Oomycetes during 120,000 years of temperate rainforest ecosystem development

Ian A. Dickie[1*], Angela M. Wakelin[2], Laura Martinez-Garcia[3], Sarah J. Richardson[4], Andreas Makiola[2], Jason M. Tylianakis[1,5]

*Corresponding author: Bio-Protection Research Centre, School of Biological Sciences, Canterbury University, Christchurch, New Zealand.

[1] Bio-Protection Research Centre, School of Biological Sciences, University of Canterbury, Christchurch, New Zealand.

[2] Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand.

[3] Department of Soil Quality, Wageningen University, Wageningen, The Netherlands.

[4] Landcare Research, Lincoln, New Zealand.

[5] Department of Life Sciences, Silwood Park Campus, Imperial College London, Ascot, UK.

author emails: Ian.Dickie@canterbury.ac.nz; Angela.Wakelin@lincoln.ac.nz; LauraBeatrizMartinez@gmail.com; RichardsonS@landcareresearch.co.nz; Andreas.Makiola@lincolnuni.ac.nz; Jason.Tylianakis@canterbury.ac.nz

Running title: **Oomycetes and ecosystem development**

Suggested subject category: **Microbial ecology and functional diversity of natural habitats**

Keywords: *Forest diversity, molecular ecology, Oomycetes, pathogen ecology, pedogenesis, plant–soil (below-ground) interactions, retrogression, succession*

Abstract

The occurrence of plant-associated oomycetes in natural ecosystems and particularly during long-term ecosystem development is largely unknown, despite the importance of many oomycetes as plant pathogens. Using DNA sequencing from roots, we investigated the frequency and host relationships of plant-associated oomycete communities along a 120 000 year glacial chronosequence, comprising site ages with rapid compositional change ("early succession"; 5, 15, and 70 years old soil); relatively stable higher-diversity sites ("mature", 280, 500, 1000, 5000, 12000 years); and ancient, nutrient-limited soils with declining plant diversity and stature ("retrogression", 60 000, 120 000 years). Plant-associated oomycetes were frequent in all three early successional sites, occurring in 38 – 65% of plant roots, but rare (average 3%) in all older ecosystems. Oomycetes were highly host specific, and more frequent on those plant species that declined most strongly in abundance between ecosystem ages. The results support the particular importance of plant-associated oomycetes in early succession (up to 70 years). High host specificity and correlations of abundance of oomycete inside roots with declining plant species are consistent with oomycete-driven successional change.

Introduction

As ecosystems develop, biotic communities undergo succession both in composition and in the nature of mutualistic and antagonistic interactions. Many of these changes have been well studied, including changes in plant communities and traits, competition intensity, and mutualisms (Lambers et al, 2008). Nonetheless, long-term successional changes in composition and community interactions of many other organisms, such as oomycetes, remain poorly understood. Indeed, oomycetes in natural ecosystems are often out-of-sight out-of-mind, until large-scale forest diebacks occur (Scott & Williams, 2014). This lack of information is particularly apparent in forest disease outbreaks, where it is often unclear whether the oomycete is native, from where it may have originated, or what background levels of oomycetes are typical in healthy ecosystems (Podger & Newhook, 1971). Oomycetes occur as part of healthy ecosystems and play important roles in ecosystem processes (Castello et al, 1995; Gómez-Aparicio et al, 2012; Bever et al, 2015; Geisen et al, 2015). For example, *Pythium* and other oomycetes (Oomycota = Peronosporomycota) can drive negative density dependence in plants (Packer & Clay, 2000) and hence are believed to contribute to the maintenance of forest plant diversity (Mangan et al, 2010a). Oomycetes are also an important component of soil biodiversity, as pathogens of plant roots and soil invertebrates, and as saprotrophs (Arcate et al, 2006; Geisen et al, 2015). One way to better understand the role of oomycetes in natural ecosystems is to look at gradients of ecosystem development, which have a long history of providing insight into plant communities.

A useful way to consider ecosystem development, at least from a plant-community perspective, is based on compositional change through three phases: early succession, mature, and retrogression. Early succession (both primary and secondary) can be defined as the

period in which communities undergo major compositional change. In the context of plant communities, this means that early successional plants are unlikely to regenerate under conspecifics and are instead adapted for dispersal into new sites. Mature ecosystems can be defined by compositional stability over timespans greater than one generation, such that species are regenerating in the presence of established conspecifics (Veblen, 1992). Finally, over very long time periods ecosystem retrogression eventually develops, where the progressive loss of nutrients leads to a decline in plant diversity and a loss of biomass and canopy stature (Peltzer et al. 2010). This shift to retrogression entails slow compositional change, favouring species with life history traits that are adapted to stressful, low-nutrient environments.

Although oomycetes can be saprotrophic, they have been proposed to play particularly important roles at all stages of ecosystem development as potential plant pathogens. One view of soil pathogens in relation to ecosystem development is that they play a critical role in driving compositional change in early succession (Van der Putten et al, 1993; Van Der Putten, 2003). This view is based, in part, on the assumption that newly establishing plant species will encounter only low levels of pathogens in the absence of conspecific host plants during succession. Over time, these established plants accumulate pathogens, which prevent establishment of conspecific seedlings but have less negative effects on seedlings of other plant species. Part of the role of pathogens in succession is driven by plant age, as pathogens that kill seedlings are often tolerated by established plants (Martin & Loper, 1999; Van Der Putten, 2003). This implies that early-successional (*r*-selected) plants may have limited selection for pathogen resistance. Instead, these species may accumulate and tolerate pathogens as mature plants, and rely on seed dispersal into new habitats to escape pathogens during the vulnerable seedling stage (Van Der Putten, 2003). Support for the role of

antagonists in early succession comes from studies of invertebrate antagonists in grasslands, where dominant early-successional plants are more suppressed than subordinate or late-successional species (De Deyn et al, 2003) and from studies showing negative plant-soil-feedback early in succession (Kardol et al, 2006). Oomycete pathogens have also been shown to be important in the mortality of the early-successional tree *Prunus serotina* (Packer & Clay, 2000; Packer & Clay, 2003). These examples span from short-term grassland to decadal scale forest patterns, suggesting a widely applicable ecological pattern.

