100 YEARS Journal of Ecology

British Ecological Society

Journal of Ecology 2015, **103**, 121–129 doi: 10.1111/1365-2745.12345

Novel interactions between non-native mammals and fungi facilitate establishment of invasive pines

Jamie R. Wood[1]*, Ian A. Dickie[1,2], Holly V. Moeller[3], Duane A. Peltzer[1], Karen I. Bonner[1], Gaye Rattray[1] and Janet M. Wilmshurst[1,4]

[1]Landcare Research, PO Box 69040, Lincoln 7640, New Zealand; [2]Bio-Protection Research Centre, Lincoln University, PO Box 85084, Lincoln 7647, New Zealand; [3]Department of Biology, Stanford University, Gilbert Hall, San Francisco, CA 94305-5020, USA; and [4]School of Environment, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

Summary

1. The role of novel ecological interactions between mammals, fungi and plants in invaded ecosystems remains unresolved, but may play a key role in the widespread successful invasion of pines and their ectomycorrhizal fungal associates, even where mammal faunas originate from different continents to trees and fungi as in New Zealand.

2. We examine the role of novel mammal associations in dispersal of ectomycorrhizal fungal inoculum of North American pines (*Pinus contorta*, *Pseudotsuga menziesii*), and native beech trees (*Lophozonia menziesii*) using faecal analyses, video monitoring and a bioassay experiment.

3. Both European red deer (*Cervus elaphus*) and Australian brushtail possum (*Trichosurus vulpecula)* pellets contained spores and DNA from a range of native and non-native ectomycorrhizal fungi.

4. Faecal pellets from both animals resulted in ectomycorrhizal infection of pine seedlings with fungal genera *Rhizopogon* and *Suillus*, but not with native fungi or the invasive fungus *Amanita muscaria*, despite video and DNA evidence of consumption of these fungi.

5. Native *L. menziesii* seedlings never developed any ectomycorrhizal infection from faecal pellet inoculation.

6. *Synthesis.* Our results show that introduced mammals from Australia and Europe facilitate the co-invasion of invasive North American trees and Northern Hemisphere fungi in New Zealand, while we find no evidence that introduced mammals benefit native trees or fungi. This novel tripartite 'invasional meltdown', comprising taxa from three kingdoms and three continents, highlights unforeseen consequences of global biotic homogenization.

Key-words: biological invasions, ectomycorrhiza, invasion ecology, mammals, Nothofagaceae, Pinaceae, species interactions

Introduction

Globalization has resulted in vastly increased rates of species introductions into new geographical regions. The resulting biotic homogenization has led to instances of 'invasional meltdown', where the establishment, invasion or impact of one introduced species is increased by the presence of another (Simberloff & Von Holle 1999). Simberloff (2006) noted that most case studies claiming invasional meltdown failed to show reciprocal benefit between introduced species, but rather reported benefits conferred to one species by the presence of another. However, co-invasion of mutualisms (i.e. where the invasion of mutualistic species overlaps in time and space) is one instance where mutual benefit could be strong and bidirectional. Examples include the co-invasion of ants and scale insects (Green *et al.* 2011) and symbionts in nitrogen-fixing relationships (Ndlovu *et al.* 2013). Indeed, the absence of mutualistic partners can be an important factor either in preventing invasion by some plants (Nuñez, Horton & Simberloff 2009) or in extending the lag phase between a species' introduction and subsequent invasion (Hallett 2006).

Positive interactions amongst non-native invading taxa, including between plants and their microbial associates, broadly supports the idea of invasional meltdown or synergistic interactions amongst invaders (e.g. Pringle *et al.* 2009; Dickie *et al.* 2010; Rodríguez-Echeverría 2010; Green *et al.* 2011; Nuñez *et al.* 2013; Nuñez & Dickie 2014). However,

*Correspondence author: E-mail: woodj@landcareresearch.co.nz

few studies have moved beyond investigations of pairwise partnerships between taxa, or considered the suite of potential interactions amongst non-native taxa that underpin the invasional meltdown hypothesis. Furthermore, few studies have evaluated interactions amongst co-occurring native and non-native taxa, but this is needed to determine the relative importance of invasional meltdown in ecosystems (Simberloff & Von Holle 1999; Simberloff 2006) and to avoid potentially confounding non-native status *per se* with life history or other functional attributes of taxa (e.g. Kurokawa, Peltzer & Wardle 2010; Thompson & Davis 2011). Here, we overcome these potential issues by empirically determining the importance of novel, positive interactions amongst co-occurring non-native and native species across three trophic levels.

Invasive pines (Pinaceae) and ectomycorrhizal (EM) fungi are a major, widespread mutualistic invasion (Dickie *et al.* 2010). Pines are highly depend on mutualistic EM fungi for normal growth; a lack of EM fungi can reduce establishment and naturalization of these trees and limit their spread in some regions (Nuñez, Horton & Simberloff 2009). Conversely, with co-invading EM fungi facilitating the naturalization, establishment and spread of host trees, pines are considered to be some of the most invasive trees in the Southern Hemisphere (Richardson 1998; Proches *et al.* 2012). Invasive pines can form limited associations with native EM fungi, but the vast majority of their associated EM fungi are co-invading taxa (Dickie *et al.* 2010).

Although some pioneer fungi disperse via wind (Peay *et al.* 2012; Dam 2013), it is widely acknowledged that mycophagy by mammals is a key dispersal mechanism (Cazares & Trappe 1994; Ashkannejhad & Horton 2006; Perez *et al.* 2012; Nuñez *et al.* 2013). For example, *Rhizopogon* is a truffle-like, largely hypogeous (i.e. fruits below-ground) fungus and hence does not have wind-dispersed spores, and although *Suillus* is epigeous (i.e. fruits above-ground), it has limited wind dispersal capability (Galante, Horton & Swaney 2011). The dependence of pines on fungi that require mammalian dispersal vectors raises the possibility that the pine – EM fungi invasional meltdown requires a third participant. This is particularly of interest in countries where pines and their associated fungi have been introduced, but the role of mammalian dispersal is filled by non-native mammals.

From this perspective, New Zealand presents an interesting scenario as biological invasions by trees, and invasive mammals are both major conservation issues. Many of the most common invasive trees in New Zealand are of North American origin and include lodgepole pine (*Pinus contorta*) and Douglas-fir (*Pseudotsuga menziesii*) (Ledgard 2001). Their associated EM fungi are also mostly of North American and European origin (Dickie *et al.* 2010). However, the majority of New Zealand's introduced mammal fauna is of European, Asian or Australian origin, with just two geographically restricted mammal species introduced from North America, the wapiti (*Cervus canadensis*) and white-tailed deer (*Odocoileus virginianus*). Therefore, questions remain as to whether novel associations exist in New Zealand between non-native trees, mammals and fungal species whose natural distributions do not overlap, and whether these associations result in invasional meltdown. Another key question regards whether invasive mammals also consume and disperse native fungal communities that have evolved in mammal-depauperate ecosystems.

