Biogeographic plant-microbe patterns and process: natural and anthropogenic impacts across three spatial scales

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Abstract

The plant microbiome is essential to the maintenance of plant community structure and diversity. In addition, anthropogenic forces are altering these long-standing relationships. Despite the importance of the plant microbiome and increasing human impacts, questions about how the plant microbiome drives plant biogeography and how these microbes and plant-microbe relationships change in response to anthropogenic forces remain understudied. Here, I aim to clarify the microbial contribution to biogeographical patterns of plants, biogeographical patterns of plant-associated microbes themselves, and the nature of the plant-microbe relationship, with patterns and processes contrasted in native systems and in those altered by anthropogenic impacts. The specific aims are to (1) clarify how ubiquitous plant symbionts - mycorrhizal fungi - influence global island biogeographical patterns, as well as subsequent shifts in these patterns due to plant naturalizations, (2) determine how different mycorrhizal types with varying life-history traits may differentially contribute to these patterns, (3) describe the response of plant pathogens to climate and land use impacts using a natural Midwestern precipitation and temperature gradient, and (4) investigate the evolution of the plant-mycorrhizal relationship in native plant species as a result of novel plant-mycorrhizal interactions in Kansas. Together, this work contributes to our understanding of plant-microbe associations across different scales in native systems as well as the consequences of anthropogenic impacts, with implications for conservation and management.

Chapter 1 addresses how the limited dispersal of mycorrhizal fungi on oceanic islands act as a colonization filter for plants. This hypothesis was tested using global-scale analyses of ~1.4 million plant occurrences including ~200,000 plant species across ~1100 regions. The results support the operation of a mycorrhizal filter (i.e. the filtering out of mycorrhizal plants on

islands), with mycorrhizal associations less common among native island plants than native mainland plants. In addition, the proportion of native mycorrhizal plants in island floras decreased with isolation from mainlands, consistent with a decline in symbiont establishment. Mycorrhizal plants are also shown to contribute disproportionately to the classic latitudinal gradient of plant species diversity, with the proportion of mycorrhizal plants being highest in lower latitudes and decreasing towards higher latitudes. Anthropogenic pressure and land use alter these plant biogeographic patterns, as naturalized floras showed a greater proportion of mycorrhizal plant species on islands than in mainland regions, as expected from anthropogenic co-introduction of plants with their symbionts to islands and anthropogenic disturbance of symbionts in mainland regions. Overall, this work identifies the mycorrhizal association as an overlooked driver of global plant biogeographic patterns with implications for contemporary island biogeography and our understanding of plant invasions.

Chapter 2 expands on Chapter 1 by analyzing biogeographical patterns of plants associating with three major types of mycorrhizal fungi. Plant colonization of islands may be limited by the availability of arbuscular mycorrhizal (AM) fungi in particular, which have limited dispersal ability compared to ectomycorrhizal (EM) and orchid mycorrhizal (OM) fungi. We tested for such differential island colonization within contemporary floras worldwide. We found evidence that AM plants experience a stronger mycorrhizal filter than other mycorrhizal or non-mycorrhizal (NM) plants, with decreased proportions of native AM plant species on islands relative to mainlands. This effect intensified with island isolation, particularly for non-endemic plant species. The proportion of endemic AM plant species increased with island isolation, consistent with diversification filling niches left open by the mycorrhizal filter. Naturalized floras featured higher proportions of AM plant species than native floras, a pattern that increased

with increasing isolation and land-use intensity. This work provides evidence that the biology of fungal symbionts shapes plant colonization of islands, subsequent diversification and anthropogenic impacts.

Chapter 3 uses a natural precipitation and temperature gradient across the Midwestern United States (regional scale) to examine soil-borne pathogen response to climate and land use. Soil-borne pathogens structure plant communities, shaping their diversity, and through these effects may mediate plant responses to climate change and disturbance. Little is known, however, about the environmental determinants of plant pathogen communities. Therefore, this work explored the impact of climate gradients and anthropogenic disturbance on root-associated pathogens – fungal pathogens and oomycetes – in grasslands. In undisturbed grasslands, precipitation and temperature gradients were important predictors of pathogen community richness and composition. Oomycete richness increased with precipitation, while fungal pathogen richness depended on an interaction of precipitation and temperature, with precipitation increasing richness most with higher temperatures. Disturbance altered plant pathogen composition and precipitation and temperature had a reduced effect on pathogen richness and composition in disturbed grasslands. Because pathogens can mediate plant community diversity and structure, the sensitivity of pathogens to disturbance and climate suggests that degradation of the pathogen community may mediate loss, or limit restoration of, native plant diversity in disturbed grasslands, and may modify plant community response to climate change.

Chapter 4 focuses on the evolution of the plant-AM fungal relationship at a local scale, to highlight changes to the relationship in novel environments. Arbuscular mycorrhizal fungi (AMF) play an essential role in structuring plant communities, especially in native systems. Nonetheless, increasing anthropogenic disturbance will lead to novel plant-AMF interactions,

altering a longstanding co-evolutionary trajectory between plants and their associated AMF. Although emerging work shows that plant-AMF response can evolve over short time scales due to anthropogenic change, little work has evaluated how plant AMF response specificity may evolve due to novel interactions. Therefore, changes in plant-AMF interactions in novel grassland systems were examined by comparing the mycorrhizal response of plant populations from unplowed native prairies with populations from post-agricultural grasslands to inoculation with both native prairie AMF and non-native AMF. Across four plant species, results support evolution of mycorrhizal response specificity consistent with expectations of local adaptation, with plants from native populations responding most to native AMF and plants from post-agricultural populations responding most to non-native AMF. Evolution of mycorrhizal response in two of the four plant species was also found, as overall responsiveness to AMF changed from native to post-agricultural populations. Finally, across all four plant species, roots from native prairie populations had lower levels of mycorrhizal colonization than those of post-agricultural populations. These results highlight that widespread anthropogenic disturbance can have unintended impacts on the genetic propensities of native plant species' association with AMF, causing rapid evolutionary change in the benefit native plant species gain from native symbioses.

Accumulating evidence supports the important role of the plant microbiome in mediating plant community structure and diversity, yet many basic questions about how the plant microbiome drives plant biogeography and how these microbes may indirectly affect plant communities through anthropogenic change remain understudied. The work in this thesis leveraged global datasets, molecular tools, and greenhouse experiments to begin to answer such questions, contributing substantially to our understanding of two major plant-associated microbes – mutualists and pathogens – at different scales in both native and anthropogenically-

altered systems. Together, the research in this thesis improves our understanding of how plant-associated microbes influence plant distribution (biogeography), how climate influences these plant-associated microbes, and the consequences of anthropogenic forces – including land use change, plant introduction, and novel environments – on these patterns and processes.

Introduction

Just as the human microbiome is integral to our health and functioning, plant microbiomes are essential to plant community functioning, diversity, and structure. Two important groups of microbes that contribute to structuring plant communities and are essential to the plant microbiome are mycorrhizal fungi and soil pathogens. Mycorrhizal fungi form a typically beneficial symbiotic relationship with most terrestrial plant species, conferring several plant and ecosystem benefits that influence plant fitness, including increased nutrient uptake and resistance to pathogens. These fungi have been shown to be important in plant community structure and succession. Soil-borne pathogens have also been shown to maintain plant diversity, as accumulation of species-specific pathogens around a plant suppresses recruitment of that species' offspring, preventing any one species from dominating and allowing a diversity of species to establish. Despite the importance of these plant-microbe relationships, there are significant gaps in our understanding of these relationships in both co-evolved native systems and anthropogenically-altered systems.

Major research gaps in plant-microbe interactions in native systems exist in understanding microbiome-mediated plant biogeography and the biogeography of key functional groups of plant-associated microbes. Although mycorrhizal fungi are known to influence plant community diversity and structure, there is a prominent geographical bias in related studies, with most work occurring in temperate mainland systems. This bias results in a major gap in understanding how these symbionts may be implicated in global plant biogeography as well as island biogeography. A second important gap is understanding the biogeography of functional groups of plant-associated microbes across environmental gradients. Given the important role of

mycorrhizal fungi and plant pathogens in plant community structure and diversity, the responses of plant communities to environmental drivers may be mediated by the sensitivities of these communities.

