Plant root pathogens over 120,000 years of temperate rainforest ecosystem development

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running title: **Plant pathogens and ecosystem development**

Summary

1. The role of pathogens, including oomycetes, in long-term ecosystem development has remained largely unknown, despite hypotheses that pathogens drive primary succession, determine mature ecosystem plant diversity, or dominate in retrogressive, nutrient-limited ecosystems.
2. Using DNA sequencing from roots, we investigated the frequency and host relationships of 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).
3. Oomycetes were frequent in early successional sites occurring in 38 – 65% of plant roots, but rare (average 3%) in all older ecosystems.
4. Oomycetes were highly host specific, and more frequent on those plant species that declined most strongly in abundance between ecosystem ages.
5. In contrast, oomycetes were not correlated with plant abundance or plant root traits associated with retrogression.
6. *Synthesis.* The results support the potential importance of oomycete pathogens in early succession, but not thereafter, suggesting oomycete pathogen driven dynamics may be important in driving succession but not long-term diversity maintenance.

Keywords: *Determinants of plant community diversity and structure, forest diversity, molecular ecology, Oomycetes, pathogen ecology, pedogenesis, plant–soil (below-ground) interactions, retrogression, succession*

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 pathogens, remain poorly understood. Indeed, plant pathogens in natural ecosystems are often out-of-sight out-of-mind, until large-scale forest diebacks (Scott and Williams, 2014) occur. This lack of information is particularly apparent in forest disease outbreaks, where it is often unclear whether the pathogen is native, from where it may have originated, or what background levels of pathogens are typical in healthy ecosystems (Podger and Newhook, 1971). Pathogens occur as part of healthy ecosystems and play an important role in ecosystem processes (Castello *et al.*, 1995; Gomez-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 and Clay, 2000) and hence are believed to contribute to the maintenance of forest plant diversity (Mangan *et al.*, 2010a). Pathogenic organisms, including 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 pathogens 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 persepective, 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 to dispersion 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 a compositional change, favouring species with life history traits that are adapted to stressful, low-nutrient environments. While these concepts are well developed for plants, the ecological literature is highly divided in predicting when pathogens might be important in ecosystem succession and development.

One view of soil pathogens in relation to ecosystem development is that they play a particularly 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 and 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). Pathogens have also been shown to be important in the mortality of the early-successional, shade intolerant tree *Prunus serotina* (Packer and Clay, 2000; Packer and Clay, 2003). These examples span from short-term grassland to decadal scale forest patterns, suggesting a widely applicable ecological pattern.

A second view is that pathogens are of little importance early in succession, but increase in importance in mature ecosystems (Reynolds *et al.*, 2003; Peltzer *et al.*, 2010; Bardgett *et al.*, 2005). 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, 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). Density dependence may occur within a cohort, driven by a high density of co-occurring conspecific seedlings near seed sources, or cross-cohort, with mature plants supporting high levels of pathogens on their roots and hence reducing establishment of conspecific seedlings (Connell, 1970), but most studies do not distinguish these two possibilities. Evidence for negative density dependence has come from both tropical and temperate forests (Mangan *et al.*, 2010b; Packer and Clay, 2003), although other studies have found positive density dependence or no effect (Reinhart *et al.*, 2012).

121 One of the signatures of density dependence should be a correlation between plant abundance
122 and plant-soil feedback strength, where soil feedbacks are defined as the growth of seedlings
123 of a species in a soil community developing under con-specific plants relative to other species
124 in the same soil (Bever *et al.*, 2010). Several studies suggest that the correlation between plant
125 abundance and seedling survival rates should be negative, with more abundant species
126 showing lower seedling survival rates (Queenborough *et al.*, 2007). However, positive
127 correlations of plant abundance and soil feedbacks, with abundant species exhibiting weaker
128 negative density dependence, have also been taken as evidence supporting pathogen driven
129 density-dependence as a mechanism for supporting mature forest plant diversity (Xu *et al.*,
130 2015). A logical argument can be made for either direction of correlation, depending on
131 whether you assume that observed abundances are the outcome of prior negative density
132 dependence (Xu *et al.*, 2015) as opposed to drivers of current negative density dependence.
133 Direct measurement of pathogen frequency is not typically part of plant-soil feedback studies,
134 but strong negative feedback is often assumed to indicate high pathogen loads. Nonetheless,
135 other soil organisms, including mycorrhizas, can drive similar outcomes (Bever *et al.*, 2010).
136
137 Finally, a recently proposed third view of the role of pathogens in ecosystem development is
138 that soil pathogens may be particularly critical in retrogressive ecosystems (Laliberté *et al.*,
139 2015), where declining soil nutrient availability results in declining vegetation stature and
140 diversity (Richardson *et al.*, 2004; Porder *et al.*, 2007; Peltzer *et al.*, 2010). Laliberté and colleagues
141 (2015) suggest that the highly weathered, P-limited soils of retrogressive ecosystems favour
142 species with ephemeral roots to maximize nutrient uptake, but that this imposes a trade-off
143 with increased susceptibility to pathogens. Ecosystem retrogression has been linked to
144 increased specific root length (length per unit mass), thinner roots, and increased root
145 branching (Holdaway *et al.*, 2011), all of which may increase susceptibility to root pathogens

146 (Laliberté *et al.*, 2015). This hypothesis makes no explicit prediction about how plant
147 abundance will be correlated with pathogens.
148
149 We formalized each of these views from the literature into three testable, non-mutually
150 exclusive, sets of hypotheses. Each set makes specific predictions about the occurrence of
151 pathogens at different ecosystem stages (H1.1, H2.1, H3.1) and the occurrence of pathogens
152 on different hosts based on plant strategies and root traits (H1.2, H2.2, H3.2). We test these
153 hypotheses by focusing on infection rates (the presence or absence within fine roots) of plants by
154 oomycetes, a taxon that, when found inside living plant tissue, is frequently associated with a
155 pathogenic lifestyle, and that is known to have large ecological impacts on forest ecosystems. We do
156 not attempt here to demonstrate pathogenicity, but rather use the frequency of infection as a measure
157 of pathogen incidence in the plant community.
158
159 H1. Early-successional ecosystem hypotheses
160 H1.1 Pathogen frequency and diversity are high in early-successional ecosystems relative
161 to later stages.
162 H1.2 Pathogen-driven succession will be evident as plant species that decline in
163 abundance during succession having higher pathogen loads than plant species that
164 increase or maintain their abundance.
165 H2. Mature ecosystem hypotheses
166 H2.1 Pathogen frequency and diversity are high in mature ecosystems relative to other
167 stages.
168 H2.2 Negative density-dependent mortality in mature ecosystems will be evident as a
169 correlation between plant abundance and pathogen frequency, either positively
170 (Queenborough *et al.*, 2007; Bever *et al.*, 2015), or negatively (Xu *et al.*, 2015).
171 H3. Retrogression hypotheses:

172 H3.1 Pathogen frequency is high in retrogressive ecosystems relative to earlier stages.
173 H3.2 Trade-offs between nutrient acquisition and pathogen resistance will be evident as
174 plant species with high specific root length, thinner roots, and abundant root hairs having
175 higher pathogen loads than species without these traits.
176
177 In addition, we tested the hypothesis (H4) that oomycete communities show host-specificity
178 or at least a degree of host-preference in their plant associations, as host specificity may
179 contribute to pathogens driving early-successional vegetation change (H1) or to maintaining
180 plant diversity in mature ecosystems (H2).
181
182 All of our hypotheses are structured around treating ecosystem age on the basis of vegetation
183 dynamics, either as three stages (H1.1, 2.1, 3.1) or as processes (1.2,2.2,3.2). An alternative
184 view would be that oomycetes respond to environmental changes associated with ecosystem
185 age, rather than these three stages. We therefore also tested the hypothesis (H5) that
186 oomycete frequency would be linearly related to ecosystem age across the 10 sites. We did
187 not test the effect of particular soil drivers (e.g., pH, phosphorus fractions, nitrogen) as these
188 are too strongly correlated with ecosystem age to be disentangled without much greater
189 replication at the site level.
190
191 We tested our hypotheses using an extensively studied soil and ecosystem development
192 chronosequence created by the Franz Josef glacier in New Zealand, where glacial advances
193 and retreats have created a series of soils of varying age in close proximity (Walker and Syers,
194 1976; Richardson *et al.*, 2004), allowing space-for-time substitution as an approach to infer
195 change over long time periods (Pickett, 1989). We focused on oomycetes as widespread
196 pathogens that have been directly linked to plant succession and negative density-dependence

in forest ecosystems, as well as forest die-back (Packer and Clay, 2000). Using direct DNA sequencing from roots, we provide the first study of pathogen dynamics throughout ecosystem development from early-succession to retrogression, and find strong support for the role of oomycetes in early-succession but not in later stages of ecosystem development.

Methods

Study site and sampling

The Franz Josef chronosequence includes early successional (5, 15, 70 years of development), mature (280, 500, 1000, 5000, 12 000 years), and retrogressive (60 000, 120 000 years) sites. As soils age, there are dramatic changes in nutrient availability (declining P, increasing and then decreasing N), pH, and physical properties (Walker and Syers, 1976). These changes are linked to changes in plant communities, with plant biomass increasing through succession to mature stages, and then entering retrogression where declining soil nutrients result in a concomitant decline in plant biomass, stature, and diversity (Richardson *et al.*, 2004) and shifts in root traits (Holdaway *et al.*, 2011). Vegetation shifts from a rock field with sparse herbaceous plants and sub-shrubs (5 years) to shrub land (15 years) to angiosperm forest (70 years), followed by an increasing dominance of large gymnosperm trees (Podocarpaceae) through mature stages, with an eventual decline in plant biomass, canopy height, and canopy closure in retrogression. Rainfall is high along the entire chronosequence, ranging from 3.5 to 6.5 m, and all sites are below 365 m elevation (Richardson *et al.*, 2004).