Prior to initial human settlement of New Zealand approximately 750 years ago (Wilmshurst *et al.* 2008), the terrestrial mammal fauna consisted of just three species of bat, with birds, reptiles and invertebrates dominating the forest fauna (Worthy & Holdaway 2002). The New Zealand flora had evolved for millions of years in the near complete absence of mammals, and a variety of adaptations relating to avian herbivory and dispersal are recognized in native plants (Atkinson & Greenwood 1989; Lee, Wood & Rogers 2010). The same is true of native fungi. While most hypogeous basidiomycetes around the world are dull in colour, New Zealand examples are often brightly coloured; an adaptation that may mimic fallen fruit and therefore attract avian dispersers (Bougher & Lebel 2001; Johnston 2010). The dominant ectomycorrhizal trees in New Zealand, the southern beeches (Nothofagaceae *Fuscospora* and *Lophozonia*, formerly *Nothofagus*; Heenan & Smissen 2013), are strongly limited by a lack of EM fungi distant from trees (Dickie, Davis & Carswell 2012). With the post-settlement decimation of New Zealand's native bird communities and in particular the large terrestrial herbivore guild (Worthy & Holdaway 2002), it remains to be tested whether introduced mammals provide some degree of surrogacy in dispersing native fungi (Johnston 2010).

We investigated novel interactions amongst plants, mycorrhizal fungi and animals (i.e. across three trophic levels, and in this case, kingdoms) to test the generality and importance of the invasional meltdown hypothesis (Simberloff 2006; Gurevtich 2006). More specifically, because plants and their mycorrhizal fungi are now known to frequently co-invade (Pringle *et al.* 2009; Dickie *et al.* 2010; Nuñez & Dickie 2014), most often with animal-dispersed fungal taxa (Nuñez *et al.* 2013), we predict that positive interactions amongst invasive non-native plants, fungi and animals occur. Furthermore, because native plant and mycorrhizal fungal species form distinct and separate assemblages from non-native species, and there is no evolutionary history of mammaliam dispersal of native fungi, we predict that these complex positive interactions do not also occur for native taxa. Our study thus expands on the invasional meltdown hypothesis by testing whether novel positive interactions amongst non-native species occur across trophic groups differently to interactions involving native species. Here, we examine mycophagy and spore dispersal by introduced mammals in New Zealand using a combination of video monitoring, faecal DNA analysis and palynology, and greenhouse bioassay methods. We show that the invasion of invasive North American Pinaceae and EM fungi is facilitated through novel associations with two introduced mammals of Australian (brushtail possum, *Trichosurus vulpecula*) and European (red deer, *Cervus elaphus*) origin but that these mammals are ineffective dispersers of New Zealand's native EM fungi. These complex interactions, although more difficult to demonstrate empirically than interactions within trophic groups, shed new insights into the mechanisms conferring invasion success and ecosystem impacts of invasive non-native species.

Materials and methods

STUDY SITE AND FAECAL SAMPLES

We collected 31 faecal pellet groups from 2 distinct vegetation communities in the Craigieburn Forest Park, South Island, New Zealand, during Autumn 2012 (27 March–19 May). The collected pellets had all been recently deposited (estimated <2 weeks old based on presence of mucous layer, uniform moisture from interior to exterior, texture and colour; Wegner 1992) and were identified to mammal species on the basis of their size and shape (Triggs 2010). Fourteen pellet groups deposited by red deer were collected from an area dominated by native southern beech (*Fuscospora* spp.) forest, with small areas of grassland and exotic plantings (including *Pinus*, *Eucalyptus*, and *Pseudotsuga*) (c. 43°09′00″S, 171°42′52″E). No possum-deposited pellets were found at this site. In contrast 17 pellet groups deposited by brushtail possums were collected from a nearby area (1–1.5 km to the east) dominated by plantations of exotic trees (*Pinus*, *Alnus*, *Betula, Eucalyptus* and *Pseudotsuga*) (c. 43°08′48″S, 171°44′03″E). No deer pellet groups were found at this site. The surface 1–2 mm of each pellet was removed using a scalpel to control for environmental contamination since deposition. For pellet groups consisting of more than one pellet (n = 30; 14 deer and 16 possum), single faecal pellets were selected for DNA, spore count and pollen analyses, and subsamples were then taken from the interior of the scraped pellets (0.23–0.85 g and 0.25–1 mL (equating to 0.09–1.18 g) for DNA, spore count and pollen analyses, respectively).

FAECAL DNA ANALYSES

We extracted DNA from the subsamples, using the MoBio Power Soil DNA Isolation Kit and following the manufacturer's protocol. The fungal ITS region was amplified from the extracts using fusion primers that included the 454 emulsion PCR adapter, 4-bp key sequence, a 10-bp sample-specific identifier (MID), and the primers ITS1-F (forward) and ITS4 (reverse). Two PCRs were performed independently on different Veriti® thermal cyclers (Applied Biosystems, Foster City, CA, USA) and later combined to reduce the bias associated with PCR stochasticity. PCRs (25 μL) contained 10 μg BSA, 40 μM dNTPs, 1.25 U Roche FastStart High Fidelity Taq (Roche, Mannheim, Germany), 1× PCR buffer, 0.4 μM each primer and 1 μL DNA extract. Cycling conditions were as follows: 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 52 °C, and 40 s at 72 °C, with a final step of 7 min at 72 °C. PCR products were purified using Agencourt Ampure XP magnetic beads (Beckman Coulter Genomics, Danvers, MA, USA). Purified DNA was quantified using a Fluorometer (QuantiFluor, Promega, WI, USA) and checked using the Bioanalyser for the absence of PCR primer dimers. Equimolar volumes of DNA were pooled to create 3 batches of 10 samples, and each batch was sequenced separately on a 454 GS Junior (Roche, Basel, Switzerland). Quality control and sequence analysis was performed using QIIME 1.7.0, with the scripts described in the Data S1. Taxonomic assignments were based on the UNITE 12_11 ITS alpha release reference OTU data base (accessed at http://qiime.org/home_static/dataFiles.html, April 2013). We excluded singleton sequences from our analyses.

PALYNOLOGY AND SPORE COUNTS

Subsamples of faecal pellets (see faecal sampling methods above) were processed for palynological analysis using the following steps: hot 10% potassium hydroxide for 10 min, 10% hydrochloric acid wash, acetolysis, heavy liquid separation of pollen/spores using lithium polytungstate (specific gravity 2.2), staining with fuchsin red and mounting on glass microscope slides in glycerine jelly. Samples were spiked with a known number of *Lycopodium* spores to allow quantification of fungal spores and pollen grains. We counted and identified a minimum of 200 pollen grains from each faecal pellet, except for three samples where pollen concentration was low (where counts of 174, 146 and 97 grains were made). Pollen identifications were based on a reference collection held at Landcare Research, Lincoln, New Zealand, and using reference keys and images (Moore, Collinson & Webb 1991; Moar 1993). Construction of pollen diagrams and principal component analyses were performed using C2 1.7.2 (www.staff.ncl.ac.uk/staff/stephen.juggins/software/code/C2.pdf). Fungal spores were quantified at the same time as the pollen counts were performed.