Anthropogenic impacts may alter these longstanding plant-microbe relationships and biogeographical patterns through species introductions, land use change and climate change. Nonetheless, significant gaps remain in understanding how anthropogenic forces shift microbiome-mediated plant biogeography, alter biogeography of plant-associated functional groups and evolution consequences for the plant-microbe relationship. Plant introductions are impacting the ecology of native systems worldwide, with human-lead introduction of non-native plants posing a large threat to conservation of native flora and ecosystem properties of invaded sites, but the consequences to plant-microbe interactions remain understudied. Further, land use disturbances such as tillage, fertilizer additions, heavy grazing, and row crop monocultures have been shown to alter microbial communities in general, but the sensitivity of plant-associated microbes to disturbance not well understood. Finally, contemporary and future changes in climate, including temperature, and changes in the intensity and frequency of precipitation events, make understanding plant-associated microbial responses to environmental climate gradients particularly important. Combining information on how anthropogenic drivers impact microbiome-mediated plant biogeographical patterns as well as how the plant microbiome responds to climatic variables, land use change, and species' introductions will be important to understanding human impact on these microbiome communities, and ultimately, on the plant communities they sustain.

Here, I present work focused on co-evolved mycorrhizal and pathogen plant relationships as well as the consequences of human disturbance, through plant introductions, climate shifts,

and land use. First (1) I test for evidence of a global mycorrhizal filter driving differential plant colonization of islands and how anthropogenic forces alter these patterns. Then (2), I investigate differences in island colonization patterns across the major types of mycorrhizal associations. Next (3), I present work on how land use and climate impact pathogen community structure and diversity using a natural Midwestern precipitation and temperature gradient. Finally (4), I present evidence for the rapid evolution of mycorrhizal response in post-agricultural disturbed grassland systems in Eastern Kansas due to novel plant-mycorrhizal relationships. Together, this work finds novel ways in which co-evolved microbial-plant relationships are important to native plants and the myriad consequences of human activity across different scales, with implications for management and restoration.

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Chapter 1: Mycorrhizal fungi influence global plant geography

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Abstract

Island biogeography has traditionally focused primarily on abiotic drivers of colonization, extinction, and speciation. Establishment on islands, however, could also be limited by biotic drivers, such as the absence of symbionts. Most plants, for example, form symbioses with mycorrhizal fungi, whose limited dispersal to islands could act as a colonization filter for plants. We tested this hypothesis using global-scale analyses of ~1.4 million plant occurrences including ~200,000 plant species across ~1100 regions. We find evidence for a mycorrhizal filter (i.e. the filtering out of mycorrhizal plants on islands), with mycorrhizal associations less common among native island plants than native mainland plants. Furthermore, the proportion of native mycorrhizal plants in island floras decreased with isolation, possibly as a consequence of a decline in symbiont establishment. We also show that mycorrhizal plants contribute disproportionately to the classic latitudinal gradient of plant species diversity, with the proportion of mycorrhizal plants being highest near the equator and decreasing towards the poles. Anthropogenic pressure and land use alter these plant biogeographic patterns. Naturalized floras show a greater proportion of mycorrhizal plant species on islands than in mainland regions, as expected from anthropogenic co-introduction of plants with their symbionts to islands

and anthropogenic disturbance of symbionts in mainland regions. We identify the mycorrhizal association as an overlooked driver of global plant biogeographic patterns with implications for contemporary island biogeography and our understanding of plant invasions.

Introduction

Classical island biogeography recognizes that species richness results from the balance of immigration, which decreases with isolation (i.e. distance to the mainland), extinction, which decreases with island size (MacArthur and Wilson 1967), and speciation, which increases with island size (Losos and Schluter 2000, Kisel and Barraclough 2010). Subsequent work has identified that environmental heterogeneity and geologic and climatic history also have important effects on the diversities of island biotas (Kreft et al. 2008, Whittaker et al. 2008, Weigelt et al. 2016, Borregaard et al. 2017). Individual case studies show that biotic interactions can also influence species colonization and extinction probabilities on islands, but the generalizability of these effects is uncertain (Losos and Ricklefs 2009, Onstein et al. 2017). Order of arrival, resulting in priority effects (Fukami 2015), is likely to be particularly important for mutualistic symbioses. The mycorrhizal symbiosis formed between soil fungi and most plant species is a prime candidate for priority effects because plant species vary in their dependence on the association, while mycorrhizal fungi are unlikely to establish first because they are obligately dependent on their hosts (Bever et al. 1997, van der Heijden et al. 2008).

Mycorrhizal fungi are mainly known for their role in nutrient acquisition, but also provide additional benefits for their associated plants, including pathogen resistance and soil aggregation (Delavaux et al. 2017). Associating with mycorrhizal fungi is the ancestral state of

plants, but this trait has been lost repeatedly over evolutionary history (Redecker et al. 2000, Maherali et al. 2016). There are several ecological contexts in which the independence of mycorrhizal symbioses confers a competitive advantage to plants (Smith and Read 2008, Maherali et al. 2016). One is when mycorrhizal fungal *presence is unreliable*, such as in newly formed habitats (Davison et al. 2016, Dickie et al. 2017). With their obligate plant host dependence, mycorrhizal fungi are not likely to establish on islands, particularly isolated oceanic islands, before their host plants. Therefore, the absence of these fungi may act as a biotic habitat filter leading to disproportionate colonization by plant species that do not rely on mycorrhizal fungi. A second context where the independence of the symbioses may confer an advantage to plants is when mycorrhizal fungi cannot grow due to *environmental constraints*, including anoxic soils and extreme cold (Miller et al. 1999, Brundrett 2009, Tedersoo 2017, Brundrett and Tedersoo 2018a). The latter suggests that there should be fewer mycorrhizal plant species at high latitudes and altitudes. A third context that may lead to independence of mycorrhizal fungi is when the costs of the symbioses outweigh the benefits due to particularly high or low soil fertility (Lambers et al. 2008, Steidinger and Bever 2014, Abbott et al. 2015, Jiang et al. 2018). These later two environmental forces may be more important where dispersal limitation is relatively unimportant, such as in mainland regions. Although a recent analysis of the distribution of arbuscular mycorrhizal fungi suggested that these fungi are not likely to be limited by dispersal (Davison et al. 2015), this study did not consider islands on which dispersal limitation would be strongest. Moreover, limited dispersal of mycorrhizal fungi is supported by distribution patterns of ectomycorrhizal fungi (Peay et al. 2012, Tedersoo et al. 2014), and both arbuscular and ecto- mycorrhizal plants performance can be limited by the absence of appropriate symbionts (Koziol et al. 2018a, Peay 2018).

These expected patterns of proportion of mycorrhizal species for native plant species, may differ for anthropogenically driven introductions of plant species (Redecker et al. 2000, Maherali et al. 2016). On islands, plant introductions by humans may overcome the mycorrhizal filter, as agricultural or ornamental perennial plants are commonly brought with soil and associated microbes (Richardson et al. 1999). This may result in naturalized floras with a greater reliance on mycorrhizal fungi (Callaway et al. 2004, Dickie et al. 2017). Human disturbance of soils through land use may disrupt mycorrhizal fungal communities (*unreliable presence*) (Oehl et al. 2003), thereby reducing the proportion of plants in a naturalized flora that rely on mycorrhizal fungi. This driver may be dominant in mainland regions, where soil disturbance has been shown to reduce presence of mycorrhizal fungi (Pringle et al. 2009). While individual studies have found differences in the proportion of mycorrhizal plant species between native and naturalized floras (Pringle et al. 2009, Bunn et al. 2015, Davison et al. 2015, Bueno et al. 2017, Reinhart et al. 2017), they were mostly conducted on small scales and within mainlands. A comprehensive global analysis of factors influencing the distribution of mycorrhizal plants is required to test for general patterns.