While much evidence supports the role of pathogens in early succession, there are also suggestions that pathogens, in general, increase in mature ecosystems (Reynolds et al, 2003; Bardgett et al, 2005; Peltzer et al, 2010). Soil biotic communities as a whole increase in biomass and species diversity over periods of tens to hundreds of years (Bardgett et al, 2005), and pathogen communities may follow similar patterns of increasing biomass and diversity. Further, plant competition is high in mature ecosystems, and Gilbert (2002) suggests that competition may stress plants and increase susceptibility to pathogens. Pathogen host-specificity in these systems leads to density-dependent mortality (Augspurger, 1984). Evidence for negative density dependence has come from both tropical and temperate forests (Packer & Clay, 2003; Mangan et al, 2010b), although other studies have found positive density dependence or no effect (Reinhart et al, 2012).

Finally, a recently proposed third view of the role of pathogens in ecosystem development is that soil pathogens may be particularly critical in retrogressive ecosystems (Laliberté et al, 2015), where declining soil nutrient availability results in declining vegetation stature and diversity (Richardson et al, 2004; Porder et al, 2007; Peltzer et al, 2010). Laliberté et al. (2015) suggest that the highly weathered, P-limited soils of retrogressive ecosystems favour

species with ephemeral roots to maximize nutrient uptake, but that this imposes a trade-off with increased susceptibility to pathogens. Ecosystem retrogression has been linked to increased specific root length (length per unit mass), thinner roots, and increased root branching (Holdaway et al, 2011), all of which may increase susceptibility to root pathogens (Laliberté et al, 2015).

Based on the three views from the literature, we tested the non-exclusive hypotheses that oomycetes would be associated with (1) early-successional, (2) mature, and/or (3) retrogressive ecosystems. We used an extensively studied soil and ecosystem development chronosequence created by the Franz Josef glacier in New Zealand, where glacial advances and retreats have created a series of soils of varying age in close proximity (Walker & Syers, 1976; Richardson et al, 2004), allowing space-for-time substitution as an approach to infer change over long time periods (Pickett, 1989). We focussed on oomycetes in living plant roots, in order to link oomycete communities to particular hosts. The presence of an oomycete in roots of specific plant species does not necessarily indicate that it causes disease in that plant. Indeed, mature plants may often tolerate organisms that would kill conspecific seedlings. It is also conceivable that oomycetes are living within the roots of plants on which they would never cause disease, regardless of plant age. Nonetheless, while the presence of oomycetes does not necessarily indicate disease, the absence of oomycetes from a plant can be taken as an indication of absence of oomycete-driven disease.

Materials and Methods

Study site and sampling

144 The Franz Josef chronosequence includes early-successional (5, 15, 70 years of
145 development), mature (280, 500, 1000, 5000, 12 000 years), and retrogressive (60 000, 120
146 000 years) sites. As soils age, there are dramatic changes in nutrient availability (declining P,
147 increasing and then decreasing N), pH, and physical properties (Walker & Syers, 1976).
148 These changes are linked to changes in plant communities, with plant biomass increasing
149 through succession to mature stages, and then entering retrogression where declining soil
150 nutrients result in a concomitant decline in plant biomass, stature, and diversity (Richardson
151 et al, 2004) and shifts in root traits (Holdaway et al, 2011). Vegetation shifts from sparse
152 herbaceous plants and sub-shrubs (5 years) to shrub land (15 years) to angiosperm forest (70
153 years), followed by an increasing dominance of large gymnosperm trees (Podocarpaceae)
154 through mature stages, with an eventual decline in plant biomass, canopy height, and canopy
155 closure in retrogression. Rainfall is high along the entire chronosequence, ranging from 3.5 to
156 6.5 m, and all sites are below 365 m elevation (Richardson et al, 2004).
157
158 In a previous study, we collected 510 individual plant roots from ten sites along the
159 chronosequence and characterised both plant identity and arbuscular mycorrhizal fungal
160 communities in those roots (Martínez-García et al, 2015). Here we use these same samples
161 but used taxon-specific primers to amplify DNA from oomycetes. The collection of samples
162 is described in full in Martinez-García *et al.* (2015). In brief, we collected 51 or 52 (at two
163 sites) individual fine root (< 2 mm diameter) fragments, taking a single root fragment
164 (approx. 15 mg dry weight) every 2 m along two parallel transects with equal sampling from
165 three depths (up to 20 cm depth max). This depth is likely representative of the established
166 roots a seedling might encounter during the first year of growth and could be sampled in a
167 standardised way at even our youngest sites, but may omit deeper roots in older sites. For the
168 5-year-old site, which comprised sparse vegetation in rocks, most soil was root free. We

169 therefore collected the nearest plant to the sample point and sampled one root from that plant.
170 Roots from all sites were thoroughly rinsed in water and dried before DNA extraction. DNA
171 was extracted using a MoBio soil DNA kit, and plant species identified by PCR amplification
172 and DNA sequencing of the tRNL gene region, except for the 5-year-old site, where the plant
173 was already known due to collection method. The one difference in the sampling between
174 this study and the prior study (Martínez-García et al, 2015) was that in the earlier paper a
175 spare ("B") sample was used if the first ("A") sample failed to yield both plant and
176 arbuscular-mycorrhizal fungal PCR products. In the present study we did not use the "B"
177 samples, as it would have made quantification difficult.
178
179 Oomycete PCR and identification
180
181 We primarily based our oomycete detection, operational taxonomic unit (OTU) clustering,
182 and identification on a nested PCR of large-subunit (28S) DNA (supplementary methods).
183 Initial results using T-RFLP suggested that most samples contained either no oomycetes or
184 only a single OTU, hence direct sequencing was used for identification. A negative (omitting
185 DNA template) and a positive control (including genomic DNA from a *Pythium* species)
186 were included in every PCR. PCR products were purified using DNA Clean and Concentrator
187 Kit (Zymo Research Corporation) prior to performing Sanger sequencing (Canterbury
188 Sequencing and Genotyping, University of Canterbury, New Zealand).
189
190 Some ecosystem ages had a high proportion of samples that failed to amplify a product of the
191 expected size in the first-round PCR. In order to ascertain whether these were true absences
192 of oomycetes or due to PCR inhibition, nine samples from each of these sites with the highest
193 failure rate (500, 100, 1200, 60 000 and 120 000 yrs) were tested for PCR inhibitors by

