 2013; Roy *et al.*, 2013). Despite this, our results show that both *A. glutinosa and S. fragilis* associate with many exotic EM fungi in New Zealand, strongly supporting the co-invasion hypothesis (Dickie *et al.*, 2010; Nuñez & Dickie, 2014). Contrary to earlier hypotheses (Pringle *et al.*, 2009), this suggests that host specificity may not represent a significant barrier to invasion by EM plants.

The absence of novel EM associations encountered in this study matches closely to the findings of several other studies that have examined the EM fungal communities associated with invasive EM plants: No novel associations were observed on *Pinus* spp. in New Zealand (Dickie *et al.*, 2010), the Seychelles (Tedersoo *et al.*, 2007), or the Hawaiian Islands (Hynson *et al.*, 2013), or on *Eucalyptus camaldulensis* on the Iberian peninsula (Díez, 2005). The frequency of co-invasion across studies suggests that the success of EM invasions is not contingent on formation of novel EM associations (Nuñez & Dickie, 2014). It remains possible, however, that novel EM associations could support exotic plants in other settings. For example, novel associations occur on non-indigenous *Pinus* plantations in Iran where planted in proximity to indigenous Fagaceae (Bahram *et al.*, 2013) and on planted *Eucalyptus* in the Seychelles where trees were introduced as seed without any fungal inoculum (Tedersoo *et al.*, 2007), although neither study examined invasive trees. Previous studies have found that the presence of neighbouring EM hosts can influence fungal species composition on a target EM host (Kennedy *et al.*, 2012; Bahram *et al.*, 2013), and also that spore-based fungal host specificity is relaxed when EM fungi are growing as hyphae (Molina *et al.*, 1997; Bogar & Kennedy, 2013). We believe this latter phenomenon may explain the presence of some overlap in the *A. glutinosa* and *S. fragilis* EM communities in our study, which do not appear to share EM fungal associates in their indigenous ranges (Roy *et al.*, 2013; Tedersoo *et al.*, 2013).

Alnus glutinosa and *S. fragilis* in New Zealand associate with exotic EM fungi that are either identical or very similar to those found in their European ranges. The patterns of community structure on both hosts also largely match those from other geographic locations. Specifically, the low alpha and beta diversity seen in the *Alnus* EM fungal communities is consistent with all other studies of *Alnus* EM to date (Põlme *et al.*, 2013 and references therein), indicating that, even outside their indigenous range, the structure of these communities remains atypical compared to other EM plants. The greater taxon richness and variation in the *Salix* EM fungal communities as well as the dominance by members of the Thelephoraceae and Pezizaceae is also similar to previous European-based studies (Ryberg *et al.*, 2011; Hrynkiewicz *et al.*, 2012; Tedersoo & Smith, 2013). While our sample-taxon accumulation estimates indicate that we were unable