Here, we use a global data set of 213,710 angiosperm plant species including 1,437,761 plant occurrences across 1103 regions to test for patterns of plant species' mycorrhizal status in island and mainland floras, for both native and naturalized plant species. To test whether a mycorrhizal filter affects island colonization, we contrast the mycorrhizal status (assigned to species in each family from each region in the same proportion as the averaged reported proportion of mycorrhizal and non-mycorrhizal species for that family) of native and naturalized species in island and mainland floras, and assess the effects of island geology, age, and distance to the mainland on the proportion of species that are mycorrhizal.

Results and Discussion

Our results show that mycorrhizal fungi influence global plant distributions and are associated with classic biogeographical patterns such as the latitudinal diversity gradient (Hillebrand 2004) and the species-isolation relationship (Weigelt and Kreft 2013). We find compelling evidence that initial colonization of islands by plants is influenced by a mycorrhizal filter; mycorrhizal species are under-represented in contemporary native island floras compared to mainland floras (Fig. 1). Specifically, we find a significant interaction between land type and mycorrhizal status, showing that the number of native mycorrhizal plant species on islands is significantly lower than on mainlands ($p < 0.0001$; $z = -7.474$, GLMM; Supplementary Information Table 1, model M1). Consistent with the operation of the mycorrhizal filter, the proportion of mycorrhizal plant species on islands declines with distance to the mainland (Fig 2a, $p < 0.01$,Supplementary Information Table 1, model M4, GLM). Diversification of early mycorrhizal colonists may increase the proportion of native mycorrhizal species in old oceanic archipelagos, as has been observed in the Hawaiian islands (Koske et al. 1992). Nonetheless, our data show no statistically significant relationship between island age and the proportion of mycorrhizal plant species ($p = 0.089$, Supplementary Information Table 1, M5, GLM).

For mainland native floras, variation in the proportion of mycorrhizal plant species is primarily predicted by latitude and correlated with environmental variables. Specifically, the proportion of mycorrhizal plants increases towards the equator (Fig. 2c, Fig. 2d; all $p < 0.001$, GLM). This strong relationship between latitude and proportion of mycorrhizal plant species indicates that mycorrhizal plants contribute disproportionately to the classic latitudinal diversity gradient, a pattern previously reported for European floras (Bueno et al. 2017). This latitudinal

change in proportion of mycorrhizal plant species may reflect the arbuscular mycorrhizal ancestral state and tropical origin of major plant clades (Maherali et al. 2016). Alternatively, the decreasing proportion of mycorrhizal plant species towards the poles may be explained by extreme environments limiting the plant fungal symbionts in these regions (Miller et al. 1999), such as extreme cold (*environmental constraints*) and extensive past glacial coverage (*unreliable presence*). Indeed, we find that there is a significant reduction in the proportion of mycorrhizal plant species with decreasing mean annual temperature (Fig 2b; $p < 0.001$, GLM). These environmental predictors of the proportion of mycorrhizal plant species are stronger for native mainland floras than for native island floras (Supplementary Information Table 1, models M3 and M4; Supplementary Fig. 2 & 3, GLM).

Naturalized island floras are disproportionately mycorrhizal compared to naturalized mainland floras (Fig. 1a; $p < 0.001$, Supplementary Information Table 1, model M2, GLMM), suggesting anthropogenic relaxation of the mycorrhizal filter. Anthropogenic relaxation is further supported by a weakening dependence of mycorrhizal status on island isolation, as distance to the mainland is no longer a significant predictor of species richness in naturalized island floras ($p = 0.225$, Supplementary Information Table 1, Model M7, GLM). These results are consistent with human movement of mycorrhizal fungi via transplants of mycorrhizal colonized material (e.g. perennial agricultural crops, horticultural plants, sand and soil transports) to islands (Vellinga et al. 2009, Dickie et al. 2017).

In mainland regions, mycorrhizal plant species are generally under-represented in naturalized floras (Fig. 1a) compared to native floras, particularly in areas with a high proportion of native mycorrhizal plants (Fig. 3a; pseudo $R^2 = 0.286$, $p < 0.0001$, GLM). This is consistent with invasions of non-mycorrhizal plant species being facilitated by the *unreliable presence* of

mycorrhizal fungi due to large scale anthropogenic disturbance (Pringle et al. 2009). Alternatively, this pattern may be a result of nutrient deposition; as nutrient levels, including nitrogen and phosphorus levels, are usually higher in human-modified areas, potentially changing the cost-benefit ratio of mycorrhizal symbiosis in favour of non-mycorrhizal species. Consistent with mainland regions, we find that the proportion of mycorrhizal plants in naturalized island floras decreases with human land-use intensity ($p < 0.001$, Fig. 3b, GLM). This may be a result of islands becoming more mainland-like, with human land use disturbing soil microbes, resulting in an *unreliable presence* or reduced benefit of mycorrhizal fungi. Anthropogenic influence on biogeographical patterns is also evident as environmental variables are weaker predictors for naturalized than for native floras (see Supplementary Information Fig. 2 & Fig. 4 for all graphs of naturalized plant results, GLM).

We find consistent global patterns in the distribution of mycorrhizal plant species: mainland and island floras differ in their proportions of mycorrhizal plant species and this relationship changes with human induced plant introductions. Further, we show that in native island floras, the proportion of mycorrhizal plant species decreases with isolation (distance from mainland). These findings are consistent with the limited dispersal of mycorrhizal fungi to islands, reducing the amount of native plant species that are mycorrhizal on these islands. Finally, we find a latitudinal relationship for native mainland floras, where the proportion of mycorrhizal plant species is highest at the equator and decreases toward the poles, which is consistent with extreme cold limiting the functioning of mycorrhizal fungi (Brundrett 2009, Tedersoo 2017, Brundrett and Tedersoo 2018a). We suggest that these patterns are mediated by the mycorrhizal symbiosis. Alternatively these biogeographic patterns might be caused in part by traits that co-vary with mycorrhizal status of plants (Cornelissen et al. 2001, Powell et al. 2017).

For these co-varying traits to explain our results, non-mycorrhizal plants would need to exhibit greater dispersal ability or greater cold tolerance as compared to their mycorrhizal counterparts. To date, we know of no evidence of such covariance. As there is clear evidence for fungal dispersal limitation (Peay et al. 2012, Koziol et al. 2018a, Peay 2018) and limits of fungal efficiency under cold environmental conditions (Brundrett 2009, Tedersoo 2017, Brundrett and Tedersoo 2018a), we contend that our results are likely mediated by mycorrhizal fungal availability and functioning.

We show that native and naturalized floras worldwide differ in their composition in conjunction with mycorrhizal association, with differing proportions in island versus mainland regions. Proportions of mycorrhizal plant species in mainland regions vary strongly along climatic and latitudinal gradients, with the proportion of mycorrhizal plant species being highest near the equator and decreasing toward the poles, suggesting that mycorrhizal species contribute disproportionately to the classical diversity-latitude relationship (Hillebrand 2004). For islands, we find that the composition of native floras reflects the mycorrhizal filter of plant colonization success; with the initial absence of their mycorrhizal symbionts, mycorrhizal plants are disproportionally filtered during island colonization. Human influenced movement of naturalized species with their mycorrhizal symbionts to islands may alleviate mycorrhizal dispersal limitation, thereby weakening isolation-by-distance effects. While further work examining mycorrhizal status of plants, particularly in the tropics, is necessary to confirm these patterns, our results suggest that mycorrhizal fungi influence global plant biogeography, including island biogeography and the species richness latitudinal gradient, and influence patterns of human-mediated plant introductions.