194 repeating PCR reactions in duplicate for each sample, with one of the duplicate reactions
195 spiked with 20 ng positive control DNA. Only one out of the 45 samples spiked with positive
196 control DNA failed to amplify, suggesting PCR inhibitors were not likely to be causing low
197 detection rates.
198
199 To confirm identities of sequenced oomycetes, samples that produced positive large subunit
200 PCR products were sequenced for the internal transcribed spacer (ITS) 1 region
201 (supplementary material). As DNA sequences were obtained from environmental samples
202 (root fragments) potentially containing multiple oomycetes, the ITS sequence may or may not
203 represent the same species as the 28S sequence. ITS sequences were therefore used to help
204 inform identification, but frequency analyses were based on the 28S results.
205
206 *Vegetation cover*
207
208 The percent cover of all vascular plants within sites was measured following the standard
209 relevé (or 'recce') method for describing New Zealand vegetation (Hurst & Allen, 2007) in a
210 20 x 20 m area located within 50 m of the root sample transects. For testing the correlation of
211 plant cover change with oomycete frequency we used the mid-point of vegetation cover
212 classes summed across height tiers as a measure of the abundance of each plant species.
213
214 *Sequence bioinformatics*
215
216 All DNA sequences were matched against GenBank using BLAST to find the closest
217 matching sequence, and, where the closest match had no reliable taxonomic identity, the
218 closest matching sequence associated with a taxonomic identity. Sequences that matched

219 non-oomycete specimens were discarded. Three sequences had a closest match to a
220 *Spongospora* (Cercozoa) sequence, but only at 78% identity. We therefore considered these
221 three sequences to likely be root-associated protists (Bulman & Braselton, 2014) and retained
222 them in the analysis. Out of 510 root samples, 122 had positive PCR products with oomycete
223 primers. After filtering for sequence quality and matching to an oomycete sequence, the final
224 dataset contained 91 DNA sequences of oomycetes with a mean sequence length of 310 bp.
225 Plant IDs were obtained for 458 samples overall and 86 of the 91 samples with oomycete
226 products through DNA sequencing of the trnL gene (Martínez-García et al, 2015). We
227 clustered sequences into OTUs using BLASTn to merge any sequences with > 97% similarity
228 over at least 95% of the shorter sequence length based on single-linkage clustering. This
229 allowed us to form species concepts for testing host specificity, resolved some matches to
230 "uncultured oomycete" and suggested where the gene region used lacked sufficient resolution
231 to distinguish species.
232
233 *Statistics*
234 Most of our analyses are based on the frequency of roots with oomycetes, testing the
235 prevalence of oomycetes within different plant species and sites, rather than quantifying
236 abundance within any particular root fragment. Changes in the frequency of oomycetes as a
237 function of successional stage were tested by treating age as a three-level factor. Sites were
238 allocated to successional stage levels on the basis of soil nutrient concentrations, vegetation
239 height, species richness and biomass, and plant traits (Walker & Syers, 1976; Richardson et
240 al, 2004). Early-successional sites (5, 15, 70 years) had abundant N-fixing trees; vascular
241 plant traits of high foliar nutrient concentrations, low leaf mass per unit area; very high soil P,
242 rapidly accumulating biomass and species richness of vascular plants, and increasing nitrate-
243 N. Mature ecosystems (280 to 12 000 years) had slow rates of change in species richness,

244 height and biomass among sites. These sites also had the highest biomass and plant diversity.
245 Retrogressive sites (60 000, 120 000 years) had a decline in vegetation height, richness and
246 biomass and exceptionally proficient phosphorus resorption (Richardson et al, 2005; Peltzer
247 et al, 2010; Vitousek et al, 2010).

248 We tested changes in the frequency of oomycetes as a function of successional stage
249 as a factor and log-transformed ecosystem age as a linear variate using a binomial glm, fitted
250 with quasi-likelihood to account for over-dispersion. The difference between the successional
251 stage and ecosystem age models was tested using analysis of deviance, using an F test as
252 recommended for quasibinomial fit. For these analyses n = 10 based on 512 individual
253 samples.

254 We wanted to test whether declining plant species had higher than random oomycete
255 levels, as would be expected if oomycetes are an important driver of community changes
256 with ecosystem development. To do so, we calculated the change in cover of each plant
257 species from one time point (t_0) to the next (t_1) as a log ratio (log((cover t_0 +1) / (cover t_1
258 +1))) and then used a binomial mixed effects model to test whether the presence / absence of
259 oomycetes in root fragments at t_0 could be predicted by change in cover from t_0 to t_1 across
260 the nine intervals between site ages. This test was carried out using lmer in the R package
261 lme4, with site age and plant species (to account for species occurring across multiple sites)
262 as random effects and the canonical logit link function. Host-specificity was tested within
263 ecosystem age using chi-square tests.

264

265 **Results**

266

267 *Oomycete frequency as a function of stage of ecosystem development and ecosystem age*

268

269 In early-successional ecosystems (5, 15 and 70 years), 38 to 65% of root samples had
270 oomycete DNA detected (Figure 1). In contrast, in mature and retrogressive ecosystems (280
271 to 120 000 years) an average of only 2% and never more than 8% of roots had oomycete
272 DNA found (Figure 1). Treating the sites as representing three stages, the early successional
273 stages had a significantly higher frequency of oomycetes than mature stages (quasibinomial
274 family glm; $t = -6.6$, $P = 0.00050$) or retrogressive stages ($t = -4.6$, $P = 0.0025$), and the
275 difference between mature and retrogressive ecosystems was not significant ($t = -1.1$, $P =$
276 0.32).
277
278 Oomycete frequency was also significantly related to log-transformed ecosystem age
279 (quasibinomial family glm; $t = -2.75$, $P = 0.025$). While statistically significant, this
280 relationship was a poor fit to the observed pattern of an increase for the first 3 site ages
281 followed by uniformly low levels of oomycetes at all subsequent site ages (Figure 1).
282 Analysis of deviance strongly supported the ecosystem-stage categorical model over the
283 linear ecosystem-age model ($F = 35.11$, $P = 0.00058$).
284
285 Those plant species that showed the largest decline in cover between ecosystem ages also had
286 the highest frequency of oomycetes in their roots (Figure 2, $z = -2.4$, $P = 0.018$).
287
288 *Oomycete diversity, host specificity and ecosystem age*
289
290 A total of 37 different OTUs were found, with 10 OTUs found more than once (Table 1). The
291 most frequent OTU occurred 17 times, and had DNA-sequence affinities to *Lagena*
292 *radicicola* and an uncultured oomycete from New York, USA agricultural soils (Table 1).
293