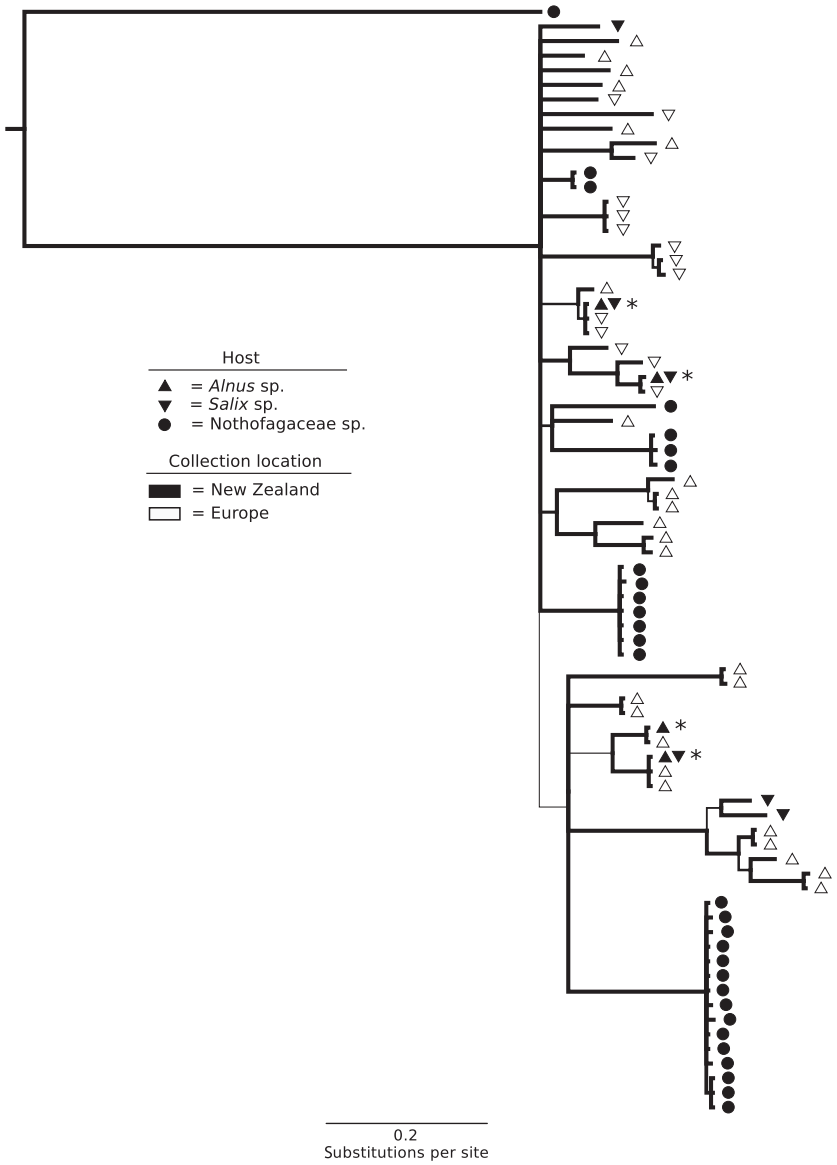

Figure 3 Phylogenetic tree of *Alnus*- and *Salix*-associated ectomycorrhizal (EM) fungi belonging to the family Thelephoraceae in New Zealand, where the hosts are invasive, and Europe, the indigenous range of the plants. This topology was generated by applying Bayesian methods to an alignment of fungal internal transcribed spacer region (ITS) sequences. Thelephoraceae associated with native New Zealand tree hosts in the Nothofagaceae were included as representative indigenous New Zealand fungal taxa. Symbols at the tips of the tree indicate the host plant and collection location associated with each fungal taxon. Branch widths indicate branch support, with thicker branches having higher posterior probabilities (all clades have posterior probabilities of at least 0.56). Specific branch support information and an ML topology produced from the same alignment are available in Fig. S3. Asterisks indicate New Zealand taxa that cluster tightly with European taxa, leading us to classify them as exotic to New Zealand. From top to bottom, these taxa are *Tomentella coerulea*, *Tomentella* sp. 3, *Thelephora alnii*, and *Tomentella testaceogilva*.

to capture all of the EM fungi associated with these hosts on either island, given the consistency of the community patterns at large geographic scales, we believe the general results observed are not likely to be significantly altered with additional sampling.

Despite an overall similarity to the European range fungal communities and our observations from New Zealand, we found one key difference, particularly in the South Island data. These two plant species are not known to associate with similar EM fungi in their native ranges (Tedersoo *et al.*, 2009, 2013). On the South Island, however, we found that all fungal associates of *A. glutinosa* were also present on *S. fragilis*. *Alnicola escharoides* has previously been considered as strictly host specific to *Alnus* (Moreau *et al.*, 2006). *Alnus*-associated EM fungi may relax their host preferences when the *Alnus* root system overlaps with that of other hosts (Bogar & Kennedy, 2013), which could explain the appearance of *Alnus*-associated EM fungi on *S. fragilis* in these mixed host sites. It has also been suggested, however, that fungi may dramatically increase their host breadth when introduced into a novel habitat (Wolfe & Pringle, 2012). The apparent host shift of *Alnicola escharoides* in New Zealand may represent an example of this phenomenon. Alternatively, the use of direct sequencing from roots may result in a broader view of host preference than traditional sporocarp surveys. Regardless of the mechanism, shared symbionts could create the potential for *Salix*, which is already widespread both in New Zealand and in many other countries, to facilitate the more recent and expanding invasion of *Alnus* by providing compatible fungal symbionts.