Methods

Plant distribution data and floristic status

Plant species occurrence data across 1103 regions around the globe (mostly administratively defined regions such as countries and provinces or islands), native status (native versus naturalized), and all additional parameters associated with regional characteristics were extracted from the Global Alien Naturalized Flora (GloNAF(Van Kleunen et al. 2015b); SINote1) and from the Global Inventory of Floras and Traits (GIFT(Weigelt 2017)) databases. Naturalized is defined as non-native species that "form self-sustaining populations in new regions(Van Kleunen et al. 2015a). From the GloNAF database, we included only regions for which the available species lists had a completeness level of 2 (50 -90% of naturalized species included) and 3 (> 90% naturalized species included). From the GIFT database, we only used regions for which checklists of native angiosperms were available. When there were overlapping regions, the smaller regions were kept if greater than 100 km^2 for mainland regions; for islands, the smaller units were always preferred. Finally, we removed islands for which island geology (i.e. volcanic, floor, shelf, fragment, etc.) was undetermined. After all cleaning of the data, we had a total of 1,437,761 plant occurrences across 1103 unique regions. Our final data included 133,491 plant occurrences in 574 regions from the GloNAF dataset and 1,304,270 plant occurrences in 979 regions from the GIFT dataset.

Mycorrhizal status

The mycorrhizal status of the 1,437,761 plant occurrences included in this study was determined by assigning each species to its plant family according to theplantlist.org, incorporating classification from APG IV (Byng et al. 2016). We relied on family proportions of known mycorrhizal and non-mycorrhizal species to assign mycorrhizal status to species in this study. Specifically, within each region, species in a family were assigned mycorrhizal or non-mycorrhizal in the same proportion as that family (Supplementary Information Table 2). Given the geographic bias in knowledge of species-level mycorrhizal status (bias towards heavily studied temperate systems), this family level assignment was the most rigorous method we could employ. We used three review papers to determine plant family consensus proportion mycorrhizal status (Gerdemann 1968, Brundrett 2009, Maherali et al. 2016). While concerns have been raised over incorrect classification in these review papers (Brundrett and Tedersoo 2018a) which cannot be addressed at this time due to lack of species-specific corrections, potential errors are not likely to have large effects on a global database. If species in a family were AM (arbuscular mycorrhizal), EM (ectomycorrhizal), ERM (ericoid mycorrhizal), ORM (orchid mycorrhizal) or AMEM (half AM and half EM), we classified these as 'mycorrhizal' (M). Different classifications and proportions between the reference papers were accounted for by using the average consensus proportions for each mycorrhizal category (M, NM, AMNM; see below) across the three references (excluding data where values were not reported). We determined the consensus proportion of sampled species in each family that were mycorrhizal (M), non-mycorrhizal (NM) or ambiguous (AMNM; equally split arbuscular mycorrhizal and non-mycorrhizal); we ran each of our analyses twice, either putting all ambiguous species (AMNM) as M or NM. Our initial distribution dataset was reduced to species for which we had family level mycorrhizal data, resulting in the data described in the "*Plant distribution data and*

floristic status" section above. Specifically, 142,164 unique observations (unique species–location combinations) out of ~2,000,000 were removed due to lack of mycorrhizal data. Nonetheless, we do find a slightly higher proportion of omitted data points at highest latitudes; these omissions are from Compositae (46) or Leguminosae (3). The full table of families and corresponding consensus proportions of mycorrhizal status can be found in our Supplementary Information (Supplementary Information Table 2).

Explanatory variables

Explanatory variables for each of the regions were extracted from the GIFT database. For details of environmental data collection, see Weigelt et al. (2017). Explanatory variables include land type (mainland or island), latitude and longitude of the region's centroid, area (km²), mean annual temperature (°C) and mean annual precipitation (mm) (Karger et al. 2017), maximum elevation range (difference between lowest and highest elevation in m) (Danielson and Gesch 2011), human population density (n/km²)(Center for International Earth Science Information Network - CIESIN - Columbia University 2005) and two land-use metrics, cultivated and managed vegetation, and urban land use area (combined as a sum following log transformation to form the new variable human land use in our analyses) (Tuanmu and Jetz 2014). For islands, we also included their distance to the nearest mainland (km) as a measure of island isolation (Weigelt and Kreft 2013), geological origin (referred to in model results as geology; oceanic or non-oceanic), and island age (millions of years; only meaningfully quantified for oceanic islands). We included non-oceanic islands in the group of oceanic islands if they were covered with ice (at least 80%) during the last glacial maximum (Tuanmu and Jetz 2014) because the

land would have characteristics of a newly formed oceanic island after the plant and fungal communities were destroyed by glaciation.

Statistical analysis

To test broad-scale patterns of mycorrhizal-plant distributions, we modeled regional plant species richness (counts) for mycorrhizal (M) and non-mycorrhizal (NM) plants in two analyses (separately for native and naturalized plants; Fig 1 A). For these analyses, we used generalized linear mixed effects models (GLMM). We chose a Poisson distribution, as the response variable, species richness, is count data. The fixed effects were mycorrhizal status, land type (mainland or island) and their interaction. The random effects were region nested within land type (mainland or island) and mycorrhizal status nested within region nested within land type (mainland or island). We ran this model separately for native (model M1) and naturalized (M2) plant species richness as response variables. The sample size (N) in these two models represents a unique regional combination of native status (native or naturalized) and mycorrhizal status (mycorrhizal or non-mycorrhizal). Of the 4492 observations in this dataset, 2246 involved native, 2246 involved naturalized floras; this corresponded to 1123 regions with native data, and 1123 regions with naturalized data. To create Fig. 1, we converted our count estimates from the model to proportions.

To investigate drivers of mycorrhizal status of native and naturalized plants in mainland and island floras, we used the proportion of mycorrhizal (M) species at each region as the response variable. For this analysis, we used generalized linear models (GLMs) with a logit link function, assuming a binomial distribution of the response variable. For these models, we took

the natural log of area, human population density, distance to the nearest mainland, elevation range and island age to normalize distributions. For the native mainland model (M3), we included area, mean annual precipitation, mean annual temperature and elevation range. For the native island model (M4), we included area, distance to the mainland, precipitation, temperature, elevation range and geology as well as the interaction between distance to the mainland and geology (oceanic or non-oceanic). Variables tested were informed through prior study of these variables with this dataset(Kreft et al. 2008) as well as other island biogeographic studies (Kueffer et al. 2010, Triantis et al. 2015). As the presence of naturalized species is likely to be driven by human activities, the naturalized mainland model (M6) and the naturalized island model (M7) included human population density in addition to the explanatory variables included in the corresponding models for native species. Finally, to account for the effect of island age, we additionally analyzed the subset of oceanic islands for which we had age data. For the models of native floras including island age (M5, N = 246), we included area, distance to the mainland, age, precipitation, temperature and elevation range. For the models of naturalized island floras including age (M8, N = 97), we additionally included human population density. Results of these main models are presented in Supplementary Information Table 1 (M1-M8). N in these and all models excluding M1 and M2 are true N representing unique regions. Prior to any further subsetting, this dataset had a total of 1103 regions (same as observations); 979 regions had data for native flora and 574 regions had data for naturalized flora.

To explore linear and non-linear latitudinal patterns in mycorrhizal distribution in more detail, we reran all models including only absolute latitude and absolute latitude squared. We also ran models to investigate anthropogenic drivers of mycorrhizal status in naturalized plants only. For these models, we included a combined variable of urban land use area and cultivated

and managed vegetation, 'human land use' (sum of both variables). To assess the robustness of our results in the face of the uncertainty in mycorrhizal status assignment, we reran all models to assign ambiguous (AMNM) plants to NM instead of M assumed in the original models. Here, we report statistics from models in which AMNM plants were assigned to M (Supplementary Information Table 1, Supplementary Information Fig. 2-4). For models where AMNM plants were assigned to NM, see Supplementary Information Table 3 and Supplementary Information Fig. 5-7).