294 Within ecosystem ages, there was a significantly non-random distribution of oomycete OTU
295 identity across plant species at 5 (5 oomycete OTUs with 20 occurrences across 4 plant
296 species, $\chi^2 = 42.96$, df = 12, p-value = 0.000022) and 15 years (15 oomycete OTUs, 28
297 occurrences, 14 plant species, $\chi^2 = 225.87$, df = 182, p-value = 0.015), but only marginally
298 significant at 70 years (16 oomycete OTUs, 30 occurrences, 7 plant species, $\chi^2 = 109.85$, df =
299 90, p-value = 0.076; Figure 3). There were too few observations at the ecosystem stages after
300 70 years to meaningfully test for plant by OTU host-specificity at later ecosystem stages.
301
302 **Discussion**
303
304 Our results strongly support the importance of ecosystem age as a determinant of oomycete
305 communities in plant roots, with high frequencies of oomycetes observed in the three earliest
306 ecosystem stages (5, 15, and 70 years) but only very low levels thereafter. Although the
307 importance of oomycetes as pathogens is often discussed in the ecological literature (Bagchi
308 et al, 2014), relatively few studies have actually quantified oomycete frequency in natural
309 ecosystems (Gómez-Aparicio et al, 2012). Further, we believe our results are the first study of
310 oomycetes throughout a complete ecosystem development sequence. The community of
311 oomycetes was diverse, including multiple *Pythium* and *Phytophthora* species and,
312 surprisingly, a very frequent sequence matching *Lagena* on two small sub-shrubs: *Raolia*
313 *hookeri* (Asteraceae) and *Epilobium glabellum* (Onograceae). *Lagena* has previously only
314 been reported as a pathogen on grass (Blackwell, 2011).
315 In terrestrial ecosystems, oomycetes occur as pathogens and commensals of plants
316 and other eukaryotes and, less commonly, as saprotrophs (Marano et al, 2016). By sampling
317 plant roots, our results focussed primarily on endophytes. Our observation of high host-
318 specificity is also consistent with a biotrophic interaction and the correlation of oomycete

319 frequency with subsequent plant decline is consistent with pathogenicity. However, while we
320 believe that the oomycetes we observed were primarily biotrophic, the gene regions we
321 sequenced lacked sufficient resolution to rule out the possibility that some of the
322 *Phytophthora* we observed could be in clade 9, which contains some known saprotrophs
323 (Marano et al, 2016). The other clades of oomycetes where saprotrophy is known to occur
324 (Marano et al, 2016) were not observed.
325
326 *Ecological role of oomycetes in plant roots in early successional ecosystems*
327 It has been suggested that pathogens, including oomycetes, drive early-successional
328 change in plant communities (Kardol et al, 2006). The high frequency of oomycetes in early
329 successional ecosystems is consistent with this hypothesis. In addition, those plant species
330 that had the highest frequency of oomycetes at a given ecosystem age declined in percent
331 cover most strongly before the next age. The higher frequency of oomycetes on plant roots in
332 early-successional ecosystems compared to mature and retrogressive ecosystems was not due
333 to PCR inhibition in older sites, as samples spiked with positive control oomycete DNA
334 showed no evidence of inhibition.
335 Oomycetes, particularly *Pythium*, are often tolerated as endophytes by established
336 plants, but prevent seedling establishment of the same plant species (Martin & Loper, 1999;
337 Van Der Putten, 2003). On that basis, we do not argue that the observed oomycetes were
338 necessarily having any direct negative effect on established plants. Instead, we suggest that
339 the correlation of oomycete frequency with decline in cover between ecosystem ages is
340 congruent with the suggestion that these pathogens are primarily preventing re-establishment
341 of plants and hence contributing to vegetation change across cohorts (Van Der Putten, 2003).
342 Our definition of "early succession" comprises the major transition from rock field (5
343 years) to shrub land (15 years) to forest (70 years). Therefore, our finding of high pathogen

levels is potentially consistent with Packer & Clay (2000), who found strong negative feedback in *Prunus serotina* in a forest described as "at least 70 years old" (Packer & Clay, 2000), despite the differences between our primary succession and what was likely a secondary succession in the Packer & Clay study.

We believe most of the change in oomycete frequency across ecosystem stages reflects changes in plant species composition. While oomycete frequency was significantly correlated with site age as a linear predictor, treating sites as three distinct stages resulted in a much stronger model fit. Nonetheless, changes in oomycete frequency may also, in part, reflect a substantial site-age effect and direct effects of changing soil environments on oomycete populations (akin to the “habitat” hypothesis for mycorrhizal fungi of Zobel & Opik 2014). Meaningful testing of soil variables as predictors of oomycetes was not possible, as soil properties were strongly correlated with soil age.

Absence of oomycetes in plant roots in older ecosystems

It has been suggested that oomycetes are important in mature (Bagchi et al, 2010; Bagchi et al, 2014) and retrogressive ecosystems. While Packer and Clay (2000) clearly demonstrated oomycetes determined seedling dynamics in a relatively young temperate forest, a more recent study by Bagchi and colleagues (2014) in a tropical seasonal forest found that eliminating fungi alone had a stronger effect on negative density-dependence than eliminating both fungi and oomycetes. Laliberté et al. (2015) suggested that root traits adapted to highly P-limited retrogressive ecosystems would increase susceptibility to root pathogens, and that oomycetes could drive plant diversity in these ecosystems (Albornoz et al, 2017).

Our results do not support an important role of root associated oomycetes in this retrogressive sequence, which contrasts with the recent findings from a western Australian

369 system where oomycetes play key roles in older ecosystems (Albornoz et al, 2017). In part
370 this may reflect the importance of non-mycorrhizal Proteaceae in Australia. The very fine
371 roots of the Proteaceae may make this plant family particularly susceptible to pathogens
372 (Laliberté et al, 2015). Further comparative studies across multiple retrogressive ecosystems
373 are needed to resolve the circumstance under which each pattern is more common.
374
375 *Novel organisms or native?*
376 Having baseline data on oomycete ecology may be important for understanding and
377 managing future pathogen outbreaks. For example, *Phytophthora agathidicida* (=
378 *Phytophthora* taxon *Agathis*) has been implicated as a cause of forest dieback of the iconic
379 New Zealand-endemic *Agathis australis*, but, like many other oomycete disease outbreaks,
380 the origin of *Phytophthora agathidicida* remains uncertain (Weir et al, 2015). The single-
381 direction sequencing we performed directly from environmental samples was not designed
382 for accurate phylogenetic classification, even with two gene regions sequenced per sample,
383 but it does suggest considerable diversity of *Phytophthora*. We found sequences with
384 affinities to a number of species already known to be present in New Zealand, including *P.*
385 *cinnamomi*, *P. cactorum*, *P. infestans* and *P. kernoviae* (Scott & Williams, 2014). Given that
386 our samples came from relatively pristine ecosystems, we believe these OTUs are likely to be
387 from native *Phytophthora*.
388 More surprising was that the most common OTU in our data had affinities to *Lagena*
389 *radicicola* in both 28S and many of the ITS sequence results. *Lagena* is presently considered
390 to be a monotypic genus widespread as a pathogen of grasses in North America (Blackwell,
391 2011), but Barr and Désaulniers (1990) suggest it may be under-reported due to being
392 morphologically very similar to *Pythium*, and not being easily culturable. Spores described as
393 "resembling *Lagenocystis* [syn. *Lagena*] spp." were noted by Skipp and Christensen (1989) in

New Zealand *Lolium perenne* pastures, but our finding is the first report of a putative *Lagena* species outside of grasses. We believe this most likely reflects a lack of prior knowledge, as very few prior studies have used molecular methods to detect oomycetes from healthy forest ecosystems, and none of those have taken place in temperate southern hemisphere rainforest.