221 In a previous study we collected 510 individual plant roots from ten sites along the
222 chronosequence and characterized both plant identity and arbuscular mycorrhizal fungal
223 communities in those roots (Martinez-Garcia *et al.*, 2015). Here we use these same samples but
224 used taxon-specific primers to amplify DNA from oomycetes. The collection of samples is
225 described in full in Martinez-Garcia *et al.* (2015). In brief, we collected 51 (or 52 due to a
226 slight error at 2 sites) individual fine root (< 2 mm diameter) fragments, taking a single root
227 fragment (approx. 15 mg dry weight) every 2 m along two parallel transects with equal
228 sampling from three depths (up to 20 cm depth max). This depth is likely representative of
229 the established roots a seedling might encounter during the first year of growth (and hence
230 most relevant to our hypotheses) and could be sampled in a standardised way at even our
231 youngest sites, though it omits deeper roots in older sites. For the 5-year-old site, which
232 comprised sparse vegetation in rocks, we collected the nearest plant to the sample point and
233 sampled one root from that plant. This was a necessary given that most of the soil at the 5-
234 year-old site was root free. Roots from all sites were thoroughly rinsed in water and dried
235 before DNA extraction. DNA was extracted using a MoBio soil DNA kit, and plant species
236 identified by PCR amplification and DNA sequencing of the tRNL gene region, except for
237 the 5-year-old site, where the plant was already known due to collection method. The one
238 difference in the sampling between this study and the prior study (Martinez-Garcia *et al.*, 2015)
239 was that in the earlier paper a spare ("B") sample was used if the first ("A") sample failed to
240 yield both plant and arbuscular-mycorrhizal fungal PCR products. In the present study we did
241 not use the "B" samples, as it would have made quantification difficult.
242
243 Oomycete PCR and identification
244

245 We primarily based our oomycete detection, operational taxonomic unit (OTU) clustering,
246 and identification on a nested PCR of large-subunit (28S) DNA (supplementary methods).
247 Initial results using T-RFLP suggested most samples contained either no oomycetes or only a
248 single OTU, hence direct sequencing was used for identification. A negative (omitting DNA
249 template) and a positive control (including genomic DNA from a *Pythium* species) were
250 included in every PCR. PCR products were purified using DNA Clean and Concentrator Kit
251 (Zymo Research Corporation) prior to performing Sanger sequencing (Canterbury
252 Sequencing and Genotyping, University of Canterbury, New Zealand). I
253
254 Some ecosystem ages had a high proportion of samples that failed to amplify a product of the
255 expected size in the first-round PCR. In order to ascertain whether these were true absences
256 of oomycetes or due to PCR inhibition, nine samples from each of these sites with the highest
257 failure rate (500, 100, 1200, 60 000 and 120 000 yrs) were tested for PCR inhibitors by
258 repeating PCR reactions in duplicate for each sample, with one of the duplicate reactions
259 spiked with 20 ng positive control DNA. Only one out of the 45 samples spiked with positive
260 control DNA failed to amplify, suggesting PCR inhibitors were not likely to be causing low
261 detection rates.
262
263 To confirm identities of sequenced oomycetes, samples that produced positive large subunit
264 PCR products were sequenced for the internal transcribed spacer (ITS) 1 region
265 (supplementary material). As DNA sequences were obtained from environmental samples
266 (root fragments) potentially containing multiple oomycetes, the ITS sequence may or may not
267 represent the same species as the 28S sequence. ITS sequences were therefore used to help
268 inform identification, but frequency analyses were based on the 28S results.
269

270 *Sequence bioinformatics*

271

272 All DNA sequences were matched against GenBank using BLAST to find the closest
273 matching sequence, and, where the closest match had no reliable taxonomic identity, the
274 closest matching sequence associated with a taxonomic identity. Sequences that matched
275 non-oomycete specimens were discarded. Three sequences had a closest match to a
276 *Spongospora* (Cercozoa) sequence, but only at 78% identity. We therefore considered these
277 three sequences to likely be root-associated pathogens (Bulman and Braselton, 2014) without
278 making any strong claim as to their actual identity, and retained them in the analysis. Out of
279 510 root samples, 122 had positive PCR products with oomycete primers. After filtering for
280 sequence quality and matching to an oomycete sequence, the final dataset contained 91 DNA
281 sequences of oomycetes with a mean sequence length of 310 bp. Plant IDs were obtained for
282 458 samples overall and 86 of the 91 samples with oomycete products through DNA
283 sequencing of the trnL gene (Martinez-Garcia *et al.*, 2015). We clustered sequences into OTUs
284 using BLASTn to merge any sequences with > 97% similarity over at least 95% of the
285 shorter sequence length based on single-linkage clustering. This allows us to form species
286 concepts for testing host specificity (H4), resolved some matches to "uncultured oomycete"
287 and suggested where the gene region used lacked sufficient resolution to distinguish species.
288 Finally, we assembled all of our environmental DNA sequences and a selection of named
289 sequences from GenBank to examine how our OTUs were reflected in phylogenetic
290 clustering. Phylogenetic clustering was determined using Clustal Omega for alignment and
291 ClustalW2 phylogeny based on both UPGMA clustering (ebi.ac.uk). The single-linkage
292 BLAST clustering and phylogenetic clustering were broadly consistent.

293

294 *Root traits*

Root trait data (root diameter, root hairs per mm, and specific root length (SRL)) had been previously obtained for most of our plant species in Holdaway *et al.* (2011). Intraspecific root trait variability across sites is minimal (Holdaway *et al.* (2011). We collected new trait data for two species that were abundant in our data but missing from Holdaway *et al.* (2011), *Raoulia hookeri* and *Epilobium glabellum*. Additional trait data were obtained for *Nertera depressa* root diameter from Johnson (1976). Roots that could not be identified to species (e.g., *Coprosma* spp.) were excluded from root trait analysis due to high variability of traits within genus (Holdaway *et al.*, 2011).

Vegetation cover

The percent cover of all vascular plants within sites was measured following standard protocols (Hurst and Allen, 2007) in a 20 x 20 m area located within 50 m of the root sample transects. For testing H1.2 and 2.2 we used the mid-point of vegetation cover classes summed across height tiers as a measure of the abundance of each plant species. Vegetation data are archived in the New Zealand National Vegetation Survey Databank (https://nvs.landcareresearch.co.nz; last accessed 8 Feb 2016).

Statistics

Most of our analyses are based on the frequency of roots with oomycetes, testing the prevalence of oomycetes within different plant species and sites, rather than quantifying abundance within any particular root fragment. Changes in the frequency of oomycetes as a function of successional stage were tested by treating age as a three-level factor. Sites were allocated to successional stage levels on the basis of soil nutrient concentrations, vegetation

320 height, species richness and biomass, and plant traits (Walker and Syers, 1976; Richardson *et al.*,
321 2004). Early-successional sites (5, 15, 70 years) had abundant N-fixing trees; vascular plant
322 traits of high foliar nutrient concentrations, low leaf mass per unit area; very high soil P,
323 rapidly accumulating biomass and species richness of vascular plants, and increasing nitrate-
324 N. Mature ecosystems (280 to 12 000 years) had slow rates of change in species richness,
325 height and biomass among sites. These sites also had the highest biomass and plant diversity.
326 Retrogressive sites (60 000, 120 000 years) had a decline in vegetation height, richness and
327 biomass and exceptionally proficient phosphorus resorption (Richardson *et al.*, 2005; Peltzer *et*
328 *al.*, 2010; Vitousek *et al.*, 2010).

329 We tested changes in the frequency of oomycetes as a function of successional stage
330 as a factor (H1.1, 1.2 and 1.3) and log-transformed ecosystem age as a linear variate (H5)
331 using a binomial glm, fitted with quasi-likelihood to account for over-dispersion. The
332 difference between the successional stage and ecosystem age models was tested using
333 analysis of deviance, using an F test as recommended for quasibinomial fit. For these
334 analyses n = 10 based on 512 individual samples. To test whether declining plant species had
335 higher than random oomycete levels (H1.2), we calculated the change in cover of each plant
336 species from one time point (t_0) to the next (t_1) as a log ratio (log((cover t_0 +1) / (cover t_1
337 +1))) and then used a binomial mixed effects model to test whether the presence / absence of
338 oomycetes in root fragments at t_0 could be predicted by change in cover from t_0 to t_1 across
339 the nine intervals between site ages. This test was carried out using lmer in the R package
340 lme4, with site age and plant species (to account for species occurring across multiple sites)
341 as random effects and the canonical logit link function. For trait correlations, only plant
342 observations with more than three root samples total were included in order to have some
343 degree of confidence in oomycete frequency estimation. For the test of H1.2 this results in a
344 total of 63 occurrences of oomycetes across 317 root fragments, with n = 34 representing 17

345 plant species and 9 age intervals. We similarly tested the correlation between oomycete
346 frequency and vegetation abundance as cover scores (H2.2) and root traits (H3.2), again using
347 mixed effects models and including random terms for plant species and site. For these tests n
348 = 36 observations, representing 19 plant species and 10 ages, except for root diameter for
349 which we were able to include *Nertera depressa* for n = 37 with 20 plant species and 10 ages.
350 For all binary tests, over dispersion was tested using the Pearson residuals divided by degrees
351 of freedom, tested against a chi-square distribution (from http://glmm.wikidot.com/start;
352 accessed 10 July 2015). Host-specificity (H4) was tested within ecosystem age using chi-
353 square tests.
354
355 **Results**
356
357 *Oomycete frequency as a function of stage of ecosystem development (H1.1, 2.1 and 3.1) and*
358 *ecosystem age (H5)*
359
360 In early-successional ecosystems (5, 15 and 70 years), 38 to 65% of root samples had
361 oomycete DNA detected (Figure 1). In contrast, in mature and retrogressive ecosystems (280
362 to 120 000 years) an average of only 2% and never more than 8% of roots had oomycete
363 DNA found (Figure 1). Treating the sites as representing three stages following hypotheses
364 1.1, 2.1, and 3.1, the early successional stages had a significantly higher frequency of
365 oomycetes than mature stages (quasibinomial family glm; t = -6.6, P = 0.00050) or
366 retrogressive stages (t = -4.6, P = 0.0025), and the difference between mature and
367 retrogressive ecosystems was not significant (t = -1.1, P = 0.32).
368

369 Oomycete frequency was also significantly related to log-transformed ecosystem age
370 (quasibinomial family glm; t = -2.75, P = 0.025). While significant, this relationship was a
371 poor fit to the observed pattern of an increase for the first 3 site ages followed by uniformly
372 low levels of oomycetes at all subsequent site ages (Figure 1). Analysis of deviance
373 supported the ecosystem-stage factorial model (above) strongly over the linear ecosystem-age
374 model (F = 35.11, P = 0.00058).
375
376 *Oomycete frequency as a function of plant strategies and traits (H1.2, 2.2, 3.2)*
377 The frequency of oomycetes was significantly correlated with the decline of plant species
378 cover between ecosystem ages (Figure 2a, z = -2.4, P = 0.018), supporting hypothesis 1.2.
379 There was no correlation between oomycete frequency and plant abundance (z = 0.84, P =
380 0.40). Of the root traits tested, only root hair abundance was significantly correlated with
381 oomycete frequency (Figure 2b, z = 3.0, P = 0.003), providing partial support for hypothesis
382 3.2, while other root traits were not significant (SRL, z = 0.013, P = 0.99; root diameter, z =
383 0.82, P = 0.42). The significant root hair correlation was largely driven by *Raoulia hookeri*,
384 which has very hairy roots, frequent oomycetes, and occurs only in early succession; without
385 this species the correlation would not reverse in direction but would no longer be significant.
386
387 *Oomycete diversity, host specificity and ecosystem age (H4)*
388
389 A total of 37 different OTUs were found, with 10 found more than once (Table 1). The most
390 frequent OTU occurred 17 times, and had DNA-sequence affinities to *Lagena radicicola* and
391 an uncultured oomycete from New York, USA agricultural soils (Table 1).
392