Before running all models, we removed regions where plant coverage was unreliable; we considered this to be the case when there is a zero in total calculated mycorrhizal or non-mycorrhizal species counts in this region. The main cause of this was the incomplete family coverage of mycorrhizal status; if we had no information on the mycorrhizal status of all species' families in a region, these regions would result in an incorrect sum of zero mycorrhizal and zero non-mycorrhizal plant species. We removed these regions because this zero value was not representative of the entire region. We also removed island regions where geology was undetermined prior to analyses. We corrected for overdispersion in GLMs using a quasi-binomial or quasi-Poisson family model. In addition, most of our model residuals showed spatial autocorrelation as tested using Moran's I, which is expected in global scale models with spatially clustered geographic regions. We corrected for this spatial autocorrelation by creating a new variable (spatial autocovariate) that incorporates a matrix of longitude and latitude coordinates of the regions (Crase et al. 2012) in the spdep package in R (Bivand and Piras 2015). After checking for spatial autocorrelation in our corrected models, some models still showed spatial autocorrelation (as determined through Moran's I), but all spatial autocorrelation was reduced substantially (correlograms shown in Supplementary Information Fig. 4 & 7). All models and

summary statistics were run in R 3.4.1(Team 2016) in the lme4 package (Bates et al. 2015).

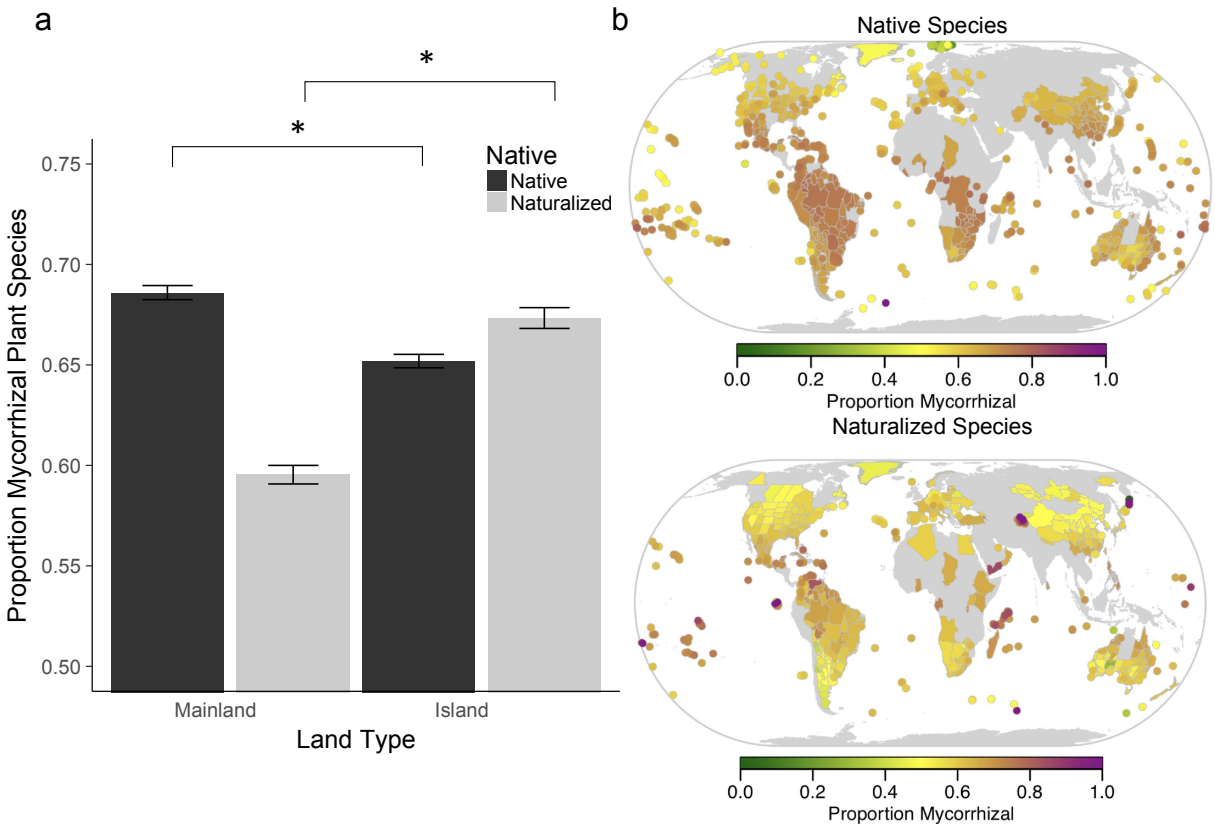


Figure 1. Proportion of mycorrhizal plant species in native and naturalized floras of island and mainland regions. (a) For native floras (black bars), the proportions of species that are mycorrhizal are significantly greater for mainland than island regions (marginal $R^2 = 0.57$, $p < 0.001$, GLMM). For naturalized floras (grey bars), the proportions of species that are mycorrhizal are significantly greater for island than mainland regions (marginal $R^2 = 0.11$; $p < 0.001$; Native: Mainland N = 1030, Island N= 930; Naturalized: Mainland N = 809; Island N = 361, GLMM). Error bars shown are standard errors; lines above graph show significantly different proportions. (b) Maps of geographic regions with their proportion of mycorrhizal plant species for native and naturalized floras included in this study.

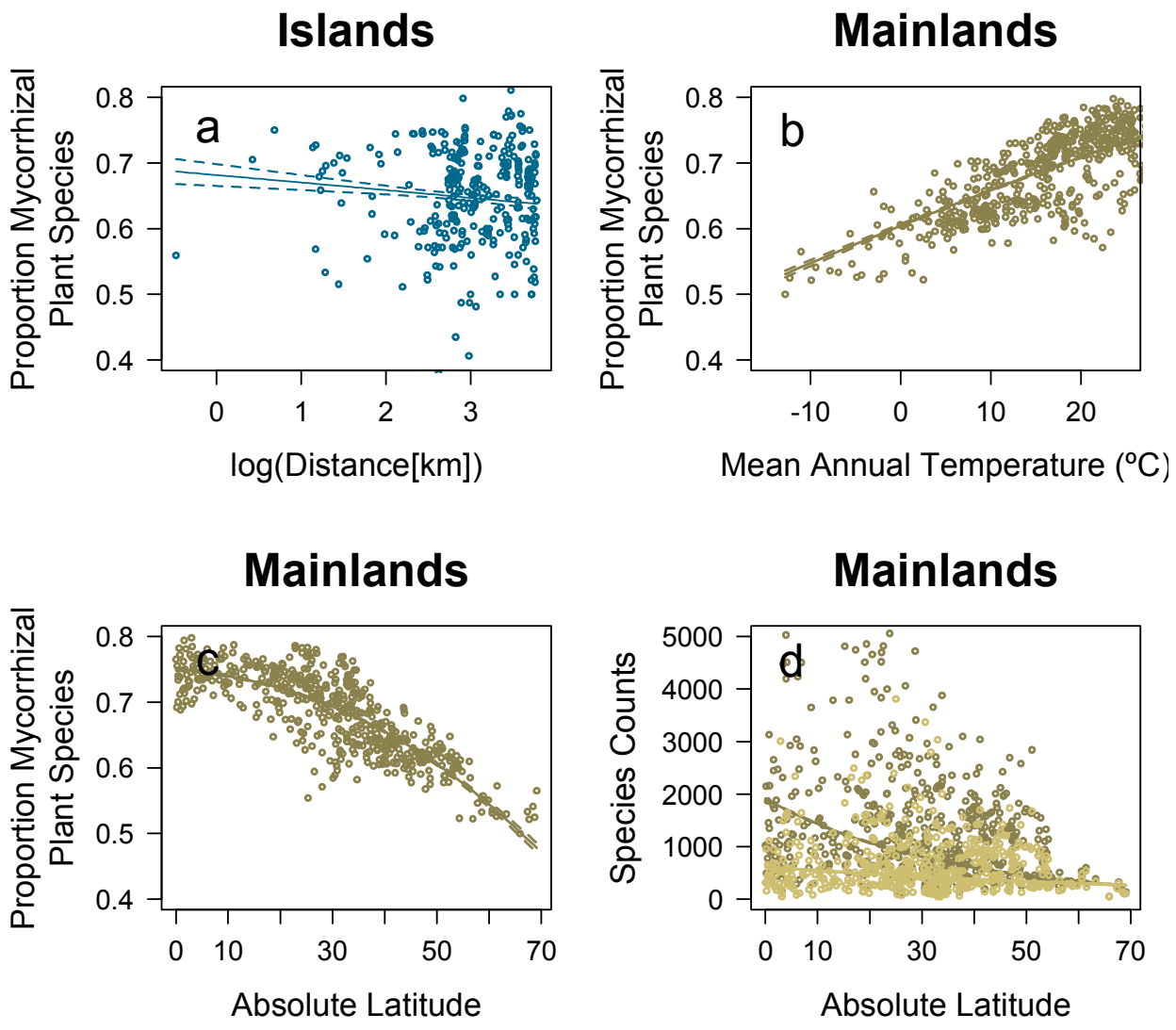


Figure 2. Mycorrhizal fungal associations affect biogeographical patterns of native plants. (a) In island regions, increasing distance to the mainland is associated with a lower proportion of mycorrhizal plant species (N = 422, $p < 0.01$, GLM). (b) In mainland regions, the proportion of mycorrhizal plant species increases with mean annual temperature (N = 515, $p < 0.001$, GLM). In mainland regions, (c) the proportion of mycorrhizal plant species decreases in a non-linear manner with increasing absolute latitude (N = 515, $p < 0.001$, GLM), a relationship primarily driven (d) by mycorrhizal species (dark brown line and points) as compared to non-mycorrhizal