Conclusions

Much of the Earth's surface is covered in young, early successional ecosystems comparable to our early successional sites of 5, 15, and 70 years (Vitousek et al, 1997; Haddad et al, 2015). The strong plant-host specificity and correlation of plant decline and oomycete frequency is consistent with the suggestion that these oomycetes as pathogens contribute to vegetation succession. While we do not find frequent oomycetes in older ecosystems, oomycete DNA was detected in all but one site. These low levels of oomycetes in roots, along with oospores in soil, may still be sufficient to prevent establishment of susceptible plant species or to drive pathogen outbreaks following external climatic stressors, but seem incompatible with density dependent mortality being driven by oomycetes on established plant roots. More broadly, our results support the concept that oomycetes are a diverse part of native ecosystems. Understanding this native diversity in is critical to better understand, prevent, and manage future oomycete disease outbreaks (Dickie et al, 2017).

Acknowledgements. We thank D. Peltzer for help with locating plots, characterising vegetation, and editorial input, R. Holdaway for helping with data and other advice, H. Ridgway for providing positive control DNA, N. Merrick for sequencing, R. Buxton and C. Morse for collecting additional plant trait data, and S. Wakelin, L. Condron, J. Bufford, S. Bulman and the Mycorum discussion group at Lincoln University who provided valued advice and input. Funding was provided by Tertiary Education Commission funding for the

Bio-Protection Research Centre (I.A.D., AMW, JMT), Lincoln University Research Fund (IAD, AMW), a Rutherford Fellowship (JMT), and Core Funding to Crown Research Institutes (LMG, SR).

Data Accessibility

- DNA reference sequences: GenBank (accession numbers in Table 1)
- Vegetation data are archived in the New Zealand National Vegetation Survey Databank (https://nvs.landcareresearch.co.nz; last accessed 8 Feb 2016).

427 **Table 1.** Identity of non-singleton OTUs as clustered based on 28S, with accession numbers and best matching 28S and best matching ITS1

428 sequences, the inferred identity, and the number of sequences in the OTU.

28S accession	**Best match based on 28S (% identity / % length; sequence length)**	**ITS1 accession**	**Best match based on ITS (% identity / % length; sequence length)**	**Inferred identity (clustering based on 28S)**	**n**
KU863596	Uncultured oomycete 98%/98% 407 bp	KU863605	*Lagena radicicola* 93%/99% 205 bp	*Lagena* sp.	17
KU863595	*Pythium dissotocum* 97%/100% 379 bp	KU863604	*Pythium intermedium* 90%/47% 302 bp	*Pythium* aff. *aquatile/dissotocum*	13
KU863588	*Phytophthora cinnamomi* 99%/100% 385 bp	KU863598	*Phytophthora cinnamomi* 99%/100% 254 bp	*Phytophthora* aff. *cinnamomi*	8
KU863589	Uncultured oomycete 98%/100% 380 bp	KU863599	*Pythium* sp. 92%/100% 203 bp	*Pythium* sp.	6
KU863594	*Pythium tracheiphilum* 98%/99% 370 bp	KU863603	*Pythium tracheiphilum* 89%/98% 309 bp	*Pythium* aff. *tracheiphilum*	6
KU863593	*Pythium junctum*	KU863602	*Pythium irregulare*	*Pythium* aff. *junctum*	4

	98%/99% 378 bp		100%/28% 307 bp		
KU863597	*Phytophthora megasperma*	KU863606	*Phytophthora infestans*	*Phytophthora* sp. 1	4
	91%/100% 383 bp		95%/53% 447 bp		
KU863590	*Spongospora* sp.	N/A	No PCR product obtained	Chromista aff. *Spongospora*	3
	75%/79% 306 bp				
KU863591	*Phytophthora polonica*	KU863600	*Phytophthora kernoviae*	*Phytophthora* sp. 2	3
	99%/99% 384 bp		96%/99% 214 bp		
KU863592	*Pythium violae*	KU863601	*Pythium* sp.	*Pythium violae*	3
	98%/100% 368 bp		99%/99% 286 bp		