DISPERSAL OF EM FUNGI ONTO SEEDLINGS

We considered formation of ectomycorrhizas on seedlings as our metric of spore viability, and so tested the viability of EM fungal spores in 31 faecal pellet groups, using a bioassay with three ectomycorrhizal tree species: native silver beech (*Lophozonia menziesii*) and two exotic (non-native) invasive conifers: lodgepole pine and Douglas-fir. Seedlings were planted in an autoclaved mix of perlite and peat. Faecal pellet groups were divided into two equal portions. Each portion was placed in a sterile glass bottle with 100 mL of distilled water and physically macerated. One of these bottles was then autoclaved for 30 min at 121 °C. The samples were agitated on a stir plate while a 5-mL pipette tip with the end removed was used to evenly divide each portion into nine sterilized vials (for three replicates of each pellet group on each of the three seedling species). Additional control seedlings were inoculated with water. The inocula were applied to seedlings between 2 and 5 July 2012. Inocula contained a mean of 8.1×10^6 spores g^{-1} (range 1.1×10^5–8.9×10^7), with no significant difference in spore density between deer and possum pellets. Seedlings were harvested between 3 and 6 Dec 2012. All root tips of harvested seedlings were examined under stereo microscopy (10–20×) to assess the level of ectomycorrhizal infection.

To test statistically the effects of autoclaving, animal species and plant species on the proportion of seedlings with ectomycorrhizas, we used a manual model simplification approach in R (Crawley 2007), starting with a full three-way interaction model and testing the significance of each term by removal and comparison of models, using AIC. Data were analysed as a binomial family GLM with autoclaving and seedling species (n = 608) nested within individual dung sample (n = 31).

IDENTITY OF DISPERSED EM FUNGI

Twelve ectomycorrhizal root tips from each infected seedling (or up to 12 if fewer were present) were sampled (by randomly selecting squares from a grid placed under the petri dish), and genomic DNA was extracted from these and amplified using REDExtract-N-AmpTM Tissue PCR Kit (REDEX; Sigma-Aldrich, St Louis, MO, USA). PCRs included 11 μL of the REDEX solution, 0.4 μL DNA extract and 1 μM of each primer (ITS1f and ITS4). Cycling conditions were as follows: 1 min 25 s at 94 °C, followed by 14 cycles of 35 s at 95 °C, 55 s at 55 °C, and 45 s at 72 °C, 15 cycles of 35 s at 95 °C, 55 s at 55 °C, and 2 min at 72 °C, 10 cycles of 35 s at 95 °C, 55 s at 55 °C, and 3 min at 72 °C, with a final step of 10 min at 72 °C.

PCR products were purified using the ZR-96 DNA Clean-up kit (Zymo Research, Irvine, CA, USA) and eluted in 30 μL H_2O. Restriction fragment length polymorphism (RFLP) digests were performed using the HpyCH4IV (New England Biolabs, Ipswich, MA, USA) and BsuR1 (Fermentas, Thermo Fisher Scientific, Waltham, MA, USA) enzymes. Digest reactions included 0.18 μL (0.18 U) of enzyme, 1 μL buffer, 5 μL PCR product and 4 μL H_2O and were incubated for 16 h at 37 °C. Enzymes were deactivated by incubation at 65 °C for 20 min (HpyCH4IV) or 80 °C for 20 min (BsuR1). 7 μL of the digested product was separated on a 2% low melt gel in 0.5× TBE at 65V for 2 h and stained with ethidium bromide for visualization under UV light. Each root tip was coded based on the combined patterns (Fig. S1) for the two enzymes (e.g. pattern 3 with BsuR1 and pattern 5 with HpyCH41V = 35). Examples of each RFLP code were bidirectionally sequenced using BigDye terminator technology on a capillary sequencer. Primer removal, sequence reorientation, generation of consensus sequences for each sample and consensus sequence alignment were performed using Geneious 6.0.5 (Biomatters, Auckland, New Zealand). A maximum-likelihood phylogeny of the aligned sequences was constructed using PHYML (HKY85, 1000 bootstrap replicates) in Geneious.

VIDEO RECORDINGS

We used motion-triggered infrared video cameras (Faunatech Scout Guard Models DTC-530V and DTC-560K) to record animals visiting fruiting bodies of the exotic *Amanita muscaria* within an area of exotic plantation forest (that includes *Pinus contorta*, *P. menziesii* and *Betula*) within our study area. Cameras were positioned adjacent to mushrooms and left for periods of 1–2 weeks, before being moved to new locations once the fruiting body had decayed. Recording periods were 10–22 April 2012 (one camera, videos of 30 s duration), 21 April–5 May 2012 (one camera, videos of 30 s duration) and 29 March–6 April 2013 (three cameras, videos of 45 s duration). A single camera was also placed beside a patch of native *Cortinarius* sp. EM fungi within a nearby mixed beech–*Eucalyptus* forest, from 27 March to 10 April 2012.

Results

FAECAL DNA ANALYSES

A total of 27 fungal genera were identified from DNA sequencing of faecal pellets (Table S1). In addition, several higher level taxa were also identified but could not be resolved to genus. Coprophilous fungi were well represented, particularly the Thelebolales, which include many cold-adapted taxa. This may, in part, reflect storage of the specimens at 4 °C prior to analysis, which could have promoted the proliferation of cold-adapted taxa post-sampling.

Fungi exhibiting a variety of fruiting body types and representing a range of trophic groups were identified from the ITS sequences, including 10 genera of EM fungi (Fig. S1; Table S1). Deer pellets contained a higher richness of EM fungal genera than possum pellets (10 and 6, respectively) (Fig. S1).

FAECAL PALYNOLOGY

Pollen assemblages from the faecal pellets (Fig. S2) were dominated by wind-dispersed pollen types, reflecting the vegetation communities within which the animals had been feeding. Possum pellets were dominated by either Pinaceae or Poaceae pollen, with *Fuscospora*, *Betula*, *Alnus*, Lactuceae (Asteraceae) pollen and monolete fern spores also being relatively important types. Deer pellets were dominated by either *Fuscospora* or Pinaceae pollen, with *Alnus*, Fabaceae, and *Lotus* pollen and monolete fern spores also being present in some samples at moderate abundances.

Fungal spores were abundant on most palynology slides and a diverse variety of spore morphologies were noted. Several of the spore morphological groups were attributable to EM taxa, including *Octaviania*, *Laccaria* and *Rhizopogon* (Fig. S3).