393 Within ecosystem ages, there was a significantly non-random distribution of oomycete OTU
394 identity across plant species at 5 (5 oomycete OTUs with 20 occurrences across 4 plant
395 species, χ^2 = 42.96, df = 12, p-value = 0.000022) and 15 years (15 oomycete OTUs, 28
396 occurrences, 14 plant species, χ^2 = 225.87, df = 182, p-value = 0.015), but only marginally
397 significant at 70 years (16 oomycete OTUs, 30 occurrences, 7 plant species, χ^2 = 109.85, df =
398 90, p-value = 0.076; Figure 3). There were too few observations at the ecosystem stages after
399 70 years to meaningfully test for plant by OTU host-specificity at later ecosystem stages.
400
401 **Discussion**
402
403 Our results strongly support the potential importance of pathogens in early succession, with
404 high frequencies observed in the three earliest ecosystem stages (5, 15, and 70 years) but
405 oomycetes being present only at very low levels thereafter. Although the importance of
406 pathogens is often discussed in the ecological literature (Bagchi *et al.*, 2014), relatively few
407 studies have actually quantified pathogen frequency in natural ecosystems (Gomez-Aparicio *et*
408 *al.*, 2012) and we believe our results are the first study of pathogenic organisms throughout a
409 complete ecosystem development sequence. The community of oomycetes was diverse,
410 including multiple *Pythium* and *Phytophthora* species and, surprisingly, a very frequent
411 sequence matching *Lagena,* a pathogen previously only reported on grass (Blackwell, 2011),
412 on *Raolia hookeri* (Asteraceae) and *Epilobium glabellum* (Onograceae).
413
414 *Pathogens as drivers in early successional ecosystems*
415 The early succession hypothesis postulates that pathogens drive early-successional
416 change in plant communities (Kardol *et al.*, 2006). The high frequency of oomycetes in early
417 successional ecosystems strongly supports this hypothesis. In addition, those plant species

418 that had the highest frequency of oomycetes at a given ecosystem age declined in percent
419 cover most strongly before the next age. The higher frequency of oomycetes on plant roots in
420 early-successional ecosystems compared to mature and retrogressive ecosystems was not due
421 to PCR inhibition in older sites, as samples spiked with positive control oomycete DNA
422 showed no evidence of inhibition.

423 Oomycetes, particularly *Pythium*, are often tolerated by established plants, but prevent
424 seedling establishment (Martin and Loper, 1999; van der Putten, 2003). On that basis, we do not
425 argue that the observed oomycetes were necessarily having any direct negative effect on
426 established plants. Instead, we suggest that the correlation of oomycete frequency with
427 decline in cover between ecosystem ages is congruent with the suggestion that these
428 pathogens are primarily preventing re-establishment of plants and hence contributing to
429 vegetation change across cohorts (van der Putten, 2003).

430 Our definition of "early succession" comprises the major transition from rock field (5
431 years) to shrub land (15 years) to forest (70 years). Therefore our finding of high pathogen
432 levels is potentially consistent with Packer & Clay (2000), who found strong negative
433 feedback in a shade-intolerant, seral species (*Prunus serotina*) in a forest described as "at
434 least 70 years old" (Packer and Clay, 2000), despite the differences between our primary
435 succession and what was likely a secondary succession in the Packer & Clay study.

436 We believe most of the change in oomycete frequency across ecosystem stages
437 reflects changes in plant species composition. Nonetheless, changes in oomycete frequency
438 may also, in part, reflect a substantial site-age effect and direct effects of changing soil
439 environments on oomycete populations (akin to the “habitat” hypothesis for mycorrhizal
440 fungi of Zobel & Opik (2014). Meaningful testing of soil variables as predictors of
441 oomycetes was not possible, as the three early-successional sites had both uniformly high
442 oomycete frequency and uniformly high (pH, total P) or low (total C) soil measures. Thus,

443 while we can conclude that oomycetes were particularly frequent in early-successional
444 ecosystems and on early-successional plants, our data are insufficient to determine whether
445 this was due to the presence of particular plant hosts, or to soil abiotic conditions driving both
446 host plants and oomycete frequency (Martin and Loper, 1999).
447
448 *Oomycetes in mature ecosystems and forest diversity*
449 The mature ecosystem hypothesis suggests that pathogens are important drivers of
450 diversity in forest ecosystems, with evidence from tropical and temperate forests (Bagchi *et*
451 *al.*, 2010; Bagchi *et al.*, 2014), although other studies have failed to support a role of pathogens
452 in mature ecosystems (Reinhart *et al.*, 2012). Our finding of only low levels of oomycetes on
453 roots in mature ecosystems suggest that any role of pathogens in maintaining mature forest
454 diversity is unlikely to be driven by oomycete populations on the roots of established plants.
455 Further, we found no significant correlation between the abundance of a particular tree
456 species and its pathogen frequency, whereas the mature ecosystems hypothesis would predict
457 either a positive (Queenborough *et al.*, 2007) or negative correlation (Xu *et al.*, 2015) depending
458 on interpretation.
459 Our findings do not rule out negative density dependent mechanisms linking pathogen
460 infection to forest diversity, but suggest an important restriction of potential mechanisms. In
461 the original formulation, negative density dependent theories distinguished between distance-
462 dependent and density-dependent agents of seed and seedling mortality (Janzen, 1970).
463 Distance-dependent agents are primarily driven by the presence of the parent tree itself, while
464 density-dependent agents are attracted by the density of seeds and seedlings around that tree.
465 While it was previously known that established plant individuals are less susceptible to
466 oomycetes than seedlings, we had limited prior knowledge of whether roots in mature
467 ecosystems tolerated oomycetes, hence supporting populations that could cause distance-

468 dependent mortality, or resisted oomycete infection altogether (c.f., Spear *et al.*, 2015). Our
469 results suggest that roots of established plants in mature ecosystems are not supporting high
470 oomycete populations, implying that distance-dependent oomycete infection is not an
471 important driver of diversity, at least in this temperate rainforest. This is consistent with a
472 number of studies showing positive, rather than negative, effects of established plants on
473 conspecific seedlings (Simard and Durall, 2004). On the other hand, our results do not rule out
474 density-dependent mechanisms driven by oomycetes on seedlings. A high density of
475 conspecific seedlings may still lead to increased risk of oomycete driven mortality, as has
476 been found in other studies (Bagchi *et al.*, 2010).

477 While Packer and Clay (2000) clearly demonstrated oomycetes driving seedling
478 dynamics in a relatively young temperate forest, a more recent study by Bagchi and
479 colleagues (2014) in a tropical seasonal forest found that eliminating fungi alone had a
480 stronger effect on negative density-dependence than eliminating both fungi and oomycetes.
481 Further research is needed, but this may suggest that fungal pathogens are more important
482 drivers of forest diversity in mature ecosystems than oomycetes.

483

484 *Oomycetes in retrogression*

485 Laliberté and colleagues (2015) suggested that root traits adapted to highly P-limited
486 retrogressive ecosystems would increase susceptibility to root pathogens. Of the root traits we
487 tested, only root hair abundance was correlated with a high frequency of oomycetes in roots.
488 While this supports the general concept that root traits can determine pathogen susceptibility,
489 species with root hair abundance shows no increase with ecosystem age at this site
490 (Holdaway et al. 2011) and hence this correlation did not drive an increase in oomycete
491 frequency during retrogression. Studies of oomycete populations in other retrogressive
492 ecosystems will be needed to confirm our findings, but at present the suggestion of Laliberté

493 and colleagues (2015) of high pathogen loads in retrogression is not supported by our
494 findings, despite part of the mechanistic basis of that hypothesis (root traits determining
495 pathogen loads) was supported at least in the case of root hairs.

496 In comparing our results to other studies, it may be important to distinguish between
497 ecosystem age, as in the pedogenic sequence studied here, and response of pathogens to
498 disturbance or secondary succession (e.g., Reinhart *et al.*, 2010). None of our older sites
499 showed any sign of stand-replacing disturbance in the past hundreds to thousand years, but
500 smaller-scale disturbance is a normal part of ecosystem development (Peltzer *et al.*, 2010). The
501 retrogressive sites, in particular, have much younger vegetation (the oldest trees being no
502 more than 1000 years old) than underlying soils, and were unlikely to have supported tall
503 forests during the Pleistocene when these sites were exposed by glacial retreat.

504

505 *Novel organisms or native?*

506 Having baseline data on oomycete ecology may be important for understanding and
507 managing future pathogen outbreaks. For example, *Phytophthora agathidicida* (=
508 *Phytophthora* taxon *Agathis*) has been implicated as a cause of forest dieback of the iconic
509 New Zealand-endemic *Agathis australis*, but, like other oomycete disease outbreaks, the
510 origin of the species remains uncertain (Weir *et al.*, 2015). The single-direction sequencing we
511 performed directly from environmental samples was not designed for accurate phylogenetic
512 classification, even with two gene regions sequenced per sample, but it does suggest
513 considerable diversity of *Phytophthora*. We found sequences with affinities to a number of
514 species already known to be present in New Zealand, including *P. cinnamomi*, *P. cactorum*,
515 *P. infestans* and *P. kernoviae* (Scott and Williams, 2014). Given that our samples came from
516 relatively pristine ecosystems, we believe these OTUs are likely to be from native
517 *Phytophthora*.

More surprising was that the most common OTU in our data had affinities to *Lagena radicicola* in both 28S and many of the ITS sequence results. *Lagena* is a monotypic genus widespread as a pathogen of grasses in North America (Blackwell, 2011), but Barr and Désaulniers (1990) suggest it may be under-reported due to being morphologically very similar to *Pythium*, and not being easily culturable. Spores described as "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.

Our sampling of washed plant roots and choice of primers that preferentially amplify the Peronosporales focused our analysis on putative plant pathogens within the Oomycota occurring in soils. This is reflected in the affinity of most of our sequences to known plant pathogens. It is likely that many of these oomycetes were causing little harm to the established plants they occurred in the roots of, but the detection from root tissue and host-specificity imply a pathogenic lifestyle. Nonetheless, it is conceivable some of the detected oomycetes were saprotrophs. Further, we note that the Oomycota also includes animal pathogens and free-living soil saprotrophs as well as foliar pathogens; potential pools of species that were not likely to be included in our sampling.