species counts (N = 515, $p < 0.001$, GLM; non-mycorrhizal species: light brown line and points). Plots a and b are based on multi-predictor models including other co-variables; we held other co-variables at their mean to predict the variable plotted here. These model statistics can be found in Supplementary Information Table 1. Plots c and d include only latitude and are simple pairwise models.

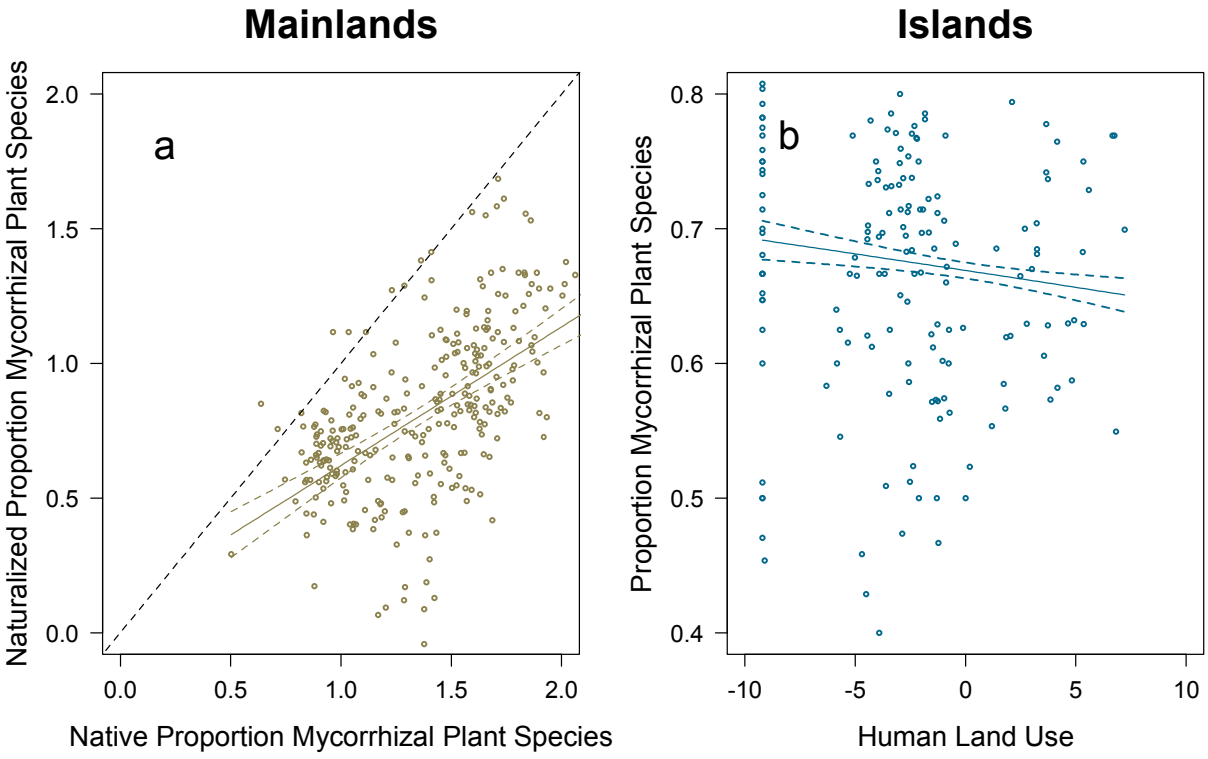


Figure 3. Mycorrhizal fungal associations differentially impact biogeography of naturalized floras. In mainland regions, the proportion of mycorrhizal plant species in the naturalized flora is lower than that in the native flora, particularly in areas with a high proportion of native mycorrhizal plants (a, Native Mainland N = 515, Naturalized Mainland N = 294, $p < 0.001$, GLM). On islands, the proportion of mycorrhizal plant species decreases with human land use

(b), a composite variable resulting from the sum of log transformed urban and managed land area ($N = 177$, $p = 0.001$, GLM).

Chapter 2: Mycorrhizal types influence island biogeography of plants

Abstract

Plant colonization of islands may be limited by the availability of symbionts, particularly arbuscular mycorrhizal (AM) fungi, which have limited dispersal ability compared to ectomycorrhizal (EM) and orchid mycorrhizal (OM) fungi. We tested for such differential island colonization within contemporary floras worldwide. We found evidence that AM plants experience a stronger mycorrhizal filter than other mycorrhizal or non-mycorrhizal (NM) plants, with decreased proportions of native AM plant species on islands relative to mainlands. This effect intensified with island isolation, particularly for non-endemic plant species. The proportion of endemic AM plant species increased with island isolation, consistent with diversification filling niches left open by the mycorrhizal filter. Naturalized floras featured higher proportions of AM plant species than native floras, a pattern that increased with increasing isolation and land-use intensity. This work provides evidence that the biology of fungal symbionts shapes plant colonization of islands, subsequent diversification and anthropogenic impacts.

Introduction

Classical island biogeography recognizes that species richness results from the net effects of immigration, which decreases with isolation (i.e., distance to source pools), extinction, which decreases with island size (MacArthur and Wilson 1967), and speciation, which increases with

island size and isolation (Losos and Schluter 2000, Kisel and Barraclough 2010). While a limited number of case studies show that biotic interactions may also influence colonization, extinction, and speciation probabilities on islands (Bush and Whittaker 1991, Losos and Ricklefs 2009, Onstein et al. 2017), generalizations are difficult. Order of arrival, resulting in priority effects (Fukami 2015), is likely to be particularly important for mutualisms. The mycorrhizal mutualisms formed between soil fungi and most plant species are prime candidates for priority effects because plant species vary in their dependence on mycorrhizal fungi (Hoeksema et al. 2018), and the absence of mycorrhizal fungi may limit the colonization of mycorrhizal plant species. Indeed, a recent global analysis of native floras found both a lower proportion of mycorrhizal plant species on islands than on continents and a decrease in the proportion of mycorrhizal plant species in island floras with increasing geographical isolation (Delavaux et al. 2019), consistent with the presence of a mycorrhizal filter on plant colonization of islands (Duchicela et al. 2020). However, whether different mycorrhizal fungal types differentially impact the composition of island floras is currently unknown. Here, we develop and test the case that the ecological and life-history differences between different types of mycorrhizal fungi influence the establishment probability of the plants with which they associate, leading to island 'disharmony' (Taylor et al. 2019) in plant species associating with different mycorrhizal types.