DISPERSAL OF EM FUNGAL SPORES

Ectomycorrhizas were observed on 21.4% of *Pinus contorta* seedlings that were inoculated with viable deer pellets, and 47.1% that were inoculated with viable possum pellets (Fig. 1), averaging 24% of total root tips infected per seedling when present. Ectomycorrhizas were observed on 11.9% of *P. menziesii* seedlings that were inoculated with viable deer pellets, and 68.6% that were inoculated with viable possum pellets (Fig. 1). Infection levels were low following deer pellet inoculation even when present (5% of root tips on infected seedlings) and higher with possum pellet inoculation (22% of root tips). No EM fungi were observed on *L. menziesii* seedlings. No EM fungi were observed on control seedlings of any species in either the autoclaved faeces or water controls. Binomial GLM models of the presence/absence of ectomycorrhizal infection including terms for autoclaving and the interaction of animal and plant species effects were both significantly better than simplified models excluding these terms ($P < 2.2 \times 10^{-16}$ and $P = 0.015$, respectively). Excluding *Lophozonia* seedlings, there was still a highly significant interaction of animal and plant species ($P = 0.0036$; again autoclaving also significant $P < 2.2 \times 10^{-16}$) indicat-

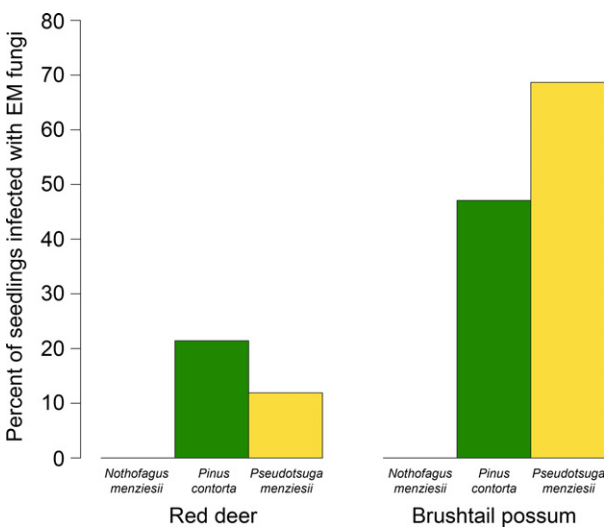

Fig. 1. Percentage of seedlings with ectomycorrhizas by plant and mammal species.

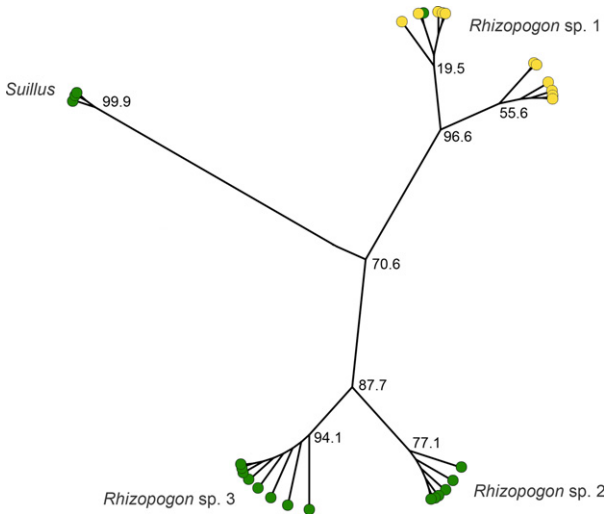

Fig. 2. Maximum-likelihood tree (1000 bootstrap replicates) of ITS sequences representing the range of RFLP patterns obtained from infected seedling root tips. Yellow circles represent sequences obtained from Douglas-fir (*Pseudotsuga menziesii*) root tips, and green circles represent sequences obtained from lodgepole pine (*Pinus contorta*) root tips. Generic identities were assigned by BLAST.

ing that the two animal species had different effects on mycorrhizal infection of the two conifers. Total spores applied in inoculum did not differ between the two animal species (means and standard errors: $1.5 \pm 0.4 \times 10^7$ spores in deer inoculum; $1.2 \pm 0.8 \times 10^7$ spores in possum inoculum; $P = 0.76$).

IDENTITY OF VIABLE EM FUNGI

For most RFLP patterns identified (Fig. S4) multiple samples were sequenced, and in all instances, these returned the same taxonomic identities (Table S2). One sequence matched the plant pathogen (*Cadophora*). The other ITS sequences obtained from the range of observed RFLP patterns clustered into four main groups (Fig. 2). One cluster (99.9% bootstrap support) had a BLAST match to *Suillus* (99% similarity to *S. luteus*). All other sequences had nearest BLAST matches to *Rhizopogon*, but formed three separate clusters (with 96.6%, 94.1% and 77.1% bootstrap support). It is likely that these represent different species or subspecies.

Each of the three *Rhizopogon* taxa exhibited a high degree of host tree specificity (Fig. 3). *Rhizopogon* sp. 1 was identified on 37 *P. menziesii* seedlings but just one *Pinus contorta* seedling, *Rhizopogon* sp. 2 was identified on 16 *P. contorta* but just one *Ps. menziesii* seedling, and *Rhizopogon* sp. 3 was identified on 14 *P. contorta* and no *Ps. menziesii* seedlings.

All *P. menziesii* seedlings that were infected with EM fungi from deer pellets had *Rhizopogon* sp. 1, and most (91.4%) *Ps. menziesii* seedlings that were infected from possum pellets also had this taxon (Fig. 3). However, there were larger differences between deer and possum pellets in the infection of *Pinus contorta* seedlings with the other two *Rhizopogon* taxa. A higher percentage of seedlings infected by deer pellets had *Rhizopogon* sp. 2 (66.7%) than those infected by possum

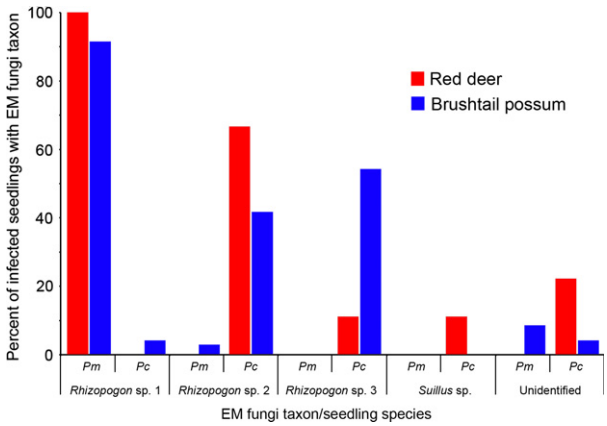

Fig. 3. Host specificity of ectomycorrhizal fungal taxa that infected seedling root tips via red deer (*Cervus elaphus*) and brushtail possum (*Trichosurus vulpecula*) faecal pellets. Pm, Douglas-fir (*Pseudotsuga menziesii*); Pc, lodgepole pine (*Pinus contorta*).

pellets (41.7%), and a higher percentage of seedlings infected by possum pellets had *Rhizopogon* sp. 3 (54.2%) than those infected by deer pellets (11.1%) (Fig. 3).

A mean of 1.045 EM fungal taxa were detected on the sampled root tips from each infected seedling (maximum of two observed on a seedling).

VIDEO RECORDINGS

Forty-one video recordings were captured by cameras beside *Amanita muscaria* mushrooms; 24 of brushtail possum, 3 of red deer, 2 of birds (one each of blackbird *Turdus merula* and dunnock *Prunella modularis*) and 12 with no animal visible. Three recordings showed instances of animals consuming *Amanita muscaria* mushrooms.

Two of the videos were of a female red deer (1–2 years old), which first fed on a mushroom after removing the stalk by rubbing it along the ground (Movie S1), before following a scent to a second *Amanita muscaria* and eating this as well (Movie S2).