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). Our results support the concept that oomycete pathogens are a diverse part of these ecosystems. Further, the strong plant-host specificity and correlation of plant decline

543 and oomycete frequency is consistent with the suggestion that these pathogens contribute to
544 vegetation succession. While we do not find frequent oomycetes in older ecosystems,
545 oomycete DNA was detected in all but one site. These low levels of oomycetes in roots,
546 along with oospores in soil, may still be sufficient to prevent establishment of susceptible
547 plant species or to drive pathogen outbreaks following external climatic stressors, but seem
548 incompatible with density dependent mortality being driven by oomycetes on established
549 plant roots.
550
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560
561 *Data Accessibility*
562 - DNA reference sequences: GenBank (accession numbers in Table 1)
563 - All other data: Landcare Research DataStore (xxxxx)
564

565 **Table 1.** Identity of non-singleton OTUs as clustered based on 28S, with accession numbers and best matching 28S and best matching ITS1
566 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* 98%/99% 378 bp	KU863602	*Pythium irregulare* 100%/28% 307 bp	*Pythium* aff. *junctum*	4
KU863597	*Phytophthora megasperma* 91%/100% 383 bp	KU863606	*Phytophthora infestans* 95%/53% 447 bp	*Phytophthora* sp. 1	4
KU863590	*Spongospora* sp. 75%/79% 306 bp	N/A	No PCR product obtained	Chromista aff. *Spongospora*	3
KU863591	*Phytophthora polonica* 99%/99% 384 bp	KU863600	*Phytophthora kernoviae* 96%/99% 214 bp	*Phytophthora* sp. 2	3
KU863592	*Pythium violae* 98%/100% 368 bp	KU863601	*Pythium* sp. 99%/99% 286 bp	*Pythium violae*	3

567

Figure 1. Frequency of oomycetes on 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. The two significant predictors of oomycete frequency on roots: vegetation cover (a) and the abundance of root hairs (b), with circles representing each plant species between site transitions (a) or within site (b). Lines indicate random effects to account for site in a binary mixed effects model with random intercepts for site and plant species. Color coding in (a) reflects the site age at t_0 for each transition, hence no points for 120 000 years are shown.

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 colors 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.

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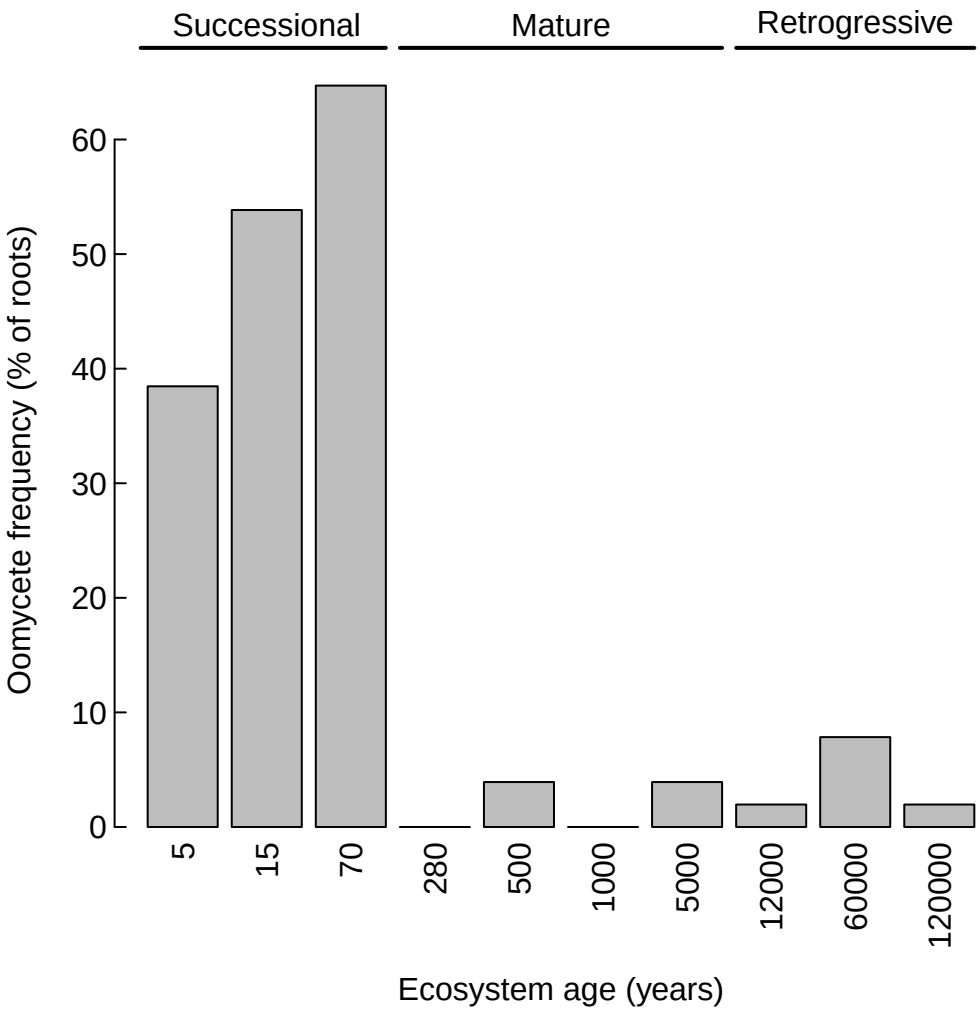
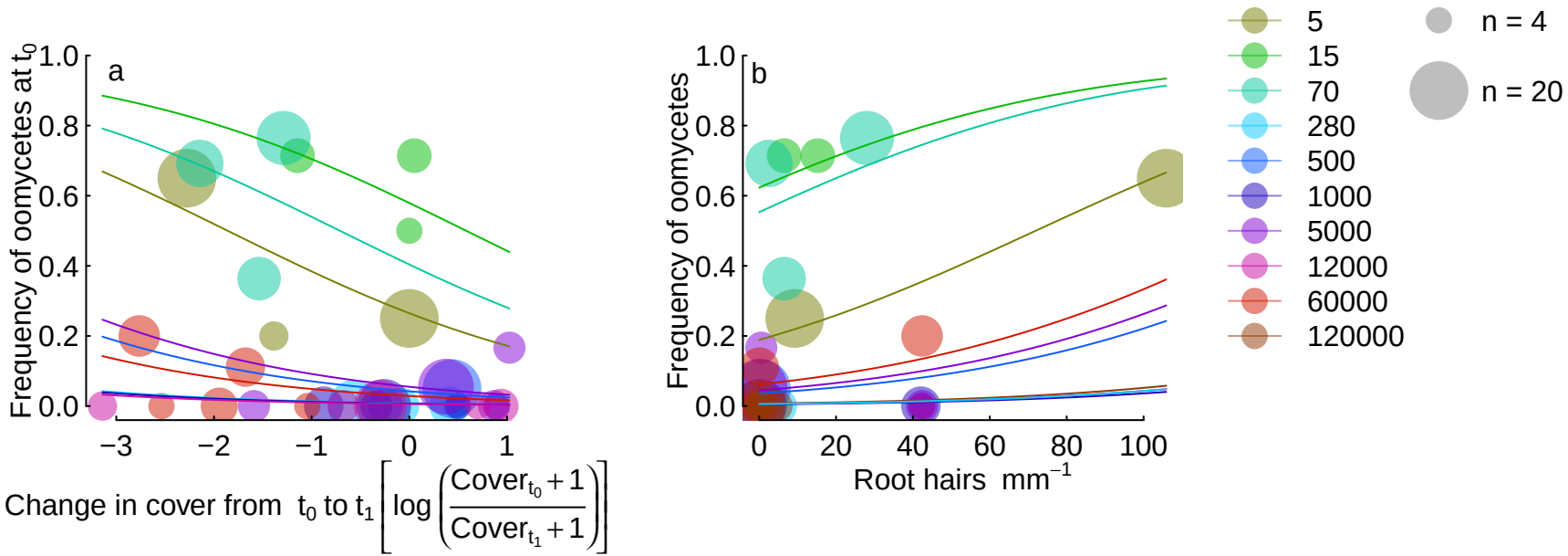
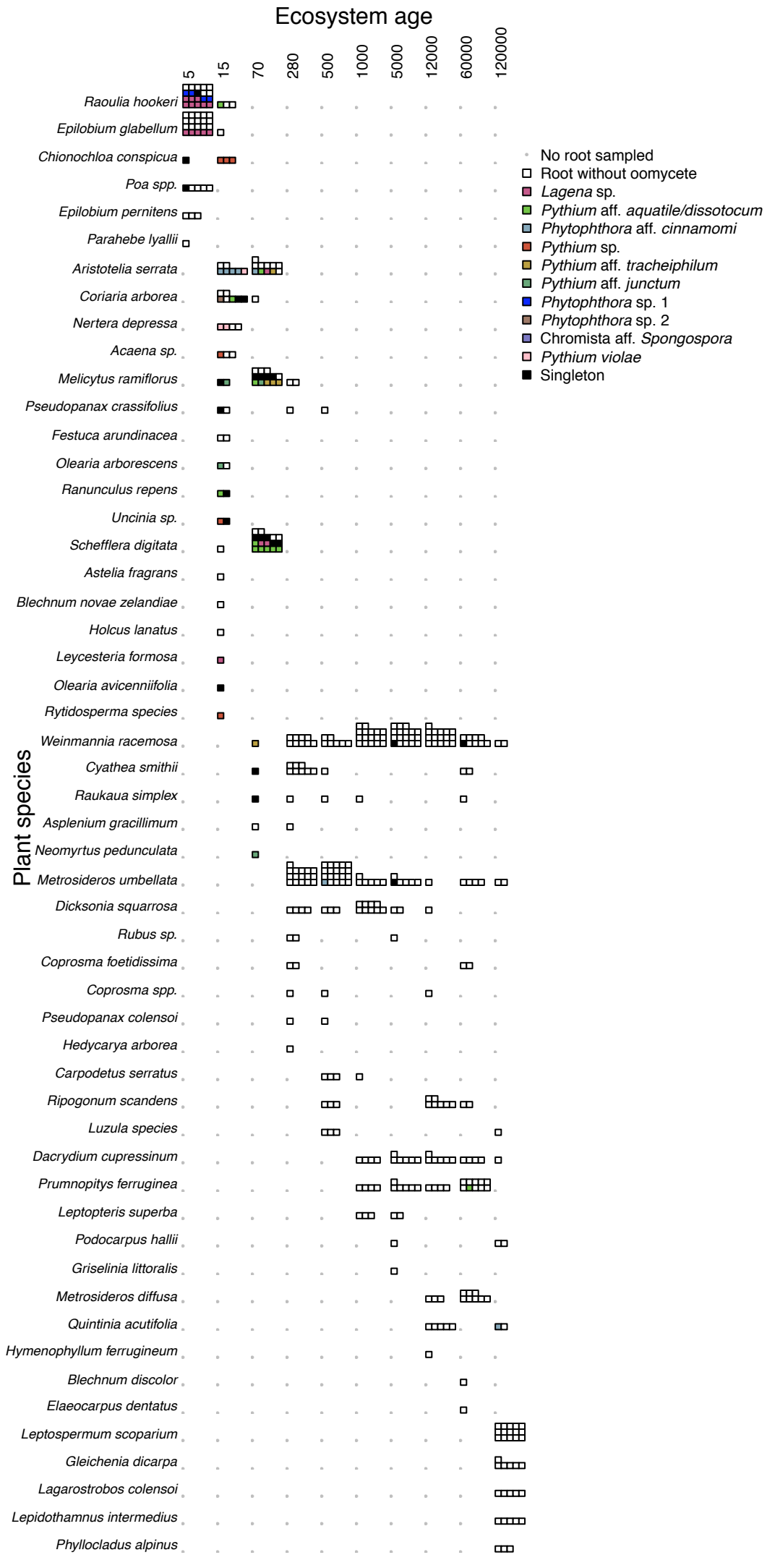