We can construct two *a priori* sets of expectations for the relative strength of the mycorrhizal filter based on differences in the biology of different groups of mycorrhizal fungi. Arbuscular mycorrhizal (AM) fungi, which form the most common type of mycorrhizae, are likely to be most limited in their ability to colonize islands prior to plants due to two life-history traits. First, AM fungi lack adaptations for aerial dispersal; while spores of small-spored species of AM fungi can disperse with wind erosion of soil (Chaudhary et al. 2020), the viability of these

aerial spores is unknown. Moreover, AM fungi are the only group of mycorrhizal fungi that cannot grow in the absence of an association with their hosts (Smith and Read 2008). In contrast, other types of mycorrhizal fungi, including ectomycorrhizal and ericoid mycorrhizal (EM), as well as orchid mycorrhizal (ORC) fungi, produce spores that are effectively aerially dispersed and can grow independently of their host, i.e., saprophytically (Smith and Read 2008). We therefore expect EM and ORC fungi to be better able to establish on islands prior to their hosts, and EM and ORC plant species to be less impacted by the mycorrhizal filter at early stages of island assembly than AM plant species (Peay et al. 2012).

Plant colonization success could also be impacted by specificity within these associations. Mycorrhizal associations with low fungal-plant specificity are less likely to limit plant establishment because the establishment of a single fungal species could enable the colonization of many plant species (Pither et al. 2018). Alternatively, in associations with high specificity, the establishment of a single fungal species may only enable colonization of a small subset of the plant species of that mycorrhizal type, limiting the potential of their host plant establishment. AM fungi have lower specificity of association than EM and ORC (Smith and Read 2008), thereby reversing expectations for the strength of the mycorrhizal filter from those based on colonization ability. Finally, the extent to which plants are obligately dependent on mycorrhizal fungi could modify the potential for mycorrhizal fungi to limit plant colonization of islands, with facultatively dependent plants colonizing islands more easily. A greater proportion of plants that associate with AM than EM fungi have been identified as facultatively dependent on mycorrhizal fungi (Pyšek et al. 2019). This would again generate patterns counter to dispersal expectations but consistent with specificity expectations, where AM plants experience a weaker filter than EM or ORC plants.

Besides acting as a filter on colonization, the types of mycorrhizal associations may influence the global distribution patterns of plant species through functional differences, providing additional hypotheses relevant to global biogeography. For instance, AM fungi are thought to be most effective at facilitating access to relatively immobile resources such as inorganic phosphorus and nitrogen, and EM fungi are commonly thought to be able to better access organic nitrogen (Phillips et al. 2013), potentially bypassing the decomposition pathway. This function is assumed to be particularly important in colder climates where decomposition is slow. These differences underlie arguments for the dominance of EM plant species in colder climates (Read and Perez-Moreno 2003, Gomes et al. 2019). Recent analyses built on assumptions of the ecological differences in AM and EM symbioses predict extant patterns of mycorrhizal types in forests, with greater dominance of AM plant species near the equator and greater dominance of EM plant species closer to the poles (Lu and Hedin 2019, Steidinger et al. 2019). Predictions based on functional differences of ORC are difficult, as the associated plants can be parasitic rather than mutualistic with their fungi (Cameron et al. 2006, Dearnaley 2007).

Here, we explore biogeographical patterns of angiosperm species that associate with different types of mycorrhizal fungi. We first analyze plant colonization patterns of two oceanic islands that have been denuded or formed within recorded history. To test for generalization across islands, we then use a global database to test for persistent legacies of the differential strength of a mycorrhizal filter in island colonization as predicted by differences in dispersal-dependence and host specificity of these fungal groups. We test for differences in the proportion of plant species that associate with different types of mycorrhizal fungi between mainland and island systems in both native and naturalized floras. We also examine endemism patterns in native island floras to confirm that colonization patterns, independent of diversification, are

consistent with our analyses. *A priori*, we expect that the mycorrhizal type most affected by the mycorrhizal filter will have higher diversification rates to fill niches left open by limited colonization, and therefore higher rates of endemism. Finally, we analyze the potential drivers of these patterns by predicting these proportions based on geographical, environmental and human impact variables.

Results

Mycorrhizal filters on recent island colonization

Analysis of plant colonization data from two islands that have formed or been denuded within the last 140 years, Rakata (Krakatau) and Surtsey (Whittaker et al. 1989, Magnússon et al. 2009), showed that in both cases that the proportion of plants that associate with mycorrhizal fungi was initially low and increased with year since the initiation of colonization, consistent with the operation of a mycorrhizal filter (fig. S1, $p < 0.001$), but not with the hypothesis of AM limitation of island ecosystem assembly. However, both islands are a mere 30 - 40 km from mainland source pools. Moreover, patterns on these two islands provided weak tests of differential limitation of mycorrhizal fungal type because of low replication, lack of information on the potential source colonists and relatively short periods of time over which they have been monitored.

Evidence of differential mycorrhizal filters in native oceanic island floras

Across oceanic islands globally, we found support for dispersal limitation of native plant species that associate with AM fungi (Fig. 1, Fig. 2, tables S1-S6). Specifically, compared to mainland floras, we found that native island floras had a significantly lower proportion of AM than EM

(EM:AM $p < 0.001$; table S2a M1) and NM plants (AM:NM $p < 0.001$; table S4a M1). Moreover, the proportion of plant species on islands that associate with AM relative to NM significantly decreased with increasing distance from the mainland ($p < 0.01$, table S4a M4, Fig. 3, and fig. S2). When examining the proportion of endemic plant species, we found a significant interaction between mycorrhizal type and distance, with the proportion of endemic AM species showing a faster rate of increase with distance compared to the other groups ($p < 0.001$, Fig. 4A and Fig. S4). Specifically, the number of non-endemic AM species decreased strongly compared to other mycorrhizal types or NM plants (Fig. 4C, $p < 0.001$), while the number of endemic species did not change with distance (Fig. 4B, $p = 0.74$). Together, these results are consistent with the hypothesis that plants relying on AM fungi are more limited by the dispersal of their mutualists than plants associated with EM fungi. Finally, the proportion of AM to EM plants varied with island area (EM:AM $p < 0.001$, table S3a M4; and EM:NM $p < 0.001$, table S5a M4) and the proportion of endemism within these groups mycorrhizal types varied with area and elevation (fig. S5 and fig. S6).

Native island floras showed a lower proportion of ORC plant species compared to mainlands (ORC:M $p = 0.06$, table S3a M1; and ORC:NM $p < 0.001$, table S6a M1), consistent with establishment limitation for orchids on islands. However, the proportion of ORC plant species increased with greater distance from the mainland as compared to both other mycorrhizal ($p < 0.001$, table S3a M4) as well as NM plant species ($p < 0.01$, table S6a M4), suggesting superior dispersal ability.

Environmental drivers of mycorrhizal species distributions in native floras

For native mainland floras, variation in the proportion of mycorrhizal plant species was primarily explained by latitude and environmental variables. The proportion of EM plant species increased non-linearly from the equator towards the poles (absolute latitude: EM:AM $p < 0.001$, EM:NM $p = 0.002$; absolute latitude squared: EM:AM $p < 0.001$, EM:NM $p < 0.001$, table S7). This may be indicative of the functional advantage of EM symbioses in colder climates. AM and ORC plant species counts showed the strongest saturating declines with latitude compared to EM and NM plant species (Fig. 5). This indicates that plants associating with AM or ORC fungi contribute more to the classical latitudinal plant species diversity gradient than EM and NM plants. We note that the latitudinal gradient was present but diminished on islands (fig. S3).

Environmental and anthropogenic drivers of mycorrhizal species distributions in naturalized floras

Human-mediated plant naturalizations affected global plant biogeographical patterns influenced by mycorrhizal fungi (Fig. 2). In the naturalized flora, we found evidence of an increase of the representation of EM relative to AM ($p < 0.001$, table S2a M2) and AM relative to NM plant species on islands ($p < 0.001$, table S4a M2). On oceanic islands, increasing urban land use was correlated with an increase in the proportion of AM plant species compared to NM plant species ($p = 0.02$), possibly due to horticultural introduction and early successional advantage of AM plant species. Further evidence of human-mediated impacts on these biogeographical patterns was evident from the shift in drivers predicting the proportion of EM plant species (EM:AM, table S2a M5, M6) in naturalized floras. In naturalized island floras, we found evidence of humans overriding initial biogeographical patterns stemming from the mycorrhizal filter. Specifically, the effect of distance was reversed, with the proportion of AM plant species

increasing with distance (AM:NM $p = 0.25$, table S4a M6). In mainland floras, increasing human land use was correlated with an increase in the proportion of EM plant species (EM:AM $p < 0.001$, EM:NM $p < 0.001$), while population density was correlated with a decrease in the proportion of EM plant species (EM:AM $p = 0.03$, table S2a M5; and EM:NM $p < 0.001$, table S5a M5). These results show that anthropogenic impacts are altering the original mycorrhizal biogeographical patterns seen in native floras.