The third video (Movie S3) shows an adult brushtail possum browsing the gills on the underside of an *Amanita muscaria* mushroom cap. In a video recorded the night prior to the mycophagy event, a possum sniffed the same mushroom but rapidly flinched away as if exhibiting an aversion to it (Movie S4).

The camera placed beside a patch of *Cortinarius* sp. mushrooms recorded a brushtail possum feeding on these (Movie S5).

Discussion

FACILITATION OF PINE ESTABLISHMENT AND EM FUNGAL CO-INVASION BY MAMMALS

Novel, non-co-evolved assemblages of species are a product of the current era of increased global movement of species, sometimes termed the 'Homogocene'. Our results suggest that the novel assemblage of fungi, mammals and trees in New

Zealand has resulted in a tri-trophic invasional meltdown, where all three partners potentially benefit. In contrast, native species were excluded from the positive interactions that we observed: native fungi were consumed by invasive mammals but did not infect either native or invasive tree species from dung, and seedlings of native trees did not form mycorrhizas with introduced fungi.

Invasional meltdown requires that the establishment, invasion or impact of each species is increased by the presence of the other (Simberloff & Von Holle 1999). In the case of the pine–fungus–mammal interaction, reciprocal facilitation occurred between plant and EM fungi as symbionts, and EM fungal invasion was enhanced by mammalian dispersal. We did not directly quantify enhanced invasion of mammals due to either fungi or plants; however, non-native fungi have been shown to comprise relatively large proportions of the diet of both deer and possums (Nugent 1990; Cochrane *et al.* 2003) in New Zealand forests, and fungi are known to be an important food resource for mammals elsewhere (e.g. Cazares & Trappe 1994; Claridge & May 1994; Johnson 1996). Hence, both invasive fungi and invasive mammals are likely receiving mutual benefits from their interaction. Although direct herbivory on trees could provide a more one-sided benefit to the herbivore, neither deer nor possums heavily browse exotic pines in New Zealand, and they may therefore have an indirect positive effect by suppressing native competitors (Relva, Nuñez & Simberloff 2010). Disentangling these direct and indirect effects would provide information on the relative magnitude and importance of the tritrophic invasional meltdown amongst invasive trees, fungi and mammals.

Our findings are consistent with Simberloff (2006), Gurevtich (2006) and others (e.g. Pringle *et al.* 2009; Green *et al.* 2011; Nuñez *et al.* 2013; Flory & Bauer 2014; Nuñez & Dickie 2014; Russo *et al.* 2014) who have highlighted the potential importance of novel, positive interactions amongst invading non-native species. We provide additional evidence for these ideas, demonstrating that invasional meltdown does not just occur between taxa within trophic levels or pairwise interactions between trophic levels, but it also occurs across kingdoms. More generally, complex interactions amongst invasive plants, fungi and their animal dispersers may be more common than previously supposed (Kempel *et al.* 2013; Nuñez *et al.* 2013) and underpin feedbacks between above- and below-ground components of ecosystems (van der Putten *et al.* 2013). The complexity of these interactions makes them more difficult to document and demonstrate empirically. However, studies of such interactions will provide new insights into invasion success, and the ecosystem impacts of invasive non-native species, because the taxa involved collectively regulate both population and ecosystem processes (Nelis & Wootton 2010; Peltzer *et al.*, 2010; Simberloff *et al.* 2013).

DISPERSAL OF INVASIVE EM FUNGI INTO OPEN HABITATS

Pollen assemblages demonstrate that mammals whose faecal pellets contained viable EM spores of fungi from exotic forest were also feeding in grassland (shift towards the Poaceae and Lactuceae vectors) (Fig. S5), suggesting that these animals were dispersing fungi between different vegetation types. Similarly, animal movement studies (Cowan & Clout 2000; Hu 2006) show that brushtail possums and red deer frequently move between areas of forest and grassland to feed and thus have the potential to disperse EM fungi from closed native forest and plantations into open habitats. Further evidence supporting this idea is shown by several of the faecal pellet groups in our study, which were collected within small (<1 ha) grasslands surrounded by forest. The pollen data (Fig. S5) also suggest that the lower infection rates by EM fungi observed for deer pellets are likely to be due to the predominantly native habitat in which they were collected, rather than a true dispersal difference between deer and possum. The deer pellets that infected seedlings with EM fungi are more dominated by exotic pollen types, while those that have a greater amount of *Fuscospora* pollen (reflecting feeding dominantly within native forest) did not infect any seedlings with EM fungi. Red deer and possum co-occur in numerous regions of New Zealand with pine plantations and therefore have the potential to rapidly facilitate the further spread and invasion of pines into new habitat across the country. Moreover, a suite of other introduced mammal species, including additional deer species, goat (*Capra hircus*), rodents (*Rattus* spp. and *Mus musculus*), hedgehog (*Erinaceus europaeus*), wallabies (*Macropus* spp.) and pig (*Sus scrofa*) also have the potential to act as potential dispersers of EM fungi further compounding the issue of assisted dispersal and spread. Pigs, in particular, have been shown to be an important disperser of invasive EM fungi in South America (Nuñez *et al.* 2013). Our findings and previous studies in combination suggest that invasive mammals can be important vectors of dispersal for non-native mutualistic fungi amongst habitats.

Numerous EM fungi have been introduced to new regions around the globe (Vellinga, Wolfe & Pringle 2009). In New Zealand, this has resulted in a moderately diverse community of EM fungi associated with pine plantations (Walbert *et al.* 2010) and elsewhere in the introduced range of pines (Hynson *et al.* 2013). Despite this fungal diversity, the *Suillus*/*Rhizopogon* clade appears to be particularly important for the establishment of invasive pines in grasslands, both in their native and invasive ranges (Cazares & Trappe 1994; Ashkannejhad & Horton 2006; Hynson *et al.* 2013; Nuñez *et al.* 2013). This may reflect introduction effort, as *Suillus* and *Rhizopogon* are the first and second most frequently deliberately introduced genera of EM fungi globally (Vellinga, Wolfe & Pringle 2009). Both also have particular adaptations that increase their importance in early succession, particularly animal-dispersed spores (Ashkannejhad & Horton 2006) and, at least in the case of *Rhizopogon*, a high spore longevity (Bruns *et al.* 2009; Nguyen, Hynson & Bruns 2012). However, neither exhibit low host specificity; a characteristic that has been suggested to be an important trait in invasive mutualisms (Richardson *et al.* 2000). Instead, *Suillus* and *Rhizopogon* are highly specific to the Pinaceae and even to individual

species within the Pinaceae. This host specificity was clearly evident in the results of our bioassay experiment (Figs 2 and 3). The high host specificity of these fungi is also likely the primary mechanism driving their absence on native *Lophozonia*.

Only one introduced EM fungus, *Amanita muscaria*, has been documented to associate with native Nothofagaceae (Orlovich & Cairney 2004; Dunk, Lebel & Keane 2012). Our video recordings showed clear evidence of consumption of *Amanita muscaria* by introduced mammals. However, DNA of this species was not found in deer or possum faeces, and *Amanita muscaria* was not recorded in the bioassay. It has also been suggested that *Amanita muscaria* may require the presence of mature plants (or high levels of exogenous sugar) to infect seedling roots (Deacon & Fleming 1992), which may contribute to its absence in the bioassay.