429

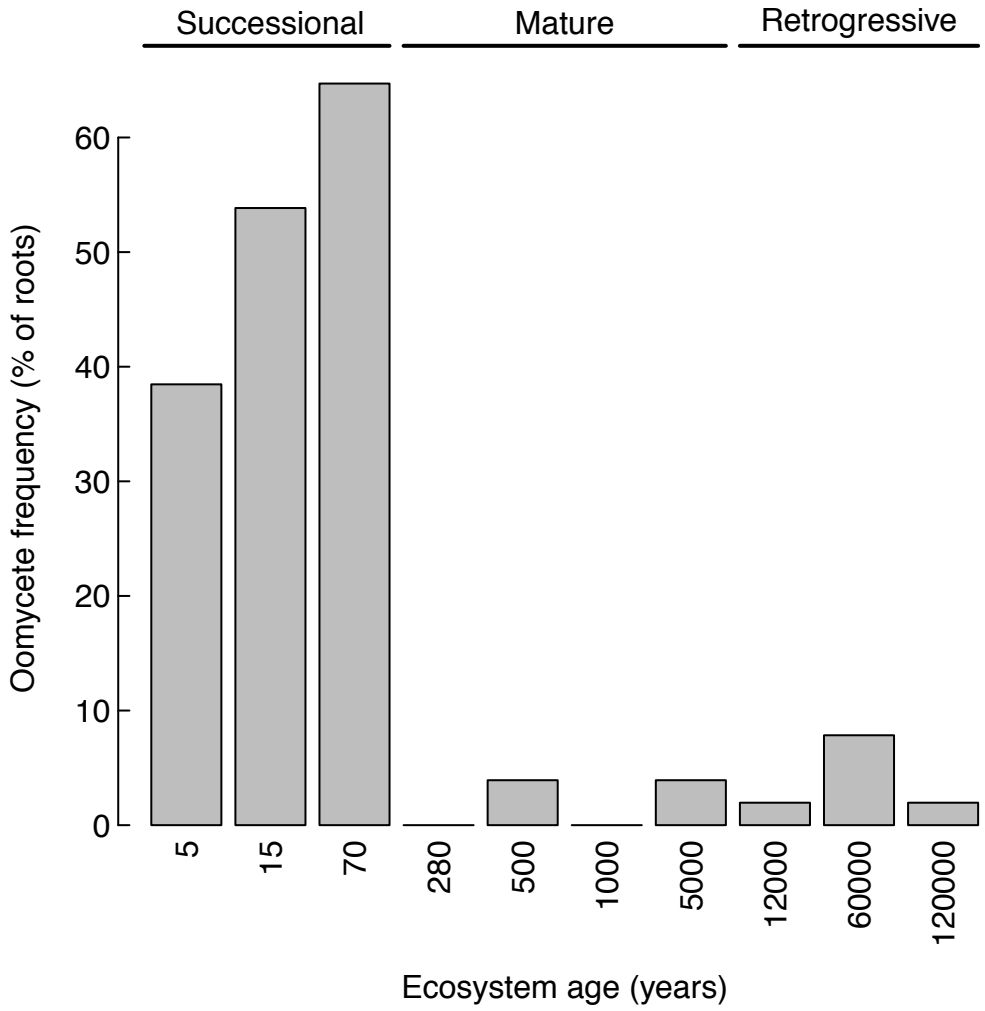

Figure 1. Frequency of oomycetes in plant roots (%) as a function of ecosystem age. The 5, 15, and 70-year-old sites represent a successional sere from rock field through shrubland to angiosperm forest. Sites 280 to 5000 years of age are mature forest with high biomass and plant diversity, while after 12 000 to 60 000 years plant biomass, stature, and diversity decline due to retrogressive P-limitation.

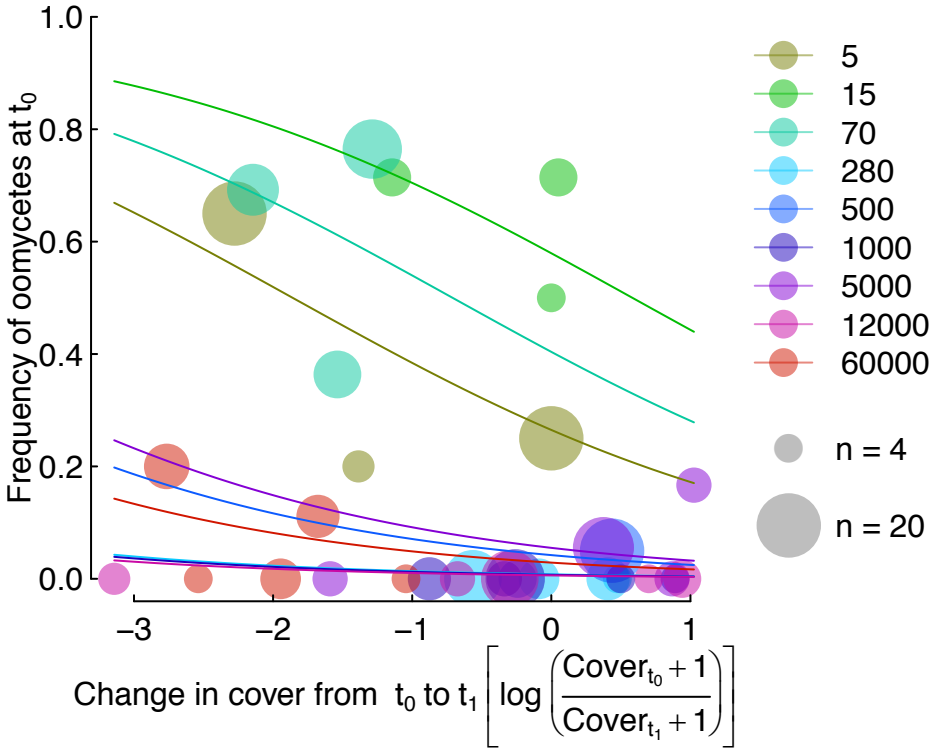

Figure 2. Oomycete frequency as a function of vegetation decline with circles representing each plant species between site transitions. Points scaled by number of samples for each plant species within site. Lines indicate random effects to account for site in a binary mixed effects model with random intercepts for site and plant species. Colour coding in reflects the site age at t_0 for each transition, hence no points for 120 000 years are shown.