Despite a data set with limited taxon coverage and poor resolution at deeper nodes, our phylogenetic analysis of *Alnus*, *Salix* and Nothofagaceae-associated Thelephoraceae revealed that the taxa associated with *Alnus* and *Salix* in New Zealand are closely related to fungi collected in Europe and

do not cluster with the indigenous Nothofagaceae-associated taxa. Although there are indigenous EM fungi in the Thelephoraceae in New Zealand (Cunningham, 1963), it appears the *A. glutinosa*- and *S. fragilis*-associated taxa may be exotic. This seems especially likely to be true of the *A. glutinosa*-associated dominant taxa in our analysis (*Tomentella testaceogilva*, *Thelephora alnii*), as *Alnus* is known for discriminating among EM fungal associates at the species level (Moreau *et al.*, 2006; Rochet *et al.*, 2011; Roy *et al.*, 2013). A key next step to validate these phylogenetic results will be the inclusion of additional Thelephoraceae samples from other indigenous New Zealand EM hosts, specifically *Leptospermum*, *Kunzea* and *Pomaderris* spp. In addition, population genetics studies of targeted *Alnus* and *Salix*-associated EM taxa using microsatellite markers would provide greater insight into possible gene flow between New Zealand and European populations (e.g. Vincenot *et al.*, 2012).

Overall, our data suggest that both *A. glutinosa* and *S. fragilis* are associating with exotic EM fungi from their indigenous ranges as they invade New Zealand. This is consistent with other studies of invasive EM plants (Díez, 2005; Nuñez *et al.*, 2009; Dickie *et al.*, 2010; Hynson *et al.*, 2013) and suggests the co-invasion pattern is robust. With regard to conservation, our findings imply that specificity to a particular set of mutualists may not present a significant barrier to invasion, in contrast to earlier hypotheses (Pringle *et al.*, 2009). This suggests that conservation resources must be applied to potential invasives, even where those invasives have highly specific mutualists such as *Alnus*. We believe future research should directly compare the EM fungal communities in the native and invasive ranges of a host plant species, to further clarify the extent to which invasion itself can shift EM fungal community composition. It will also be important to explicitly examine the potential for facilitation among invasive plants with a range of specificities for their mutualists. As entire consortia of non-indigenous species often invade at the same time and in the same place (e.g. harbours, roadsides, river beds etc.), shared EM fungal symbionts may create novel interaction networks of non-native plants and fungi and potentially contribute to invasional meltdown.

ACKNOWLEDGEMENTS

The authors thank N. Fitzgerald, R. Buxton and N. Bolstridge for field and laboratory assistance, and K. Peay and P. Vitousek for thoughtful feedback on the manuscript. The authors also thank T. Fukami and the members of the Stanford University Ecological Statistics class for their help with the statistical methods and data presentation. This work was supported by funding to P.G.K. by NSF grant 1020735 and to I.A.D. by the Bio-Protection Research Center, Lincoln University, and Core funding for Crown Research Institutes to Landcare Research from the New Zealand Ministry of Business, Innovation and Employment's Science and Innovation Group.

REFERENCES

Ashkannejhad, S. & Horton, T.R. (2006) Ectomycorrhizal ecology under primary succession on coastal sand dunes: interactions involving *Pinus contorta*, suilloid fungi and deer. *New Phytologist*, **169**, 345–354.

Avis, P.G., McLaughlin, D.J., Dentinger, B.C. & Reich, P.B. (2003) Long-term increase in nitrogen supply alters above- and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. *New Phytologist*, **160**, 239–253.