INTRODUCED MAMMALS AND NATIVE FUNGI

Fungi provide an important seasonal food resource for many species of mammal across the world (Claridge & May 1994; Johnson 1996; Katarzyte & Kutorga 2011). Mycophagy by introduced mammals within native vegetation communities has been documented in New Zealand (Harvie 1973; Warburton 1978; Cowan 1989; Cochrane *et al.* 2003; Sweetapple 2003; Medway 2004; Glen *et al.* 2012), although the species of fungi consumed are rarely identified. Mycophagy has been found in several dietary studies of brushtail possums, and these studies suggest fungi may form a greater component of possum diet within native forest compared to exotic habitats (Sweetapple 2003). Sweetapple (2003) found the importance of fungi in the diet of possums living in native beech forest was relatively high, particularly during non-mast years (18.3% mean dry weight in non-mast years compared with 8.6% in mast years). Cochrane *et al.* (2003) found fungi to represent 3.3% of the overall mean dry weight of possum stomach content from native mixed beech forest. In contrast, Warburton (1978) found that fungi represented just 0.05% of stomach content analysed during autumn from an exotic plantation forest, and Glen *et al.* (2012) reported that fungi occurred in 22% of the samples analysed but represented <1% of the dry weight of possum stomach content from an agricultural-dominated system. Fungi were also rarely observed in stomach content of possums from coastal farmland in the North Island (Harvie 1973). In addition to these stomach content studies, there is a record of putative possum mycophagy on the native ectomycorrhizal *Amanita pekeoides* (Medway 2004), and spores of the arbuscular mycorrhizal fungus *Glomus* have been reported from possum pellets (Cowan 1989).

Native fungi have also been recorded in the diet of ungulates from New Zealand. Nugent (1990) reported that stomach content of fallow deer (*Dama dama*) contained 2.8–9.3% fungi by mean dry weight and that amongst those taxa consumed were the native *Cyttaria gunnii* and EM *Cantharellus elsae*. A suite of additional dietary studies have also recorded unidentified fungal taxa in the diets of deer and goats from native habitats (summarized by Forsyth *et al.* 2002). The overall impact of introduced mammals on native fungi remains to be fully determined and may be obscured somewhat in our current study by limitations of fungal bioassay methods. We recorded a possum feeding on *Cortinarius* sp. mushrooms, and native EM fungi such as *Octaviania*, *Cortinarius* and *Gallacea* were abundant as DNA in mammal faeces. However, native EM fungi were completely absent in the bioassay on either native or invasive tree species.

Several of these native fungi (e.g. *Octaviania*, *Gallacea* and *Chamonixia*) have above-ground, sequestrate sporocarps. It has been suggested that sequestrate sporocarps are an adaptation to mammalian dispersal or dry climates. However, New Zealand has few native terrestrial mammals (i.e. two extant bats), neither of which is known to disperse fungi and a generally mesic to wet climate. Therefore, it seems likely that these fungi may be adapted for bird or insect dispersal, and further work to establish the relative roles of native birds and insects and introduced mammals in their dispersal is needed. Some taxa, such as *Cortinarius,* are considered late-stage fungi and are generally not found on seedlings inoculated by spores (Ishida *et al.* 2008; Peay *et al.* 2012; but see Chu-Chou & Grace 1982). It is possible that introduced mammalian dispersal of late-stage fungi might result in ectomycorrhizal formation on mature tree roots rather than on isolated seedlings.

The greenhouse bioassay technique is a pragmatic approach to studying fungal spore dispersal, but may discriminate against some fungal taxa. Nonetheless, it has long been noted that a lack of mycorrhizal fungi limits the establishment of native *Lophozonia* (Baylis 1980; Dickie, Davis & Carswell 2012), while the widespread invasion of Pinaceae suggests no such limitation. These prior studies are consistent with invasive mammals not providing effective dispersal of native fungi into non-forested areas.

Conclusions

The ongoing homogenization of the world's biota creates diverse opportunities for novel interactions to emerge through serendipity rather than co-evolution. Our study provides an example of how such interactions may lead to invasional meltdown, as the dispersal of North American fungi by Australian brushtail possum and European red deer appears to be a key contributing factor in the establishment of invasive North American pines in New Zealand. The identification of such interactions can greatly assist with predicting naturalization of other non-native species and for mitigating future potential impacts of invasions. Enhanced dispersal of non-native EM fungi by invasive mammals and the resulting accumulation of spores in soils could also lead to increased invasiveness of currently non-spreading EM trees (Dickie *et al.* 2010). The coincidence of invasive trees, fungi and mammals is a global phenomenon, and we suggest that the tritrophic facilitation demonstrated here may be a more general phenomenon than previously supposed. Consideration should be given to co-managing invasions across trophic

boundaries to avoid the propagation of unexpected meltdowns or other synergistic effects amongst invasive species.

Acknowledgements

We thank Duckchul Park, Kat Trought and Nicola Bolstridge for laboratory assistance and Nic Rawlence and Maria Zammit for field assistance, Rob Allen for providing infrared video cameras and Christine Bezar for manuscript editing. Jerry Cooper provided samples of EM fungi from which we made reference-spore microscope slides. We also acknowledge financial support from Core funding for Crown Research Institutes from the New Zealand Ministry of Business, Innovation and Employment's Science and Innovation Group.

Data accessibility

Supporting data deposited in the Dryad repository (Wood *et al.* 2014). DNA sequences deposited in GenBank (KM596867-KM596896).

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.

Atkinson, I.A.E. & Greenwood, R.M. (1989) Relationships between moas and plants. *New Zealand Journal of Ecology*, **12**, 67–96.

Baylis, G.T.S. (1980) Mycorrhizas and the spread of beech. *New Zealand Journal of Ecology*, **3**, 151–153.

Bougher, N.L. & Lebel, T. (2001) Sequestrate (truffle-like) fungi of Australia and New Zealand. *Australian Systematic Botany*, **14**, 439–484.

Bruns, T.D., Peay, K.G., Boynton, P.J., Grubisha, L.C., Hynson, N.A., Nguyen, N.H. & Rosenstock, N.P. (2009) Inoculum potential of *Rhizopogon* spores increases with time over the first 4 yr of a 99-yr spore burial experiment. *New Phytologist*, **181**, 463–470.

Cazares, E. & Trappe, J.M. (1994) Spore dispersal of ectomycorrhizal fungi on a glacier forefront by mammal mycophagy. *Mycologia*, **86**, 507–510.

Chu-Chou, M. & Grace, L.J. (1982) Mycorrhizal fungi of *Eucalyptus* in the North Island of New Zealand. *Soil Biology and Biochemistry*, **14**, 133–137.

Claridge, A.W. & May, T.W. (1994) Mycophagy among Australian mammals. *Australian Journal of Ecology*, **19**, 251–275.

Cochrane, C.H., Norton, D.A., Miller, C.J. & Allen, R.B. (2003) Brushtail possum (*Trichosurus vulpecula*) diet in a north Westland mixed-beech (*Nothofagus*) forest. *New Zealand Journal of Ecology*, **27**, 61–65.