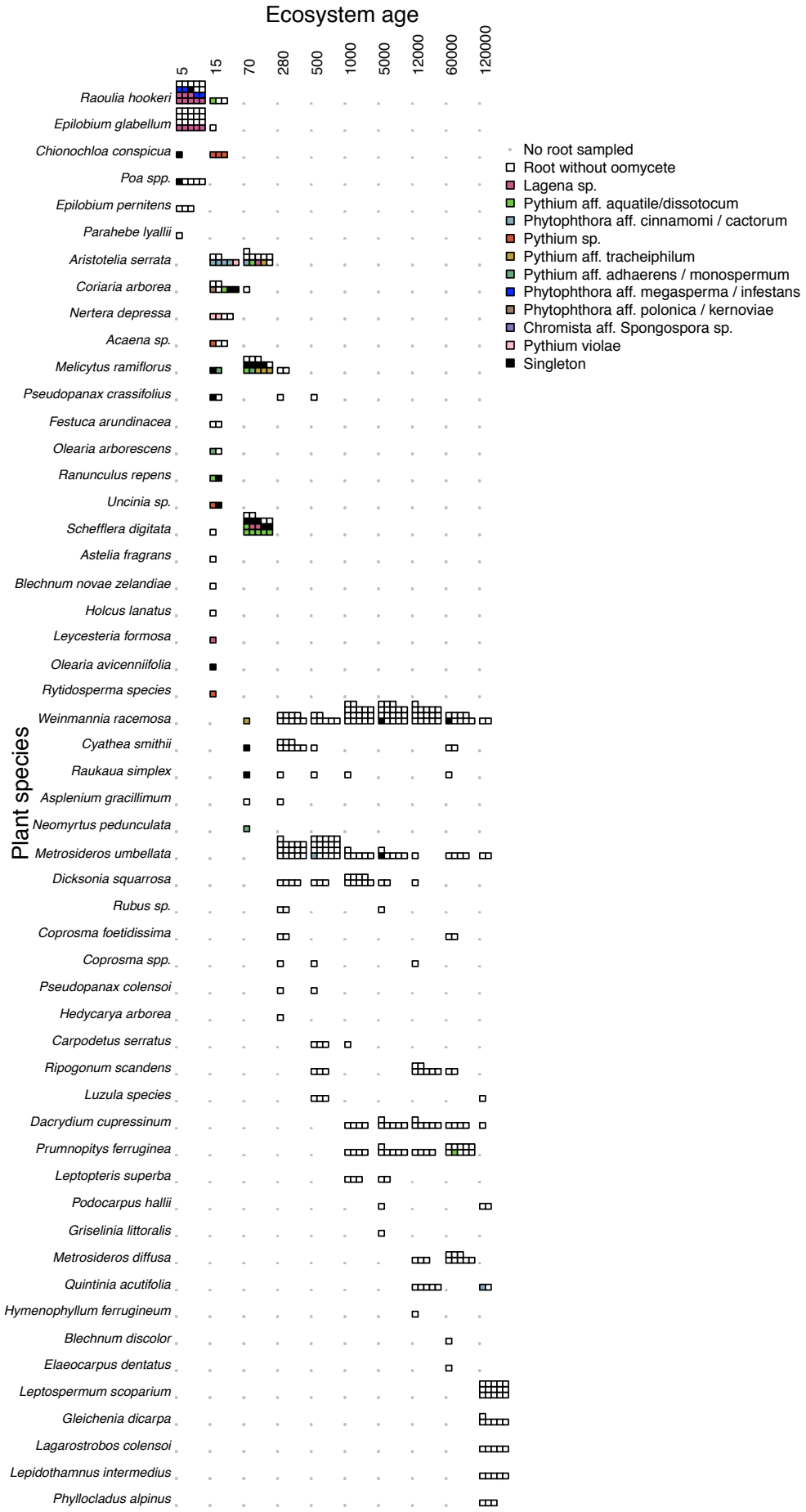
Ecosystem age
5
15
70
280
500
1000
5000
12000
60000
120000
Plant species
Raoulia hookeri
Epilobium glabellum
Chionochloa conspicua
Poa spp.
Epilobium pernitens
Parahebe lyallii
Aristotelia serrata
Coriaria arborea
Nertera depressa
Acaena sp.
Melicytus ramiflorus
Pseudopanax crassifolius
Festuca arundinacea
Olearia arborescens
Ranunculus repens
Uncinia sp.
Schefflera digitata
Astelia fragrans
Blechnum novae zelandiae
Holcus lanatus
Leycesteria formosa
Olearia avicenniifolia
Rytidosperma species
Weinmannia racemosa
Cyathea smithii
Raukaua simplex
Asplenium gracillimum
Neomyrtus pedunculata
Metrosideros umbellata
Dicksonia squarrosa
Rubus sp.
Coprosma foetidissima
Coprosma spp.
Pseudopanax colensoi
Hedycarya arborea
Carpodetus serratus
Ripogonum scandens
Luzula species
Dacrydium cupressinum
Prumnopitys ferruginea
Leptopteris superba
Podocarpus hallii
Griselinia littoralis
Metrosideros diffusa
Quintinia acutifolia
Hymenophyllum ferrugineum
Blechnum discolor
Elaeocarpus dentatus
Leptospermum scoparium
Gleichenia dicarpa
Lagarostrobos colensoi
Lepidothamnus intermedius
Phyllocladus alpinus
No root sampled
Root without oomycete
Lagena sp.
Pythium aff. aquatile/dissotocum
Phytophthora aff. cinnamomi / cactorum
Pythium sp.
Pythium aff. tracheiphilum
Pythium aff. adhaerens / monospermum
Phytophthora aff. megasperma / infestans
Phytophthora aff. polonica / kernoviae
Chromista aff. Spongospora sp.
Pythium violae
Singleton

Figure 3. Plant by site age visualisation of oomycete frequency within sampled roots. Each square represents a single root sample, with coloured squares indicating the presence of oomycete pathogens with colours indicating OTU and black indicating an OTU found only once. See Table 1 for OTU information. Grey dots indicate species not found in root samples for that site.

Supplemental files S1: Detailed molecular methods and discussion.

453 **References**

454 Albornoz FE, Burgess TI, Lambers H, Etchells H, Laliberté E. (2017). Native soilborne
455 pathogens equalize differences in competitive ability between plants of contrasting
456 nutrient-acquisition strategies. *Journal of Ecology* 105: 549–557.

457 Arcate JM, Karp MA, Nelson EB. (2006). Diversity of peronosporomycete (oomycete)
458 communities associated with the rhizosphere of different plant species. *Microbial*
459 *Ecology* 51: 36–50.

460 Augspurger CK. (1984). Seedling survival of tropical tree species: interactions of dispersal
461 distance, light-gaps, and pathogens. *Ecology* 65: 1705–1712.

462 Bagchi R, Gallery RE, Gripenberg S, Gurr SJ, Narayan L, Addis CE *et al.* (2014). Pathogens
463 and insect herbivores drive rainforest plant diversity and composition. *Nature* 506: 85–
464 88.

465 Bagchi R, Swinfield T, Gallery RE, Lewis OT, Gripenberg S, Narayan L *et al.* (2010).
466 Testing the Janzen-Connell mechanism: pathogens cause overcompensating density
467 dependence in a tropical tree. *Ecology Letters* 13: 1262–1269.

468 Bardgett RD, Bowman WD, Kaufmann R, Schmidt SK. (2005). A temporal approach to
469 linking aboveground and belowground ecology. *TRENDS IN ECOLOGY &*
470 *EVOLUTION* 20: 634–641.

471 Barr DJS, Désaulniers NL. (1990). The life cycle of Lagena radicicola, an oomycetous
472 parasite of wheat roots. *Canadian Journal of Botany* 68: 813–824.

473 Bever JD, Mangan SA, Alexander HM. (2015). Maintenance of plant species diversity by
474 pathogens. *Annual review of ecology, evolution, and systematics* 46: 305–325.

475 Blackwell WH. (2011). The genus Lagena (Stramenopila: Oomycota), taxonomic history and
476 nomenclature. *Phytologia* 93: 157–167.

477 Bulman S, Braselton JP. (2014). 4 Rhizaria: Phytomyxea. eds. *Systematics and evolution*.

Springer, 99–112.

Castello JD, Leopold DJ, Smallidge PJ. (1995). Pathogens, patterns, and processes in forest ecosystems. *Bioscience* 45: 16–24.