Successional
Mature
Retrogressive
Oomycete frequency (% of roots)
0
10
20
30
40
50
60
5
15
70
280
500
1000
5000
12000
60000
120000
Ecosystem age (years)


a
b
Frequency of oomycetes at t_0
Frequency of oomycetes
1.0
0.8
0.6
0.4
0.2
0.0
−3
−2
−1
0
1
Change in cover from t_0 to t_1 $\left[\log\left(\frac{\text{Cover}_{t_0}+1}{\text{Cover}_{t_1}+1}\right)\right]$
0
20
40
60
80
100
Root hairs mm^{-1}
5
15
70
280
500
1000
5000
12000
60000
120000
n = 4
n = 20


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
Pythium sp.
Pythium aff. tracheiphilum
Pythium aff. junctum
Phytophthora sp. 1
Phytophthora sp. 2
Chromista aff. Spongospora
Pythium violae
Singleton

Detailed molecular methods and discussion

Molecular methods

We primarily based our oomycete detection, operational taxonomic unit clustering, and identification on a nested PCR of large-subunit (28S) DNA. The primer pair LR0R: ACCCGCTGAACTTAAGC (LoBuglio *et al.* 1991) and LR5: TCCTGAGGGAAACTTCG (Vilgalys & Hester 1990) was used for first-round PCR amplification, followed by using 1µL of a 10-fold dilution from this PCR product as the template for the second-round PCR amplification with Oom1F: GTGCGAGACCGATAGCGAACA and Oom1R: TCAAAGTCCCGAACAGCAACAA (Arcate *et al.* 2006) primers, fluorochrome-labelled with VIC and 6FAM respectively. PCR reactions for LR0R-LR5 were carried out in a 20 µl volume containing 2 µl 10X PCR Buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, 0.2 mg ml^{-1} BSA, 0.8 U Roche Faststart Taq DNA Polymerase, and 1 µl DNA extract; with thermocycling of 94°C for 1 min, then 35 cycles at 94 °C for 30s, 47 °C for 30s and 72 °C for 60s, final extension at 72 °C for 7 min. PCR reactions for the Oom1F-Oom1R primer pair were the same, with the exception that $MgCl_2$ was 2.5 mM, annealing temperature of 57 °C, and extension time was 45 s with final extension of 10 min. A negative (omitting DNA template) and a positive control (including genomic DNA from a *Pythium* species) were included in every PCR. Our intention in using fluorochrome-labelled primers was to provide the option of performing tRFLP analysis. However, initial results suggested that most samples contained a single tRFLP type. We therefore moved to a direct-sequencing technique. PCR products were purified using DNA Clean and Concentrator Kit (Zymo Research Corporation) prior to performing Sanger sequencing (Canterbury Sequencing and Genotyping, University of Canterbury, New Zealand).

To confirm identities of sequenced oomycetes, samples that produced positive large subunit PCR products were sequenced for the ITS region. The oomycete specific primer pair OOMUP18Sc: TGCGGAAGGATCATTACCACAC (Lievens *et al.* 2004) and ITS2-OOM: GCAGCGTTCTTCATCGATGT (Lievens *et al.* 2006) was used to amplify the ITS1 gene region (~ 240 bp). PCR reactions were carried out in a 25 µl volume containing 2.5 µl 10X Ex Taq Buffer, 2 µl Ex Taq dNTP Mixture (2.5 mM each), 0.5 µl of 10 µM of each primer, 5 µl of 10 mg ml-1 RSA, 1 U TaKaRa Ex Taq DNA Polymerase, and 1 µl DNA extract, and sterilized distilled water up to 25 µl; with thermocycling of 94 °C for 3 min, then 35 cycles at 94 °C for 30s, 60 °C for 30s and 72 °C for 60s, final extension at 72 °C for 7 min.

Additional root trait data

Two species were present on the 5 year site that had no previously measured root traits: *Epilobium glabellum* and *Raoulia hookeri*. Root traits were measured using the same methods as for the earlier data set (Holdaway *et al.* 2011). Diameters = 0.22 ± 0.015 mm and 0.28 ± 0.018 mm; specific root lengths = 1437 ± 147 and 2354 ± 387 cm g^{-1}; and root hairs = 9.3 ± 4.8 and 106 ± 3.4 hairs cm^{-1} for *Epilobium glabellum* and *Raoulia hookeri,* respectively.

Supplemental discussion of methods

Using direct Sanger sequencing from environmental samples results in somewhat short and lower quality sequences than might be achievable with other methods, such as clone libraries. Nonetheless, direct sequencing is a cost-effective and efficient way of detecting and

identifying the oomycetes in samples with a single dominant DNA sequence. This greater cost-effectiveness allows greater investment in replication; a critical factor in ecological studies of cryptic organisms (Prosser 2010).

Most samples that produced a positive PCR product in large subunit sequencing also produced a positive PCR product with ITS primers. In most cases, the two sequences appear to represent the same species (Figure 3). The exceptions tended to be samples at the base of the phylogeny, matching *Spongospora, Albugo*, and *Sapromyces* in 28S and either failing to produce ITS PCR products at all, or producing ITS sequences that were distantly related. This suggests that the ITS primers were more specific to a subset of the Oomycota, and may omit some basal groups; while the 28S primers better amplify basal Oomycota but potentially amplify other Chromalveolata as well. Neither gene region was sufficient to identify all OTUs to species, largely reflecting the relative paucity of oomycota sequences in GenBank compared to better studied groups, such as fungi. Large subunit sequences also lacked resolution within *Phytophythora* compared to ITS sequences.

References

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Revision Notes 26 July 2016

This document list the revisions we have made between the first and second versions of the manuscript. These have been made in response to a thorough peer review process at a top tier journal, where three reviewers gave more the 3500 words of review for a 5000 word manuscript. While the outcome of the review was rejection, we found the reviews helpful in improving the document and provide this document as an indication of how we responded to the reviewer comments (as we did not have the opportunity to do so directly). A senior editor of the journal in question agreed, in principle, that reprinting the anonymous reviewer comments and our replies was acceptable. We have copied every comment from the reviewers without omission or editing. For each comment we first try to analyse the comment, then note the actual changes we made.

Rev 1: Summary: This manuscript proposes to test a series of very interesting hypotheses about the diversity and abundance of oomycete pathogens in plant communities across a long glacial chronosequence. The study system is very interesting and have previously provided views to understanding plant and fungal community changes along a chronosequence, and the questions about how host abundance, host traits, and ecosystem attributes interact with disease dynamics are broadly interesting. However, I do not think that the data from this study provide robust tests of most of the proposed hypotheses. In addition, the structure of some of the hypotheses is overly narrow and inappropriately supported in the introduction, and some of the statistical analyses have important issues. Although I think the data presented here will be an interesting addition to the literature (first attempt across a long chronosequence), I do not think that they provide the claimed answers to the larger ecological questions posed here.

Analysis of reviewer comment: The summary was nice in bringing the reviewer comments together. The reviewer then broke the comments down in detail, so we respond to them in the following.

Rev 1. Overall the introduction is quite effective at developing a clear set of testable hypotheses. However, it is too "either A or B" for my taste, since multiple mechanisms and patterns may be present in the same systems. In addition, some of the distinctions made here (successional vs. mature, host specificity) either overly categorical or are restricted to particular ecosystems, and so by the end of the introduction I felt like the I had been manipulated to a particular way of looking at the world that was ideally tested using the system at hand, but that was overselling the broader applicability to disease dynamics in wild systems."

Analysis of reviewer comment: This reviewer hit a theme that also emerged from other comments, that our divisions were too categorical. We hadn't intended our categories to be seen as perfect or entirely exclusive. Indeed, when we tested the mechanistic hypotheses (1.1, 2.2, 3.2), one reason was to look at continuous processes WITHOUT being categorical about which ages they might occur at. I view this as a failure of presentation on our part.

Changes made: We moved our definitions of the three phases into a new paragraph, and tried to make it clear that we are using these phases (early-succession, mature,

retrogression) as one way to view the system, but also now explicitly include a more continuous-variable approach (H5).

Rev 1: Conspecific abundance and strength of soil feedback

112-115: "Several studies suggest that correlation between plant abundance and feedback strength should be negative, with more abundant species showing stronger negative feedback (Queenborough et al. 2007)." Queenborough et al. show that the correlation should be POSITIVE. "However, positive correlations of plant abundance and the strength of negative soil feedbacks … (Xu et al. 2015)." Xu et al. actually report a NEGATIVE correlation between plant abundance and strength of negative soil feedback. The directions are correct, however, in H2.2. This presentation is confused.

Analysis of reviewer comment: In our manuscript we had to deal with an impossible situation, where both negative and positive correlations have both been taken as confirmatory evidence that Janzen-Connell dynamics occur. Obviously one of those views is wrong, but that isn't an argument we want to explore too deeply in this paper.

We wrote "with more abundant species showing stronger negative feedback (Queenborough *et al.* 2007)." What Queenborough wrote was "seedling survival was inversely related to the relative basal area of trees (Fig. 1)." The figure shows seedling survival rate being negatively correlated with tree abundance. This seems consistent with our phrase "more abundant species showing stronger negative feedback".

We then wrote "positive correlations of plant abundance and the strength of negative soil feedbacks…(Xu et al. 2015)". What Xu et al. wrote was "more abundant tree species exhibited weaker conspecific negative density dependence". The correlation in Queenborough is negative, the correlation in Xu is positive. The reviewer agrees with our wording later, but not here.

The important point we take from this reviewer comment is that "this presentation is confused". The confusion is because a positive correlation of a negative value means that it gets weaker, not stronger. It is our wording "the strength of negative soil feedbacks" that is the problem here. A "stronger" "negative" effect is probably a more negative effect, whereas it would have been more correct to write "positive correlations of plant abundance and soil feedback".

Changes made: We carefully re-examined the papers we cited. The criticism from the reviewer makes it clear that it was confusing even to an expert in the field, and we can understand where that confusion arose. We have therefore re-written the section as:

"Several studies suggest that correlation between plant abundance and seedling survival rates should be negative, with more abundant species showing lower seedling survival rates (Queenborough *et al.* 2007). However, positive correlations of plant abundance and soil feedbacks, with abundant species exhibiting weaker negative density dependence, have also been taken as evidence supporting pathogen driven density-dependence as a mechanism for supporting mature forest plant diversity (Xu *et al.* 2015)."

It will be interesting to see if that is more successful -- it is just a very tricky concept to get right.

Rev 1: There is a ready willingness to draw on studies of seedlings of trees (ephemeral developmental stages with regular population renewal in the same location following each new crop of seeds) and studies of grasslands (with either perennial or annual individuals with much less developmental change in susceptibility and much less temporal distinction between mortality and measures of community structure). I recognize that given the limited universe of studies in wild systems it is necessary to draw from the studies that are available, but at times I found the juxtaposition of results from very different systems to make points a bit jarring.