Cowan, P.E. (1989) A vesicular-arbuscular fungus in the diet of brushtail possums, *Trichosurus vulpecula*. *New Zealand Journal of Botany*, **27**, 129–131.

Cowan, P. & Clout, M. (2000) Possums on the move: activity patterns, home ranges, and dispersal. *The Brushtail Possum* (ed. T.L. Montague), pp. 24–34. Manaaki Whenua Press, Lincoln, New Zealand.

Crawley, M.J. (2007) *The R Book*. John Wiley & Sons, West Sussex, UK.

Dam, N. (2013) Spores do travel. *Mycologia*, **105**, 1618–1622.

Deacon, J.W. & Fleming, L.V. (1992) Interactions of ectomycorrhizal fungi. *Mycorrhizal Functioning: An Integrative Plant–Fungal Process* (ed. M.F. Allen), pp. 249–300. Chapman & Hall, New York, NY, USA.

Dickie, I.A., Davis, M. & Carswell, F.E. (2012) Quantification of mycorrhizal limitation in beech spread. *New Zealand Journal of Ecology*, **36**, 210–215.

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.

Dunk, C.W., Lebel, T. & Keane, P.J. (2012) Characterisation of ectomycorrhizal formation by the exotic fungus *Amanita muscaria* with *Nothofagus cunninghamii* in Victoria, Australia. *Mycorrhiza*, **22**, 135–147.

Flory, S.L. & Bauer, J.T. (2014) Experimental evidence for indirect facilitation among invasive plants. *Journal of Ecology*, **102**, 12–18.

Forsyth, D.M., Coomes, D.A., Nugent, G. & Hall, G.M.J. (2002) Diet and diet preferences of introduced ungulates (Order: Artiodactyla) in New Zealand. *New Zealand Journal of Zoology*, **29**, 323–343.

Galante, T.E., Horton, T.R. & Swaney, D.P. (2011) 95% of basidiospores fall within 1 m of the cap: a field- and modeling-based study. *Mycologia*, **103**, 1175–1183.

Glen, A.S., Byrom, A.E., Pech, R.P., Cruz, J., Schwab, A., Sweetapple, P.J. *et al.* (2012) Ecology of brushtail possums in a New Zealand dryland ecosystem. *New Zealand Journal of Ecology*, **36**, 29–37.

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

Gurevitch, J. (2006) Commentary on Simberloff (2006): Meltdowns, snowballs and positive feedbacks. *Ecology Letters*, **9**, 919–921.

Hallett, S.G. (2006) Dislocation from coevolved relationships: a unifying theory for plant invasion and naturalization? *Weed Science*, **54**, 282–290.

Harvie, A.E. (1973) Diet of the opossum (*Trichosurus vulpecula* Kerr) on farmland northeast of Waverley, New Zealand. *Proceedings of the New Zealand Ecological Society*, **20**, 48–52.

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.

Hu, Q. (2006) Temporal variation and inter-relationship of movement and resource selection of red deer (*Cervus elaphus*) with respect to climate: a case study. Unpublished MSc thesis, University of Canterbury, Christchurch, New Zealand.

Hynson, N.A., Merckx, V.S.F.T., 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.

Ishida, T.A., Nara, K., Tanaka, M., Kinoshita, A. & Hogetsu, T. (2008) Germination and infectivity of ectomycorrhizal fungal spores in relation to their ecological traits during primary succession. *New Phytologist*, **180**, 491–500.

Johnson, C.N. (1996) Interactions between mammals and ectomycorrhizal fungi. *Trends in Ecology and Evolution*, **11**, 503–507.

Johnston, P.R. (2010) Causes and consequences of changes to New Zealand's fungal biota. *New Zealand Journal of Ecology*, **34**, 175–184.

Katarzyte, M. & Kutorga, E. (2011) Small mammal mycophagy in hemiboreal forest communities of Lithuania. *Central European Journal of Biology*, **6**, 446–456.

Kempel, A., Nater, P., Fischer, M. & van Kleunen, M. (2013) Plant-microbe-herbivore interactions in invasive and non-invasive alien plant species. *Functional Ecology*, **27**, 498–508.

Kurokawa, H., Peltzer, D.A. & Wardle, D.A. (2010) Plant traits, leaf palatability and litter decomposability for co-occurring woody species differing in invasion status and nitrogen fixation ability. *Functional Ecology*, **24**, 513–523.

Ledgard, N. (2001) The spread of lodgepole pine (*Pinus contorta*, Dougl.) in New Zealand. *Forest Ecology and Management*, **141**, 43–57.

Lee, W.G., Wood, J.R. & Rogers, G.M. (2010) Legacy of avian-dominated plant–herbivore systems in New Zealand. *New Zealand Journal of Ecology*, **34**, 28–47.

Medway, D.G. (2004) Mycophagy of a New Zealand epigeous fungus, probably by brushtail possums (*Trichosurus vulpecula*). *Australian Mycologist*, **22**, 82–83.

Moar, N.T. (1993) *Pollen Grains of New Zealand Dicotyledonous Plants*. Manaaki Whenua Press, Lincoln, New Zealand.

Moore, P.D., Collinson, M. & Webb, J.A. (1991) *Pollen Analysis*. Blackwell Scientific, Oxford, UK.

Ndlovu, J., Richardson, D.M., Wilson, J.R.U. & Le Roux, J.J. (2013) Co-invasion of South African ecosystems by an Australian legume and its rhizobial symbionts. *Journal of Biogeography*, **40**, 1240–1251.

Nelis, L.C. & Wootton, J.T. (2010) Treatment-based Markov chain models clarify mechanisms of invasion in an invaded grassland community. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **277**, 539–547.

Nguyen, N.H., Hynson, N.A. & Bruns, T.D. (2012) Stayin' alive: survival of mycorrhizal fungal propagules from 6-yr-old forest soil. *Fungal Ecology*, **5**, 741–746.

Nugent, G. (1990) Forage availability and the diet of fallow deer (*Dama dama*) in the Blue Mountains, Otago. *New Zealand Journal of Ecology*, **13**, 83–95.

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.

Nuñez, M.A., Hayward, J., Horton, T.R., Amico, G.C., Dimarco, R.D., Barrios-Garcia, M.N. & Simberloff, D. (2013) Exotic mammals disperse exotic fungi that promote invasion by exotic trees. *PLoS ONE*, **8**, e66832.

Orlovich, D.A. & Cairney, J.W.G. (2004) Ectomycorrhizal fungi in New Zealand: current perspectives and future directions. *New Zealand Journal of Botany*, **42**, 721–738.

Peay, K.G., Schubert, M.G., Nguyen, N.H. & Bruns, T.D. (2012) Measuring ectomycorrhizal fungal dispersal: macroecological patterns driven by microscopic propagules. *Molecular Ecology*, **21**, 4122–4136.

Peltzer, D.A., Allen, R.B., Lovett, G.M., Whitehead, D. & Wardle, D.A. (2010) Effects of biological invasions on forest carbon sequestration. *Global Change Biology*, **16**, 732–746.