Azeria, E.T., Ibarzabal, J., Boucher, J. & Hérbert, C. (2011) Towards a better understanding of beta diversity: Deconstructing composition patterns of saproxylic beetles breeding in recently burnt boreal forests. *Research in biodiversity: models and applications* (ed. by I. Pavlinov), pp. 75–94. InTech, http://www.intechopen.com/books/research-in-biodiversity-models-and-applications/towards-a-better-understanding-of-beta-diversity-deconstructing-composition-patterns-of-saproxylic-b

Bahram, M., Kõljalg, U., Kohout, P., Mirshahvaladi, S. & Tedersoo, L. (2013) Ectomycorrhizal fungi of exotic pine plantations in relation to native host trees in Iran: evidence of host range expansion by local symbionts to distantly related host taxa. *Mycorrhiza*, **23**, 11–19.

Bogar, L.M. & Kennedy, P.G. (2013) New wrinkles in an old paradigm: neighborhood effects can modify the structure and specificity of *Alnus*-associated ectomycorrhizal fungal communities. *FEMS Microbiology Ecology*, **83**, 767–777.

Chazdon, R.L., Chao, A., Colwell, R.K., Lin, S.-Y., Norden, N., Letcher, S.G., Clark, D.B., Finegan, B. & Arroyo, J.P. (2011) A novel statistical method for classifying habitat generalists and specialists. *Ecology*, **92**, 1332–1343.

Collier, F.A. & Bidartondo, M.I. (2009) Waiting for fungi: the ectomycorrhizal invasion of lowland heathlands. *Journal of Ecology*, **97**, 950–963.

Colwell, R.K. (2013) EstimateS: Statistical estimation of species richness and shared species from samples. Version 9, Persistant URL purl.oclc.org/estimates.

Cunningham, G.H. (1963) The Thelephoraceae of Australia and New Zealand. *New Zealand Department of Scientific and Industrial Research Bulletin*, **145**, 1–357.

Davis, M.A. (2009) *Invasion biology*. Oxford University Press, New York.

Dickie, I.A. & Moyerson, B. (2008) Towards a global view of ectomycorrhizal ecology. *New Phytologist*, **180**, 263–265.

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

Díez, J. (2005) Invasion biology of Australian ectomycorrhizal fungi introduced with eucalypt plantations into the Iberian Peninsula. *Biological Invasions*, **7**, 3–15.

Diez, J.M., Dickie, I.A., Edwards, G., Hulme, P.E., Sullivan, J.J. & Duncan, R.P. (2010) Negative soil feedbacks

accumulate over time for non-indigenous plant species. *Ecology Letters*, **13**, 803–809.

Gardes, M. & Bruns, T.D. (1993) ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. *Molecular Ecology*, **2**, 113–118.

Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, **59**, 307–321.

Heenan, P.B. & Smissen, R.D. (2013) Revised circumscription of *Nothofagus* and recognition of the segregate genera *Fuscospora*, *Lophozonia*, and *Trisyngyne* (Nothofagaceae). *Phytotaxa*, **146**, 1–31.

Horton, T.R. & Bruns, T.D. (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology*, **10**, 1855–1871.

Hrynkiewicz, K., Toljander, Y.K., Baum, C., Fransson, P.M., Taylor, A.F. & Weih, M. (2012) Correspondence of ectomycorrhizal diversity and colonisation of willows (*Salix* spp.) grown in short rotation coppice on arable sites and adjacent natural stands. *Mycorrhiza*, **22**, 603–613.

Hynson, N.A., Merckx, V.S., Perry, B.A. & Treseder, K.K. (2013) Identities and distributions of the co-invading ectomycorrhizal fungal symbionts of exotic pines in the Hawaiian Islands. *Biological Invasions*, **15**, 2373–2385.

Kennedy, P.G., Smith, D.P., Horton, T.R. & Molina, R.J. (2012) *Arbutus menziesii* (Ericaceae) facilitates regeneration dynamics in mixed evergreen forests by promoting mycorrhizal fungal diversity and host connectivity. *American Journal of Botany*, **99**, 1–11.