Analysis of reviewer comment: We found this comment a bit confusing, as it isn't clear to us that trees have "regular population renewal" and grasslands do not. Some trees are early successional, and do not regenerate under themselves. Conversely, some grasslands are very stable over long timespans.

Notwithstanding our confusion, the reviewer's main point is that they don't like our mixture of systems in the introduction. We cited mostly grassland studies for early-successional ecosystem and primarily forests in the mature-ecosystem section (although we did cite a Bever et al. paper on grasslands). We think that perhaps the reviewer is responding in part to our citation of Kardol et al. 2006 who refer to mid- and late- successional ecosystems within grasslands, using "late" to refer to systems "abandoned >20 years ago or semi- natural matt-grass sward/heath land".

Our research system includes grasses and herbaceous plants, so we didn't want to restrict our literature review to forests. Nonetheless, the reviewer makes a valid point that this can be a bit confusing, particularly if we don't cite any forest papers for early-succession.

Changes made: We deleted part of the reference to Kardol et al. 2006, so we now only refer to the observation of negative feedback in early succession, without referencing the switch to positive feedback later in that system. We also note the Packer & Clay study on *Prunus serotina* (a shade intolerant, early-sucessional tree) here, rather than only in the discussion (as previously). There is a risk here, in that Packer & Clay treat their findings as evidence for Janzen-Connell (which we consider a mature-ecosystem process) despite *Prunus serotina* being a successional tree species (at least according to the US Forest Service).

We also inserted a sentence "These examples span from short-term grassland to decadal scale forest patterns, suggesting a widely applicable ecological pattern." to both acknowledge the fact that we are looking across systems (as the reviewer noted) while pointing out that we consider this a strength rather than a weakness.

Rev 1: I am unable to find support for this statement (L96) in the citation provided: "Further, Gilbert (2002) suggests that increasing nutrient competition in mature ecosystems may stress plants and increase susceptibility to pathogens."

Analysis of reviewer comment: What Gilbert (2002) wrote was "Competitive interactions and host-pathogen dynamics may be strongly inter- dependent. Stress from competition for scarce resources can alter susceptibility to disease so that

bottom-up competitive interactions lead to increased top-down effects of pathogens…. Competition may stress plants and make them less tolerant to infection"

What Gilbert did not say was "in mature ecosystems". Our error was in presentation, in that we took insufficient care in where exactly the citation is placed in the sentence.

Changes made: We re-wrote the sentence so that the exact part of it that we attribute to Gilbert is (hopefully) clearer, as "Further, competition is high in mature ecosystems, and Gilbert (2002) suggests that competition may stress plants and increase susceptibility to pathogens."

Rev 1. The distinction between "successional" and "mature" ecosystems seems to me a rather dated view of plant community dynamics, clearly applicable to a fairly narrow set of low-diversity ecosystems and highly dependent on the spatial and temporal scale of analysis and disturbances. Here it is defined (L92) only as "where vegetation composition is more stable", but the systems drawn on in the introduction range widely in this regard. The particular system studied here certainly fits this picture – low diversity systems that replace each other with regularity – but I find the introduction to over-sell the generality of the study.

Analysis of reviewer comment: The most important point the reviewer makes is in questioning our categorization of ecosystems. We have addressed this by adding a paragraph on processes and stages.

Otherwise this is a case where we actually agree with the reviewer, at least to a certain extent. What we consider "mature" is a virgin rainforest, where individual living trees have been present since before Māori colonization of New Zealand. Remarkably little of the world is covered in ecosystems that would be as pristine as this; and many similar forests would occur only as tiny nature reserves. Retrogressive ecosystems are even rarer, as they only occur where soils have aged without major disturbance for exceptionally long time periods. The high rainfall at our site helps accelerate this process, but soils 120,000 years in age or greater are, admittedly, very unusual. An earlier draft included a paragraph on just this, but was removed as it didn't contribute to our main points.

On the other hand, we don't agree with the reviewer that this is a low-diversity system – that wasn't stated in our paper and isn't really true. Perhaps the reviewer was expecting tropical-rainforest levels of diversity, but for a temperate forest this is a fairly diverse system.

Changes made: As above, we moved our definitions of the three phases into a new paragraph, and tried to make it clear that we are using these phases (early-succession, mature, retrogression) as one way to view the system, but also explicitly include a more continuous-variable approach.

Rev 1: "as host specificity is requisite for pathogens to either drive early-successional vegetation change (H1) or to contribute to maintaining plant diversity in mature ecosystems (H2)" is not really true. It is only required that impacts differ among hosts (e.g., Sedio BE, Ostling AM. 2013. How specialised must natural

enemies be to facilitate coexistence among plants? Ecol. Lett. 16:995—1003). Additionally, most pathogens are NOT host specific, but have phylogenetically structured host ranges, and the importance of relatives in the surrounding community can be very important (Parker et al. 2015. Phylogenetic structure and host abundance drive disease pressure in communities. Nature 520:542—44).

Analysis of reviewer comment: "Host-specificity" is a difficult topic, and the reviewer is correct that phylogenetically related species are more likely to share pathogens than phylogenetically distant species. The reviewer states that this means they are "NOT host specific" [all-caps the reviewer's]. However, this is somewhat dependent on how one defines host-specificity. Further, the reviewer correctly notes that it is conceivable that non-host specific pathogens could still drive at least some degree of succession simply through favouring plants with increasing pathogen resistance over time.

Changes made: This comment only required re-phrasing the statement to be more cautious. It now reads:
"In addition, we tested the hypothesis (H4) that oomycete communities show host-specificity or at least a degree of host-preference in their plant associations, as host specificity may contribute to pathogens driving early-successional vegetation change (H1) or to maintaining plant diversity in mature ecosystems (H2)."

Rev 1. What is the justification for handling ordered site ages as discrete factors? And testing with chi-square test? The expected differences among sites 5 and 15 y old are not equivalent to those 5 vs. 70 or 5 vs. 60000 years, a fundamental assumption of this approach.

Analysis of reviewer comment: There is no fundamental assumption of discrete factors that all treatment differences be equivalent, at least that we are aware of. If that were true, then almost all blocking designs would be invalid. However, our hypotheses were about stages, not about ages, so there was really no logical reason we actually tested ages in addition to stage. The result is so blindingly obvious that statistics are unnecessary in any case.

What the reviewer comment made us realize, however, was that if we did treat age as a continuous variable we could test (and reject) the hypothesis that oomycetes are responding to ecosystem age directly, as opposed to successional stage. We therefore added a new hypotheses (H5) and necessary methods, results and discussion to address these. The response to ecosystem age actually does come out significant, but the model is hugely worse than treating ecosystem age as a three-stage factor. Examining the graph also makes it clear that there is no suggestion of any linear response with age -- oomycete frequency goes up for the first three ages, then becomes uniformly low.

Changes made: We removed the chi-square test of individual ages, added H5, and added tests for H5.

Rev 1. I note that in the end, they have just 91 oomycetes found on 458 plant samples.

There is much to be drawn from these data, but several of the analyses seem to based on rather thin data. It often takes more work than it should on the part of the reader to figure out exactly what are the sample sizes going into each analysis.

Analysis of comment: The response variable for the vast majority of our tests is the presence/absence of oomycetes across between 458 and 512 samples (depending on whether successful plant identification was required for the test). It looks like the reviewer is misinterpreting the presence of oomycetes in 91 samples to mean that n=91, which is an incorrect interpretation of how binary response variables work.

Still, the reviewer is right that he/she shouldn't have had to work to figure the level of replication.

Changes made: we have now made certain that n is explicitly stated.

Rev 1: From my reading of the figure on P31, only 8 host species have at least 3 root samples in each of two or more ecosystem ages. Only 4 species span two stages. That seems to be spreading the analysis of whether declining plant species had higher than random oomycete levels (H1.2) (L294) pretty thin. For trait analysis they restrict the analysis to only those spp with 3 or more, but that is equally important here, since there is little confidence in estimates of oomycete abundance if only one root is sampled. I think that for this, and all the analyses, a very transparent presentation of what the sample size was like, and how that affected confidence in any measures of variation in abundance of oomycetes, needs to be presented.

Analysis of comment: The reviewer misinterpreted our statement that only observations with more than 3 root samples were included as "more than 3 in each stage" rather only requiring 3 across all stages. Again, this reflects a need on our part to better communicate the n for statistical tests.

Changes made: as above, we have now made certain that n is explicitly stated. We also rewrote the "more than 3" statement to make it more clear that this refers to the TOTAL being above 3.

Rev 1. Oomycete frequency across stages. Although it is interesting that the hosts sampled in early-successional systems more often have associated oomycetes than do the hosts in the later stages, there is no overlap among host species so it is impossible to attribute this result to ecosystem age vs. susceptibility of those particular hosts. This is not a robust test.

Analysis of comment: We agree with the reviewer that the change in oomycete frequency is very closely related to plant species change. Indeed, we thought that was clear throughout the manuscript. Our interpretation of the reviewer comment is that they believe we were suggesting the change in oomycete frequency was driven by other factors related to ecosystem stage. We addressed this already in the discussion, but the reviewer's misunderstanding highlighted that the paragraph needed revision.

Changes made: We added an explicit statement, "We believe most of the change in oomycete frequency across ecosystem stages reflects changes in plant species

composition" to the discussion.

Rev 1. Oomycete frequency and vegetation cover. Same problem as for [previous comment].

Analysis of comment. The reviewer comment essentially indicates that they believe we needed to test one plant species across all stages. What they are proposing seems to be a reciprocal transplant experiment across ecosystem stages, which would be fantastic to do. Nonetheless, we believe that measuring the actual plant species found across ecosystem stages is entirely valid for understanding these communities.

Change made: none. This is a case where the reviewer's comments indicate a complete rejection of our approach, and not something we can change in the revision.

Rev 1. L 328-330. Oomycete frequency vs. root hairs. Interesting, but it is not clear how influential one species with hairy roots from early succession (when there is also greater oomycete abundance) has on this relationship. Since the tests of species are under different oomycete abundance conditions, this is not a robust test.

Analysis of comment: The reviewer makes two suggestions. First, that a single data point is driving the pattern, and second that the different oomycete abundance conditions are making the test invalid.

The first point is a common criticism of regressions – essentially that if particular data points are omitted, the pattern might not still be found. In this case, if we delete all observations of *Raoulia hookeri*, the significant correlation of oomycete frequency and root hair abundance becomes non-significant ($P = 0.12$), although the sign of the correlation does not reverse.

The second point ignores our use of a mixed-effect model with a random effect for site. This explicitly accounts for the site level differences before testing the correlation within sites.

Changes made: We added a sentence that notes the importance of *Raoulia hookeri* to the observed correlation.

Rev 1. A number of my critiques are discussed in some ways in the discussion, but I do not think that they compensate for the overall limitations of the data.