De Deyn GB, Raaijmakers CE, Zoomer HR, Berg MP, de Ruiter PC, Verhoef HA *et al.* (2003). Soil invertebrate fauna enhances grassland succession and diversity. *NATURE* 422: 711–713.

Dickie IA, Bufford JL, Cobb RC, Desprez-Loustau M, Grelet G, Hulme PE *et al.* (2017). The emerging science of linked plant–fungal invasions. *New Phytologist* 215: 1314–1332.

Geisen S, Tveit AT, Clark IM, Richter A, Svenning MM, Bonkowski M *et al.* (2015). Metatranscriptomic census of active protists in soils. *The ISME journal* 9: 2178.

Gilbert GS. (2002). Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* 40: 13–43.

Gómez-Aparicio L, Ibánez B, Serrano MS, De Vita P, Avila JM, Pérez-Ramos IM *et al.* (2012). Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. *New Phytologist* 194: 1014–1024.

Haddad NM, Brudvig LA, Clobert J, Davies KF, Gonzalez A, Holt RD *et al.* (2015). Habitat fragmentation and its lasting impact on Earth's ecosystems. *Science Advances* 1: e1500052.

Holdaway RJ, Richardson SJ, Dickie IA, Peltzer DA, Coomes DA. (2011). Species- and community-level patterns in fine root traits along a 120 000-year soil chronosequence in temperate rain forest. *Journal of Ecology* 99: 954–963.

Hurst JM, Allen RB. (2007). The recce method for describing New Zealad vegetation: field protocols.

Kardol P, Martijn Bezemer T, Van Der Putten WH. (2006). Temporal variation in plant–soil feedback controls succession. *Ecology Letters* 9: 1080–1088.

503 Laliberté E, Lambers H, Burgess TI, Joseph, Wright S. (2015). Phosphorus limitation, soil-
504 borne pathogens and the coexistence of plant species in hyperdiverse forests and
505 shrublands. *New Phytologist* 206: 507–521.
506 Lambers H, Raven JA, Shaver GR, Smith SE. (2008). Plant nutrient-acquisition strategies
507 change with soil age. *Trends Ecol Evol* 23: 95–103.
508 Mangan SA, Herre EA, Bever JD. (2010a). Specificity between Neotropical tree seedlings
509 and their fungal mutualists leads to plant-soil feedback. *Ecology* 91: 2594–2603.
510 Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI *et al.* (2010b).
511 Negative plant-soil feedback predicts tree-species relative abundance in a tropical
512 forest. *Nature* 466: 752–755.
513 Marano AV, Jesus AL, de Souza JI, Jerônimo GH, Gonçalves DR, Boro MC *et al.* (2016).
514 Ecological roles of saprotrophic Peronosporales (Oomycetes, Straminipila) in natural
515 environments. *Fungal Ecology* 19: 77–88.
516 Martin FN, Loper JE. (1999). Soilborne plant diseases caused by Pythium spp.: ecology,
517 epidemiology, and prospects for biological control. *Critical reviews in plant sciences*
518 18: 111–181.
519 Martínez-García LB, Richardson SJ, Tylianakis JM, Peltzer DA, Dickie IA. (2015). Host
520 identity is a dominant driver of mycorrhizal fungal community composition during
521 ecosystem development. *New Phytologist* 205: 1565–1576.
522 Packer A, Clay K. (2000). Soil pathogens and spatial patterns of seedling mortality in a
523 temperate tree. *Nature* 404: 278–281.
524 Packer A, Clay K. (2003). Soil pathogens and *Prunus serotina* seedling and sapling growth
525 near conspecific trees. *Ecology* 84: 108–119.
526 Peltzer DA, Wardle DA, Allison VJ, Baisden WT, Bardgett RD, Chadwick OA *et al.* (2010).
527 Understanding ecosystem retrogression. *Ecological Monographs* 80: 509–529.

Pickett STA. (1989). Space-for-time substitution as an alternative to long-term studies. *Long-term studies in ecology: approaches and alternatives* 110–135.

Podger FD, Newhook FJ. (1971). Phytophthora cinnamomi in indigenous plant communities in New Zealand. *New Zealand Journal of Botany* 9: 625–638.

Porder S, Vitousek PM, Chadwick OA, Chamberlain CP, Hilley GE. (2007). Uplift, Erosion, and Phosphorus Limitation in Terrestrial Ecosystems. *Ecosystems* 10: 159–171.

Reinhart KO, Johnson D, Clay K. (2012). Conspecific plant-soil feedbacks of temperate tree species in the southern Appalachians, USA. *PloS one* 7: e40680.

Reynolds HL, Packer A, Bever JD, Clay K. (2003). Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *ECOLOGY* 84: 2281–2291.

Richardson SJ, Peltzer DA, Allen RB, McGlone MS. (2005). Resorption proficiency along a chronosequence: responses among communities and within species. *Ecology* 86: 20–25.

Richardson SJ, Peltzer DA, Allen RB, McGlone MS, Parfitt RL. (2004). Rapid development of phosphorus limitation in temperate rainforest along the Franz Josef soil chronosequence. *Oecologia* 139: 267–276.

Scott P, Williams N. (2014). Phytophthora diseases in New Zealand forests. *NZ Journal of Forestry* 59: 15.

Skipp RA, Christensen MJ. (1989). Fungi invading roots of perennial ryegrass (Lolium perenne L.) in pasture. *New Zealand Journal of Agricultural Research* 32: 423–431.

Van der Putten WH, Dijk CV, Peters BAM. (1993). Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature* 362: 53–56.

Van Der Putten WH. (2003). Plant defense belowground and spatiotemporal processes in natural vegetation. *ECOLOGY* 84: 2269–2280.

Veblen TT. (1992). Regeneration dynamics. *Plant succession: Theory and prediction* 11:

553 152–187.

554 Vitousek PM, Porder S, Houlton BZ, Chadwick OA. (2010). Terrestrial phosphorus
555 limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological*
556 *Applications* 20: 5–15.

557 Vitousek PM, Mooney HA, Lubchenco J, Melillo JM. (1997). Human domination of Earth's
558 ecosystems. *Science* 277: 494–499.

559 Walker TW, Syers JK. (1976). The fate of phosphorus during pedogenesis. *Geoderma* 15: 1–
560 19.

561 Weir BS, Paderes EP, Anand N, Uchida JY, Pennycook SR, Bellgard SE *et al.* (2015). A
562 taxonomic revision of Phytophthora Clade 5 including two new species, Phytophthora
563 agathidicida and P. cocois. *Phytotaxa* 205: 21–38.

564