Lafay, B. & Burdon, J.J. (2006) Molecular diversity of rhizobia nodulating the invasive legume *Cytisus scoparius* in Australia. *Journal of Applied Microbiology*, **100**, 1228–1238.

Larena, I., Salazar, O., González, V., Julián, M.C. & Rubio, V. (1999) Design of a primer for ribosomal DNA internal transcribed spacer with enhanced specificity for ascomycetes. *Journal of Biotechnology*, **75**, 187–194.

Lilleskov, E.A., Bruns, T.D., Horton, T.R., Taylor, D. & Grogan, P. (2004) Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. *FEMS Microbiology Ecology*, **49**, 319–332.

McMurdie, P.J. & Holmes, S. (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE*, **8**, e61217.

Mitchell, C.E., Agrawal, A.A., Bever, J.D., Gilbert, G.S., Hufbauer, R.A., Klironomos, J.N., Maron, J.L., Morris, W.F., Parker, I.M., Power, A.G., Seabloom, E.W., Torchin, M.E. & Vázquez, D.P. (2006) Biotic interactions and plant invasions. *Ecology Letters*, **9**, 726–740.

Molina, R.J. (1981) Ectomycorrhizal specificity in the genus *Alnus*. *Canadian Journal of Botany*, **59**, 325–334.

Molina, R.J., Massicotte, H.B. & Trappe, J.M. (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical applications. *Mycorrhizal functioning, an integrative plant-fungal process* (ed. by M. Allen), pp. 357–423. Chapman and Hall, New York.

Molina, R.J., Smith, J.E., McKay, D. & Melville, L.H. (1997) Biology of the ectomycorrhizal genus, Rhizopogon. III. Influence of co-cultured conifer species on mycorrhizal speciflcity with the arbutoid hosts Arctostaphylos uva-ursi and *Arbutus menziesii*. *New Phytologist*, **137**, 519–528.

Moora, M., Berger, S., Davison, J., Öpik, M., Bommarco, R., Bruelheide, H., Kühn, I., Kunin, W.E., Metsis, M., Rortais, A., Vanatoa, A., Vanatoa, E., Stout, J.C., Truusa, M., Westphal, C., Zobel, M. & Walther, G.-R. (2011) Alien plants associate with widespread generalist arbuscular mycorrhizal fungal taxa: evidence from a continental-scale study using massively parallel 454 sequencing. *Journal of Biogeography*, **38**, 1305–1317.

Moreau, P.-A., Peintner, U. & Gardes, M. (2006) Phylogeny of the ectomycorrhizal mushroom genus *Alnicola* (Basidiomycota, Cortinariaceae) based on rDNA sequences with special emphasis on host specificity and morphological characters. *Molecular Phylogenetics and Evolution*, **38**, 794–807.

Nara, K. (2006) Pioneer dwarf willow may facilitate tree succession by providing late colonizers with compatible ectomycorrhizal fungi in a primary successional volcanic desert. *New Phytologist*, **171**, 187–197.

Nara, K. & Hogetsu, T. (2004) Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. *Ecology*, **85**, 1700–1707.

Nilsson, R., Kristiansson, E., Ryberg, M., Hallenberg, N. & Larsson, K.-H. (2008) Intraspecific ITS variability in the kingdom fungi as expressed in the International Sequence Databases and Its Implications for Molecular Species Identification. *Evolutionary Bioinformatics*, **4**, 193–201.

Nuñez, M.A. & Dickie, I.A. (2014) Invasive belowground mutualists of woody plants. *Biological Invasions*, **16**, 645–661.

Nuñez, M.A., Horton, T.R. & Simberloff, D. (2009) Lack of belowground mutualisms hinders Pinaceae invasions. *Ecology*, **90**, 2352–2359.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2013) *vegan: Community ecology package*. R package version 2.0-10, http://CRAN.R-project.org/package=vegan

Parker, M.A., Wurtz, A.K. & Paynter, Q. (2006) Nodule symbiosis of invasive *Mimosa pigra* in Australia and in ancestral habitats: a comparative analysis. *Biological Invasions*, **9**, 127–138.