Some of the "mix and match" across systems discussed in the introduction continues here. For example, L377-381. A 70-year old forest in essentially primary succession is hardly comparable to a 70-year old forest following human-induced disturbance.

The use of "mature" for forests and ecosystems does not always mean the same thing in systems being cited (e.g., Bagchi) and the work here.

I read through, but do not provide additional comments on the discussion here, since so much of it would need to be re-evaluated given the significant limitations in the results.

Analysis of comment(s): We disagree that shade intolerant plant species are not comparable between primary and secondary succession, and adjusted the introduction to make this clear. From our viewpoint, the important point is that in both systems *Prunus serotina* grew in the absence of established conspecifics and is unlikely to self-replace.

We had cited Bagchi et al. as evidence for the statement that "pathogens are important drivers of diversity in mature forest ecosystems". In one of the Bagchi papers, the forest is described as "Forests in this area have historically been subject to hurricanes and light selective logging". It is, of course, difficult to evaluate exactly how comparable two forest sites are, but we would suggest this isn't too fundamentally different from our mature forests which have probably also undergone small scale disturbances over time. Of course Costa Rica and New Zealand differ in temperature and forest composition, but the rainfall at the two sites is very similar, and we see no reason to think that fundamentally different processes occur in the two systems.

Changes made: We added an explicit discussion of why primary and secondary succession are comparable from the perspective of changing vegetation composition (to the introduction). We removed one word ("mature") from the sentence citing Bagchi. We also added a sentence in the introduction that addresses the use of different systems.

Rev 2: In the manuscript "Plant root pathogens over 120,000 years of temperate rainforest ecosystem development" Dickie et al. investigate plant roots in a glacial chronosequence for the diversity of associated oomycetes. They find highest oomycete infections in early successions that declined over succession, leading the authors to suggest that oomycete pathogens drive succession.
The manuscript is well written and provides novel insights about oomycete diversity and association to plants. However, I have some issues with generalities drawn from this fairly limited data output: Comparably few plant species were sampled and compared, with a maximum of one sequence obtained from individual root pieces. A high-throughput sequencing would have been more appropriate to study the real diversity of oomycetes in theses roots as more than one species might have been present. The authors refer to abundances of oomycetes, but in my opinion the limited sampling of few root pieces followed by simple PCR rules out reliable abundance information (qPCR or other really quantitative information are needed here). Despite the potential to be an interesting study, I am sceptic that the hypotheses can be proven by the analyses shown here.

Analysis of comment. The reviewer suggests that we should have used next-generation sequencing on the basis that more than one species of oomycete might have been present in individual root samples. We agree with the concept that more than one species might be present, which is why we started by using T-RFLP (a much more cost-effective approach for small, highly replicated samples than next-generation sequencing which is better for larger, lower replication samples). However the preliminary results found only one T-RFLP peak per sample, so we switched to direct sequencing. That had been made clear in the supplementary

methods, but was not in the main paper.

What is a bit frustrating about the above comment is that essentially all of our findings are based on the presence or absence of oomycetes. It wouldn't have made any difference to any of these analyses if more than one species of oomycete was actually present. (i.e., regarless of whether 1 or 200 species were present, we would still record it as "present") So while we could have run 500 individual next-generation samples (at a vast increase in cost), it wouldn't have actually resulted in any better test of our hypotheses.

We had been inconsistent in our use of the words "frequency" and "abundance" for oomycetes, which was an error. What we measured was frequency, which we believe is vastly more important than abundance as a test of our hypotheses. In particular, we were explicit in stating that we don't believe the oomycetes in plant roots were necessarily causing any damage to established plants, but were rather a source of inoculum to prevent seedling establishment. For inoculum, the actual number of DNA copies of oomycetes present (as measured by qPCR) would have relatively low importance compared to knowing how frequent oomycetes are across the area.

Changes made: We have checked every use of the word "abundance" and switched to "frequency" where more accurate. We have added a sentence giving the reason for direct sequencing.

Rev 2: L40-42: the transition from the 120,000 year old chronosequence to decades of succession seems unclear from this context. Please clarify.

Analysis of comment. With a word limit of 150 for the abstract, some concepts were left a bit… well, "abstract".

Changes made: The new target journal has a longer word limit, so we were able to make the abstract less abstract and ensure that the link between the chronosequence and individual site ages was clear.

Rev 2: L57: Here I only partially agree; while it certainly is true that mostly pathogens of economic relevance are studied, those in environmental systems receive a fair amount of attention; this holds for e.g. invasion ecology (Keane and Crawley 2002, Mitchell and Power 2003) and diverse environmental (soil and rhizosphere) studies (Arcate et al. 2006, Mendes et al. 2013, Geisen et al. 2015). Please mitigate this statement.

Analysis of comment. The reviewer disagreed with the statement that "Indeed, plant pathogens are often out-of-sight out-of-mind, until large-scale forest diebacks (Scott & Williams 2014) or major crop losses (Martin & Loper 1999) occur" and provided 5 citations. We believe the reviewer was responding to the sentence out-of-context of the remainder of the paragraph. We had followed this sentence with a review of a number of examples of where oomycetes have been shown to be important. That already included the Arcate paper that the reviewer cites.

The question of citations is an odd one. It is not imperative on authors to cite every single previous paper on a subject and, indeed, journals actively discourage this by

imposing limits on the number of references or the total pages. We tried to cite every paper that influenced our thinking, and struggled to reduce the total to the 50 permitted by the original target journal. The reviewer suggests some new citations, but doesn't indicate which citations we should delete to remain within the limit of 50 or why the insights emerging from those papers would alter the interpretation of our findings. The new target journal limits the overall page count, rather than giving an exact limit on citations, which permits a slight increase.

The Geisen paper came out very recently (October 2015) and is somewhat relevant, despite not linking pathogens to ecosystem processes. Our omission was simply a case of the paper being published after the completion of the literature review for this paper. The Mendes paper is a review, and seemed less relevant than the other papers we already cite, so we did not include it. We also did not feel the papers on pathogen release of invasive species were particularly relevant.

Changes made: We tried re-writing, but found that it made the paragraph much less logical. In the end we made minor wording changes and added the Geisen reference.

Rev 2: L81: is it necessarily true that small plants when being adult can be compared with seedlings that are in an entirely different growth stage? This makes only partly sense and I would prefer to see some clarification. Especially as the next sentence mentions that these plants might actually be resistant when older but not in their seedling stage which then should apply to other plants as well.

Analysis of comment: The sentence we believe the reviewer refers to is "Over time, these established plants accumulate pathogens, which prevent establishment of conspecific seedlings but have less negative effects on other plant species." We believe the reviewer's comment is resolved by adding 2 words to what was a poorly phrased sentence.

Changes made. "seedlings of" inserted before "other plant species"

Rev 2: L195 onwards (and later e.g. L314-317): Could it be a bias in sampling as also larger plants produce fine roots, but less at the surface where samples were taken?! Could this at least in part explain the differences in pathogen loads? Some discussion about this potential would be great to see.

Analysis of comment: We hadn't actually specified (an error on our part) the depths, but our samples are from up to 20 cm depth.

Changes made: in context, we inserted: "… with equal sampling from three depths (up to 20 cm depth max). This depth is likely representative of the established roots a seedling might encounter during the first year of growth (and hence most relevant to our hypotheses), but omit deeper roots in older sites."

Rev 2: L240 onwards: some more care for literature research should be taken- Cercozoa have confidently been placed in the eukaryotic tree by many studies and belong to the supergroup SAR (Adl et al. 2012, Burki and Keeling 2014), more exactly in Stramenopiles, while Cercozoa belong to Rhizaria; therefore it is very unlikely that these sequences resemble oomycetes, which should be tested using phylogenetic placements rather than Blast results- anyways, 78% similarity does not

mean anything and certainly likely not oomycete as a comparably well covered group of organisms.

Analysis of comment: We struggled with these 3 sequences. We recovered 3 reasonably high-quality 18S sequences that form an OTU. When blasted, they essentially match nothing with any level of certainty.

Because it is increasingly recognized that there are large groups of unknown soil organisms that are overlooked when sequences are discarded, we were very reluctant to discard these, as they do form a tight cluster.

That said, we don't really want to get involved in a phylogenetic dispute. We cited a paper that reviews *Spongospora* as a root pathogen.

We had included a phylogeny, which supported the placement of these sequences as basal oomycetes, but decided to remove this due to other reviewer concerns (see below).

Changes made: section re-written as "Three sequences had a closest match to a *Spongospora* sequence, but only at 78% identity. We therefore considered these three sequences to likely be root-associated pathogens (Bulman and Braselton, 2014), without making any strong claim as to their actual identity, and retained them in the analysis.

Rev2: L248: Why were sequences clustered? I see the reason in high-throughput sequencing based approaches, but here it seems not needed and might actually reduce the true diversity (unless sequence quality was bad). Please explain.

Changes made: inserted: "This allows us to form species concepts for testing host specificity (H4), resolved some matches to "uncultured oomycete" and suggested where the gene region used lacked sufficient resolution to distinguish species"

Rev 2: L351 onwards: I am not sure if pathogen abundance is the correct term here as not the entire root systems (and soils) are analysed and only a fraction of the potential abundance is covered; furthermore, infection rate is not measured as few spores in a sampled root would be indistinguishable from a fully infected root- for real abundances qPCR might be useful; here, "presence" seems more correct, but of course means a much weaker result. Please consider this change (also in the abstract and other parts of the manuscript).

Analysis of comment: There is a widespread view that presence/absence data is "much weaker" than abundance data. This is false, as presence/absence data can be both more ecologically relevant and more statistically powerful than abundance data when abundance is generated by a different process (e.g. hyphal growth) than colonisation. Measuring "abundance" as the number of gene copies of 18S within a tiny fragment of root would not achieve any purpose we can envision being meaningful in the context of testing our hypotheses.

Changes made: As above, we switched a few mistaken uses of the word "abundance" to "frequency".

Rev 2: Furthermore, some more care should be taken as oomycetes are not necessary pathogens-I agree, most certainly are, but the potential that they might not act as pathogens (many can be cultured on standard media so live saprophytically) should at least be mentioned; in the last sentence this actually is (L476-478), but sold as these were missed in the survey rather than that the ones found could also be non-pathogenic. A less biased way of writing seems more appropriate here.

Analysis of comment: This is roughly the same argument as saying that just because you have detected DNA of a bear, it might not actually be a predator because it could be a bear eating blueberries or perhaps a panda. In our estimation, it is true, but not particularly important.

The fact that we obtained DNA from washed plant roots, and that the oomycetes we observed showed host-specificity implies fairly strongly that they were at least growing in or on plant roots – and therefore likely pathogens. Nonetheless, the reviewer is correct that it is conceivable that some of the oomycete DNA reflected entirely saprotrophic oomycetes that happen to live inside the roots of plants but don't do any harm.