Perez, F., Castillo-Guevara, C., Galindo-Flores, G., Cuautle, M. & Estrada-Torres, A. (2012) Effect of gut passage by two highland rodents on spore activity and mycorrhiza formation of two species of ectomycorrhizal fungi (*Laccaria trichodermophora* and *Suillus tomentosus*). *Botany-Botanique*, **90**, 1084–1092.

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 & Systematics*, **40**, 699–715.

Proches, S., Wilson, J.R.U., Richardson, D.M. & Rejmanek, M. (2012) Native and naturalized range size in *Pinus*: relative importance of biogeography, introduction effort and species traits. *Global Ecology and Biogeography*, **21**, 513–523.

van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T. *et al.* (2013) Plant–soil feedbacks: the past, the present and future challenges. *Journal of Ecology*, **101**, 265–276.

Relva, M.A., Nuñez, M.A. & Simberloff, D. (2010) Introduced deer reduce native plant cover and facilitate invasion of non-native tree species: evidence for invasional meltdown. *Biological Invasions*, **12**, 303–311.

Richardson, D.M. (1998) Forestry trees as invasive aliens. *Conservation Biology*, **12**, 18–26.

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

Rodríguez-Echeverría, S. (2010) Rhizobial hitchhikers from Down Under: invasional meltdown in a plant–bacteria mutualism? *Journal of Biogeography*, **37**, 1611–1622.

Russo, L., Memmott, J., Montoya, D., Shea, K. & Buckley, Y.M. (2014) Patterns of introduced species interactions affect multiple aspects of network structure in plant-pollinator communities. *Ecology*, **95**, 2953–2963.

Simberloff, D. (2006) Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters*, **9**, 912–919.

Simberloff, D. & Von Holle, B. (1999) Positive interactions of nonindigenous species: invasional meltdown. *Biological Invasions*, **1**, 21–32.

Simberloff, D., Martin, J., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J. *et al.* (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution*, **28**, 58–66.

Sweetapple, P.J. (2003) Possum (*Trichosurus vulpecula*) diet in a mast and non-mast seed year in a New Zealand *Nothofagus* forest. *New Zealand Journal of Ecology*, **27**, 157–167.

Thompson, K. & Davis, M. (2011) Why research on traits of invasive plants tells us very little. *Trends in Ecology & Evolution*, **26**, 155–156.

Triggs, B. (2010) *Tracks, Scats and Other Rraces: A Field Guide to Australian Mammals*. Oxford University Press, Melbourne, Vic., Australia.

Vellinga, E.C., Wolfe, B.E. & Pringle, A. (2009) Global patterns of ectomycorrhizal introductions. *New Phytologist*, **181**, 960–973.

Walbert, K., Ramsfield, T.D., Ridgway, H.J. & Jones, E.E. (2010) Ectomycorrhizal species associated with *Pinus radiata* in New Zealand including novel associations determined by molecular analysis. *Mycorrhiza*, **20**, 209–215.

Warburton, B. (1978) Foods of the Australian brush-tailed opossum (*Trichosurus vulpecula*) in an exotic forest. *New Zealand Journal of Ecology*, **1**, 126–131.

Wegner, R. (1992) *Deer & Deer Hunting: The Serious Hunter's Guide*. Stackpole Books, Mechanicsburg, PA, USA.

Wilmshurst, J.M., Anderson, A.J., Higham, T.F.G. & Worthy, T.H. (2008) Dating the late prehistoric dispersal of polynesians to New Zealand using the commensal Pacific rat. *Proceedings of the National Academy of Sciences USA*, **105**, 7676–7680.

Wood, J.R., Dickie, I.A., Moeller, H.V., Peltzer, D.A., Bonner, K.I., Rattray, G. & Wilmshurst, J.M. (2014) Data from: Novel interactions between non-native mammals and fungi facilitate establishment of invasive pines. *Dryad Digital Repository*, doi:10.5061/dryad.s2241.

Worthy, T.H. & Holdaway, R.N. (2002) *The Lost World of the Moa: Prehistoric Life of New Zealand*. Indiana University Press, Bloomington, IN, USA.

Received 26 January 2014; accepted 24 October 2014
Handling Editor: David Gibson

Supporting Information

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

Figure S1. Percentage occurrence of ectomycorrhizal fungal taxa in red deer (*Cervus elaphus*) and brushtail possum (*Trichosurus vulpecula*) faecal pellet samples (n = 14 and 16, respectively) from Craigieburn, South Island, New Zealand, as detected using 454 sequencing.

Figure S2. Pollen assemblages from faecal pellets of brushtail possums (*Trichosurus vulpecula*) (n = 17) and red deer (*Cervus elaphus*) (n = 14) collected from Craigieburn, South Island, New Zealand, between 27 March and 19 May 2012. Circles represent pollen types present at <2.5%.

Figure S3. Examples of ectomycorrhizal fungal spores from brushtail possum (*Trichosurus vulpecula*) and red deer (*Cervus elaphus*) faecal pellets, Craigieburn, South Island, New Zealand.

Figure S4. Virtual gel showing RFLP patterns produced by BSrR1 and HpyCH41V restriction enzymes on ITS1f-ITS4 DNA fragments amplified from seedling root tips infected with ectomycorrhizal fungi from faecal pellets in our study.

Figure S5. Principal components analysis of faecal pellet pollen assemblages. Circles represent brushtail possum (*Trichosurus vulpecula*) pellets and triangles represent red deer (*Cervus elaphus*) pellets.

Data S1. QIIME scripts used to analyse fungal ITS sequences generated from mammal faecal pellets from Craigieburn, South Island, New Zealand.

Table S1. Occurrence frequency of fungal taxa identified from 454 sequencing of red deer (*Cervus elaphus*) and brushtail possum (*Trichosurus vulpecula*) faecal pellets from Craigieburn, South Island, New Zealand.

Table S2. Identity of RFLP patterns (see Fig. S4) observed for ectomycorrhizal fungi on seedling root tips infected from faecal pellets in our study.

Movie S1. Red deer (*Cervus elaphus*) consuming *Amanita muscaria* mushroom in *Pinus contorta* plantation at Helicopter Hill, Craigieburn, South Island, New Zealand.

Movie S2. Red deer (*Cervus elaphus*) consuming *Amanita muscaria* mushroom in *Pinus contorta* plantation at Helicopter Hill, Craigieburn, South Island, New Zealand.

Movie S3. Brushtail possums (*Trichosurus vulpecula*) consuming *Amanita muscaria* mushroom in *Pinus contorta* plantation at Helicopter Hill, Craigieburn, South Island, New Zealand.

Movie S4. Brushtail possum (*Trichosurus vulpecula*) appearing to exhibit aversion behaviour towards *Amanita muscaria* mushroom in *Pinus contorta* plantation at Helicopter Hill, Craigieburn, South Island, New Zealand.

Movie S5. Brushtail possum (*Trichosurus vulpecula*) consuming *Cortinarius* sp. mushrooms in mixed beech (Nothofagaceae)–*Eucalyptus* forest at Helicopter Hill, Craigieburn, South Island, New Zealand.