Peay, K.G., Kennedy, P.G. & Bruns, T.D. (2008) Fungal community ecology: a hybrid beast with a molecular master. *BioScience*, **58**, 799.

Põlme, S., Bahram, M., Yamanaka, T., Nara, K., Dai, Y.C., Grebenc, T., Kraigher, H., Toivonen, M., Wang, P.-H., Matsuda, Y., Naadel, T., Kennedy, P.G., Kõljalg, U. & Tedersoo, L. (2013) Biogeography of ectomycorrhizal fungi

associated with alders (*Alnus* spp.) in relation to biotic and abiotic variables at the global scale. *New Phytologist*, **198**, 1239–1249.

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

Rambaut, A., Suchard, M.A., Xie, D. & Drummond, A.J. (2014) *Tracer v1.6*. http://beast.bio.ed.ac.uk/Tracer.

Ramirez, B.W. & Montero, J.S. (1988) *Ficus microcarpa* L., *F. benjamina* L. and other species introduced in the New World, their pollinators (Agaonidae) and other fig wasps. *Revista de Biología Tropical*, **36**, 441–446.

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

Ricciardi, A., Hoopes, M., Marchetti, M. & Lockwood, J. (2013) Progress toward understanding the ecological impacts of nonnative species. *Ecological Monographs*, **83**, 263–282.

Richardson, D.M., Allsopp, N., D'Antonio, C.M., Milton, S.J. & Rejmánek, M. (2000) Plant invasions–the role of mutualisms. *Biological Reviews of the Cambridge Philosophical Society*, **75**, 65–93.

Rochet, J., Moreau, P.-A., Manzi, S. & Gardes, M. (2011) Comparative phylogenies and host specialization in the alder ectomycorrhizal fungi *Alnicola*, *Alpova* and *Lactarius* (Basidiomycota) in Europe. *BMC Evolutionary Biology*, **11**, 40.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.

Roy, M., Rochet, J., Manzi, S., Jargeat, P., Gryta, H., Moreau, P.-A. & Gardes, M. (2013) What determines *Alnus*-associated ectomycorrhizal community diversity and specificity? A comparison of host and habitat effects at a regional scale. *New Phytologist*, **198**, 1228–1238.

Rundel, P.W., Dickie, I.A. & Richardson, D.M. (2014) Tree invasions into treeless areas: mechanisms and ecosystem processes. *Biological Invasions*, **16**, 663–675.

Ryberg, M., Andreasen, M. & Björk, R.G. (2011) Weak habitat specificity in ectomycorrhizal communities associated with *Salix herbacea* and *Salix polaris* in alpine tundra. *Mycorrhiza*, **21**, 289–296.

Salgado Salomón, M.E., Barroetaveña, C. & Rajchenberg, M. (2011) Do pine plantations provide mycorrhizal inocula for seedlings establishment in grasslands from Patagonia, Argentina? *New Forests*, **41**, 191–205.

Schwartz, M.W., Hoeksema, J.D., Gehring, C.A., Johnson, N.C., Klironomos, J.N., Abbott, L.K. & Pringle, A. (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology Letters*, **9**, 501–515.

Segedin, B.P. & Pennycook, S.R. (2001) A nomenclatural checklist of agarics, boletes, and related secotioid and gasteromycetous fungi recorded from New Zealand. *New Zealand Journal of Botany*, **39**, 285–348.

Shea, K. & Chesson, P. (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology and Evolution*, **17**, 170–176.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, **17**, 1105–1109.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30**, 2725–2729.

Tedersoo, L. & Smith, M.E. (2013) Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews*, **27**, 83–99.

Tedersoo, L., Suvi, T., Beaver, K. & Kõljalg, U. (2007) Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpiniaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). *New Phytologist*, **175**, 321–333.