Change made: Inserted: "It is likely that many of these oomycetes were causing little harm to the established plants they occurred in the roots of, but the detection from root tissue and host-specificity imply a pathogenic lifestyle. Nonetheless, it is conceivable some of the detected oomycetes were saprotrophs"

Rev 2: L359: Reference would be useful here

Analysis of comment: The statement needing a reference was "*Lagena,* a pathogen previously only reported on grass" This was an error on our part – we provided the citation lower down in the discussion, but not here.

Change made: reference inserted.

Rev 2: L414 onwards: I am not sure if this can be stated as oomycetes might not stay in (often wooden in trees) roots, but in soils directly and live as saprotrophs or endure in resting stages.

Analysis of comment: What we stated was "Our results suggest that roots of established plants in mature ecosystems are not supporting high oomycete populations" which seems entirely consistent with what the reviewer suggests.

We agree that coarse woody roots are less likely to harbour oomycetes. All our roots samples were non-woody fine roots, but this had not been specified (our error).

Change made: methods modified to specify fine roots.

Rev 2: L426: there is way too little evidence to draw such a broad conclusion- and it is not scope of this work.

Analysis of comment: The statement we made was, in full: "While Packer and

Clay (2000) clearly demonstrated oomycetes driving 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. Further research is needed, but this may suggest that fungal pathogens are more important drivers of forest diversity in mature ecosystems than oomycetes."

We feel it is very important to retain this statement, as we need to acknowledge fungi as potentially important drivers of the same processes we were studying. We stated clearly, "further research is needed" and "may suggest", which is basically in agreement with the reviewer that more evidence is needed.

Change made: none.

Rev 2: Figure 3: The phylogenetic placement (tree) would benefit from more commonly used methods such as maximum likelihood analyses and Bayesian inferences; bootstrap values or Bayesian posterior probabilities would indicate how reliable the tree is. Furthermore, species and genus names should be in italics (same for legend in Figure 4).
Analysis of comment: We had explored clustering as an improvement on the more typical approach of just using best-BLASTn matches. Nonetheless, a single gene region (and 18S at that!) does not form a robust phylogeny regardless of statistical methods. The reviewer comment suggests we should use "the more commonly used methods" but gives no reason why our method is wrong. The typical concern over UPGMA is generally about assumptions of fixed evolutionary rate, which seems irrelevant in this case (single non-coding gene region). On the other hand, UPGMA had placed the reference sequences into a reasonable tree, while ML trees had produced trees that were incongruent with current understanding.

Rather than get bogged down in reviewer concerns over phylogenetic methods, we removed the phylogeny and replaced it with a best-Blast match table.

Change made: Phylogeny removed, table inserted.

Rev 2: Supplementary: "Using direct Sanger sequencing from environmental samples results in somewhat short and lower quality sequences than might be achievable with other methods"; please explain why the sequences are shorter? I can see the issue if primers target more than one species to see low quality regions where overlaps occur. This is a fundamental issue in this study as the diversity of oomycetes, which in many cases might be more than one can not be determined and reliable comparisons between plants not made. This also refers to the relevant parts in the text. Why wasn't there high-throughput sequencing performed? Alternatively, a cultivation based effort could have been conducted to cultivate potentially more than one organisms which then all could have been individually sequenced.
I am also unsure if the last part of the supplementary information is needed as it is common knowledge that ribosomal regions have a lower resolution for many groups than ITS region

Analysis of comment: in context we wrote "other methods, such as clone libraries". We have now made that just "clone libraries" as direct sequencing remains more accurate than NGS.

The reviewer notes that “diversity of oomycetes … cannot be determined”, but this isn’t really a problem here. It is true that some secondary oomycetes within samples might have been omitted, but this would only lower diversity in samples with already high levels of oomycetes.

We didn’t use culturing as some oomycetes, including the most common one in our study, are not readily grown in culture.

We retained the text on species resolution as it does no harm to make our notes on the two primers available to other researchers in a supplement.

Changes made: minor edits made to supplement.

Rev 3: Here the authors seek to determine the role of potential oomycete pathogens in ecosystem development using a glacial chronosequence with 10 sites ranging from 5-120,000 years in New Zealand. The role of pathogens in succession has received increasing attention in the last couple decades and it is quite clear that pathogens can be important at both early and late stages of succession, as was nicely described in the introduction of the paper.

This study is novel in that it is apparently the first to document "pathogen dynamics" across all stages of ecosystem development. In addition, overall the paper is very well organized, well written, and the figures are quite nicely done.

Rev 3: However, I have serious concerns about the importance of the study for understanding the hypotheses laid out in the Introduction of the paper. The background provided is very thorough and the hypotheses are well developed, but this study only provides correlative information about each hypotheses. For example, although pythium and phytophthora species are commonly pathogenic I don't think it is known here if these particular species are in fact detrimental to plant fitness for the species on which they were documented. In fact they might simply occur on the species with little effect - that is, there is no evidence for cause and effect. It might seem like a tall order but it would have been helpful to have some experimental evidence that these pathogen species do in fact affect the performance of at least some of the plant species on which they were documented.

Analysis of comment: We agree completely that another research project should be done looking at actual soil feedbacks, particularly based on our findings. There isn’t really anything we can do to address this comment other than clarifying that our hypotheses do not require that the oomycetes were necessarily harming the plants we found them on.

Rev 3: In addition, although the study is clearly novel and constitutes a huge effort (and I'm probably naive to the field and lab work required), I'm concerned about the sample size. There are relatively few samples of many plant species, and few plant species sampled at old sites. This pattern partially may reflect the number of species found at retrogressive sites.

Overall I found the paper to be very interesting, easy to read, and a compelling start to a story - but lacking in enough evidence that indeed pathogens are important in driving early but not late successional dynamics, which I think is the main conclusion

of the paper.

Analysis of comment: The approach we took was to sample 500+ root samples at random, which provides a high level of replication for each site (51 or 52 roots per site), but does, indeed, reflect the natural diversity and abundance of plants rather than having a uniform replication of a uniform number of species. Having n > 50 for each ecosystem age, we feel "relatively few" per plant species needs to be considered in the context of many samples overall.

Change made: following an earlier comment we made the sample size much clearer for each test.

Rev 3: L177-189: Use of the terms "increasing" or "decreasing" or "declining" are not accurate given the chronosequence design of the study. The ecosystem was not followed over time, instead comparisons among sites with different ages have been done such that one could say "higher" or "lower" but there is not evidence of increases or decreases at particular sites. This distinction might seem pedantic but I think it is important to be clear that this is an observational study of the current condition, not a temporal study per se.

L287: instead of "had a decline" more accurate would be "had lower"

L290: "significance of change" should be "significant of difference" - since you did not track changes over time.

Also check throughout the manuscript, e.g., L366, 384, 434, 507 for similar issues.

Analysis of comments: This is a case where a seemingly semantic argument is actually vastly important. The treatment of multiple sites as representing change over time is absolutely fundamental to our approach and, indeed, to the vast majority of studies of ecosystem succession. The reviewer disagrees, but in doing so is essentially arguing not only that all studies of succession using space-for-time substitution are invalid, but also that ecology can only study processes that occur over short time frames (certainly < 100 years, most likely < 10 years).
We could modify every sentence that used “increasing” or “decreasing” but, in actuality, our hypotheses fundamentally are based on change through time – so changing the words would be window dressing at best. At worst it actually becomes inaccurate – retrogression, for example, is defined on the basis of decline over time, not on the basis of any particular level of biomass or diversity. Thus it would be impossible to define retrogression given the reviewer's suggested approach. The only meaningful change we can make is to be certain to acknowledge the assumption of inferring change on the basis of a space for time comparison.

Change made: we now note “allowing space-for-time substitution as an approach to infer change over long time periods (Pickett, 1989).”

Rev 3: Is the frequency of possible pathogens on roots equivalent to pathogen "abundance" (L355)? I don't think so, instead this measurement describes how common the pathogen is across roots in the system - in fact, pathogens could be found less frequently but be more abundant. Right?

L358-359: is "a very abundant sequence" accurate? Or was it just frequently found? Was quantitative PCR conducted?
Similarly - L486: should "while we did not find abundant oomycetes" be "while we did not frequently find oomycetes? Maybe I am not clear on the measurements.

Analysis of comment: As noted above, we incorrectly used the word abundance in a few cases where we should have used frequency. These are fixed. We use frequency rather than within-root abundance, in the same way that epidemiological studies use infection rate (rather than the viral load per patient) to understand disease dynamics.

Rev 3: L416-418: would be neat to see an experimental test of this hypothesis in this system!

Analysis of comment. We agree. Nonetheless, the first step to designing experiments is to observe the system, and we believe our data are important correlations that (if published) could provide supporting evidence to underpin applications for funding to do experimental tests.

Rev 3: L426-427: I agree, and given this idea can you confidently state that pathogens were not important at late successional sites?

Analysis of comment: This comment is refering to the suggestion that fungal pathogens may be more important in mature ecosystems. We believe the reviewer is pointing out that we often refered to "pathogens" when we should have said "oomycete pathogens in established plant roots".
Changes made: We have taken the comment as a general critique and tried to edit our phrasing to be less generic to "pathogens" which could include many organisms we didn't measure.

Editor summation:

Although my initial excitement about the study was also shared by the three referees, they all three unanimously concluded that the story has been oversold and that the data are not strong enough to support the story. That is a real pity, but I agree and do not see any other option than proposing rejection. When preparing the manuscript for submission to a more specialized journal, I recommend the authors to not promise more than the data really support and make good use of the comments provided by the three referees.

Analysis of editor comment: We believe the editor could reach no other decision than reject on the basis of the reviews.
The line between providing sufficient "selling" to get into a high impact journal and "over-selling" is, perhaps, a subtle one. Nonetheless, the process of review was helpful despite the rejection.

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Additional comments received:
The sole comment on BioRxiv was : "Will give a full read later, but one thing jumped

out at me in a skim read, check the spelling of Phytophthora kernoviae on line 460"

Reply: spelling fixed.

Email comments from colleague (partial):"... my only query is about using the LSU which we know is not much good at distinguishing species, only genera. In fact I am pretty sure there is not LSU sequence available for all Phytophthora or pythium species. I think this is giving you good presence/absence data but I would be careful about ID's e.g.. the Lagena ID as it is based on a match to an uncultured oomycete (which could be anything).

In the tree Phytophthora megasperma occurs more than once;one of the identities must be incorrect on genbank and was a species complex that has now been divided. in the ecosystem age by host figure the legend doesn't make sense to me as a Phytophthora person. P. aft megasperma is in Phytophthora clade 6 and infestans is in clade 1 – they should not fall together – similarly aff. cinnamoni would be in clade 7 and cactorum is in clade 1 – if they are together the resolution of the LSU must be very low"

Reply: We renamed our Phytophthoras to "sp. 1" and "sp. 2" -- the LSU region proved to have very poor resolution for Phytopthora species. The Lagena match is difficult -- the best match for LSU was to "uncultured" but the second-best match is a good match to Lagena, as is the ITS sequence match.