Tedersoo, L., Suvi, T., Jairus, T., Ostonen, I. & Põlme, S. (2009) Revisiting ectomycorrhizal fungi of the genus *Alnus*: differential host specificity, diversity and determinants of the fungal community. *New Phytologist*, **182**, 727–735.

Tedersoo, L., May, T.W. & Smith, M.E. (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*, **20**, 217–263.

Tedersoo, L., Bahram, M., Toots, M., Diédhiou, A.G., Henkel, T.W., Kjøller, R., Morris, M.H., Nara, K., Nouhra, E., Peay, K.G., Põlme, S., Ryberg, M., Smith, M.E. & Kõljalg, U. (2012) Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology*, **21**, 4160–4170.

Tedersoo, L., Mett, M., Ishida, T.A. & Bahram, M. (2013) Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. *New Phytologist*, **199**, 822–831.

Terwilliger, J. & Pastor, J. (1999) Small mammals, ectomycorrhizae, and conifer succession in beaver meadows. *Oikos*, **85**, 83–94.

Thiet, R.K. & Boerner, R.E.J. (2007) Spatial patterns of ectomycorrhizal fungal inoculum in arbuscular mycorrhizal barrens communities: implications for controlling invasion by *Pinus virginiana*. *Mycorrhiza*, **17**, 507–517.

Vincenot, L., Nara, K., Sthultz, C., Labbé, J., Dubois, M.-P., Tedersoo, L., Martin, F. & Selosse, M.-A. (2012) Extensive

gene flow over Europe and possible speciation over Eurasia in the ectomycorrhizal basidiomycete *Laccaria amethystina* complex. *Molecular Ecology*, **21**, 281–299.

Walker, J.K.M., Cohen, H., Higgins, L.M. & Kennedy, P.G. (2014) Testing the link between community structure and function for ectomycorrhizal fungi involved in a global tripartite symbiosis. *New Phytologist*, **202**, 287–296.

Webb, C.J., Sykes, W.R. & Garnock-Jones, P.J. (1988) Naturalised Pteridophytes, Gymnosperms, Dicotyledons. Botany Division, Department of Scientific and Industrial Research.

Wolfe, B.E. & Pringle, A. (2012) Geographically structured host specificity is caused by the range expansions and host shifts of a symbiotic fungus. *The ISME journal*, **6**, 745–755.

Zobel, M. & Öpik, M. (2014) Plant and arbuscular mycorrhizal fungal (AMF) communities – which drives which? *Journal of Vegetation Science*, **25**, 1133–1140.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Restriction patterns produced by RsaI digestion of the *trnC-trnD* region amplified from *Alnus glutinosa* and *Salix fragilis* in New Zealand.

Figure S2 Taxon accumulation curves for the host plants and sites sampled in this study.

Figure S3 Bayesian and Maximum Likelihood phylogenetic topologies relating *Alnus* and *Salix*-associated Thelephoraceae from New Zealand and Europe, with numerical branch support and specific terminal taxon identities.

Table S1 Taxon designation and fungal origins information for fungal taxa in this data set.

Table S2 Details and accession numbers for sequences used in phylogenetic analysis of *Alnus* and *Salix*-associated Thelephoraceae from New Zealand and Europe.

BIOSKETCHES

Laura M. Bogar is interested in both the mechanisms and the outcomes of mycorrhizal interactions. Her doctoral studies are focused on symbiont partner choice, host specificity, and the importance of these processes on community structure and ecosystem function.

Ian A. Dickie is an ecosystem ecologist with a strong interest in how fungi interact with plants and soils, particularly in the context of changing ecosystems. He recently moved from Landcare Research to take up the role of Professor of Invasion Ecology at the Bio-Protection Research Centre in New Zealand.

Peter G. Kennedy is a community ecologist broadly interested in plant-microbe interactions. He has worked primarily on biotic and abiotic factors controlling ectomycorrhizal fungal community structure at a range of spatial scales.

Editor: David M. Richardson