Discussion

We found evidence that oceanic islands have different proportions of mycorrhizal plant species relative to mainlands and this disharmony is consistent with the biology of mycorrhizal symbionts influencing the strength of a mycorrhizal filter during plant colonization of oceanic islands (Delavaux et al. 2019, Duchicela et al. 2020). While patterns of colonization of two recently denuded islands near mainlands were ambiguous, we found that native floras of oceanic islands worldwide had a lower proportion of plants that associate with AM fungi compared to mainland floras. Moreover, the proportion of AM species decreased with island isolation and this effect was particularly strong for non-endemic species. Together, these results are consistent with access to AM fungi limiting plant establishment on oceanic islands, as expected from their lower dispersal ability and inability to grow independently of their host. Limited AM plant colonization led to consistent island disharmony in mycorrhizal species' types relative to mainlands. We found that the proportion of endemism of AM plants increases with island isolation, consistent with an AM plant evolutionary response through increased diversification filling niche space left by initial island disharmony and AM plant filtering.

We found consistent evidence of the legacy of dispersal limitation of plant species associating with AM fungi in contemporary native plant floras. These effects were evident in oceanic island floras, but not in floras of non-oceanic islands that were once connected to mainlands (Supplementary table S12 M1a), supporting our inference that the difference is in colonization rather than in rates of extinction or speciation. The persistence of these differences in contemporary native floras is remarkable, given the clear evidence that AM fungi do eventually colonize islands (e.g. (Davison et al. 2018)) and the thousands to millions of years for secondary colonization and/or diversification to reverse initial differences. In fact, our results suggest that plants that associate with AM fungi having disproportionately high diversification rates, as they display high endemism on distant islands, consistent with expanded opportunity due to the disharmony generated by limited colonization. Our analyses support the hypothesis that dispersal limitation of AM fungi on distant islands is a stronger limiting factor in plant colonization of islands than the higher specificity of EM associations. While there is empirical evidence of dispersal limitation of symbionts being important to both EM and AM plant species (Peay et al. 2012, Tedersoo et al. 2014, Koziol et al. 2018a, Koziol and Bever 2019), our work suggests that AM plants are more susceptible to symbiont dispersal limitation. Limited AM fungal dispersal to islands is supported by analyses of AM fungal composition showing differential AM fungal species abundances on islands compared to mainland regions (Davison et al. 2018).

We found that plants associating with AM fungi and ORC fungi contributed more to the latitudinal plant species diversity gradient than EM and NM plants. This result mirrors the well-established pattern of EM trees being relatively more abundant in boreal forests, which has been associated with functional advantages such as short-circuiting the decomposition pathway

through organic N uptake of EM symbiosis in colder climates (Lu and Hedin 2019, Steidinger et al. 2019). We suggest that this same functional difference may have contributed to the differential pattern in plant species richness of EM versus AM plants across latitude. The patterns seen in ORC plant species mirror the close association orchids have with the tropics, given that 69% of orchids are epiphytic, which highly limits their distribution outside of the tropics (Zotz 2005, 2013) .

ORC plant species are generally under-represented on islands as compared to mainland regions, consistent with a previous study on global patterns in orchid richness (Taylor et al. 2019) and consistent with limitation through high specificity of this symbiosis. However, we found evidence for higher proportions of ORC plants on distant islands, consistent with high dispersal ability of ORC plant species. These contrasting results may reflect the high ORC fungal specificity limiting island colonization overall, while high dispersal ability of ORC fungi contributes to ORC plant establishment on distant islands. Alternatively, these patterns may be influenced by other aspects of the biology of the Orchidaceae. For example, ORC plants produce abundant, but very small dust-like seeds, and feature a high dependency on and specialization of pollinators, which could influence the colonization of islands (Razanajatovo et al. 2019). In contrast to ORC plants, AM and EM plant species occur across the plant phylogeny, increasing confidence that biogeographic patterns can be attributed to mycorrhizal fungal traits.

Naturalized and native floras showed distinct patterns, as the proportion of AM plant species in naturalized floras of islands was higher than in their native floras and increased with island distance from mainlands. This may result from the co-introduction of AM plants and their symbionts through the movement of agricultural and horticultural plants with soil (Davison et al. 2018). This co-introduction may overcome the barriers to the establishment of AM plants on

islands and allow them to fill in niche space left unfilled by the mycorrhizal filter (Delavaux et al. 2019). Consistent with this hypothesis, the proportion of AM plants in naturalized island floras increased with land-use intensity. On mainlands, however, higher land-use intensity led to a greater proportion of EM plant species, possibly due to ornamental street planting and plantation use of EM trees, which subsequently naturalized.

Our data are consistent with a legacy of arbuscular mycorrhizal (AM) fungi acting as a stronger filter on the initial colonization of islands compared to ectomycorrhizal (EM) or orchid (ORC) fungi, as AM plants are underrepresented in native island floras, and this effect increases with distance from the mainland, particularly for non-endemic plant species. These patterns are consistent with expectations of limited potential for colonization of new islands by AM fungi because of limited dispersal ability and obligate host-dependence. We also find evidence of higher diversification rates of AM plants in response to the disharmony generated by the AM colonization filter. In native mainland floras, AM and ORC plant species contribute more strongly to the latitudinal plant species diversity gradient than EM and NM plant species. This work provides strong evidence that the different types of mycorrhizal mutualisms differentially influence colonization of islands, diversification, plant invasion risks and global plant biogeography.

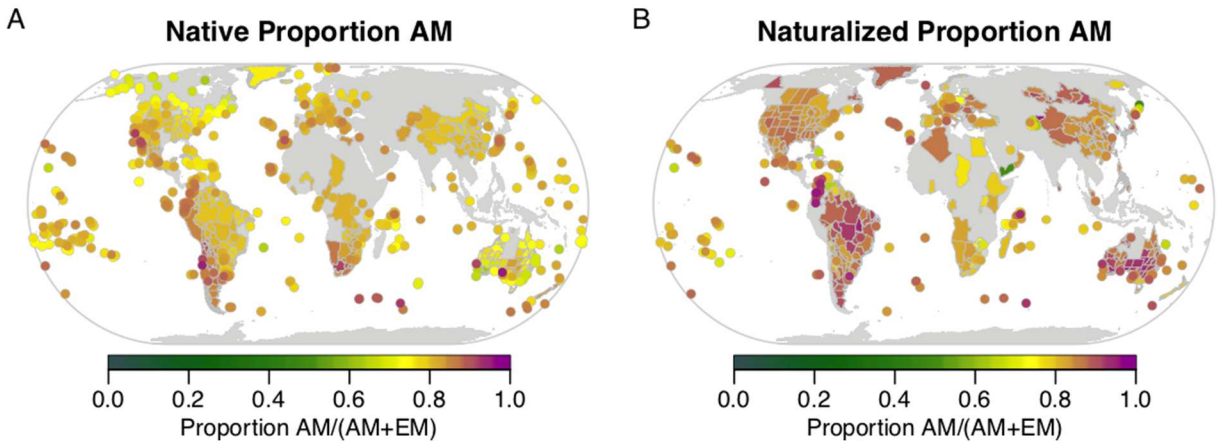


Fig. 1. Locations of regions used in study with associated proportion AM:EM.

Maps of geographical regions showing the proportion of arbuscular mycorrhizal relative to ectomycorrhizal (AM/AM+EM) plant species for native and naturalized floras included in this study (A, mainland n = 515, island n = 313; B, mainland n = 287, island n = 100).

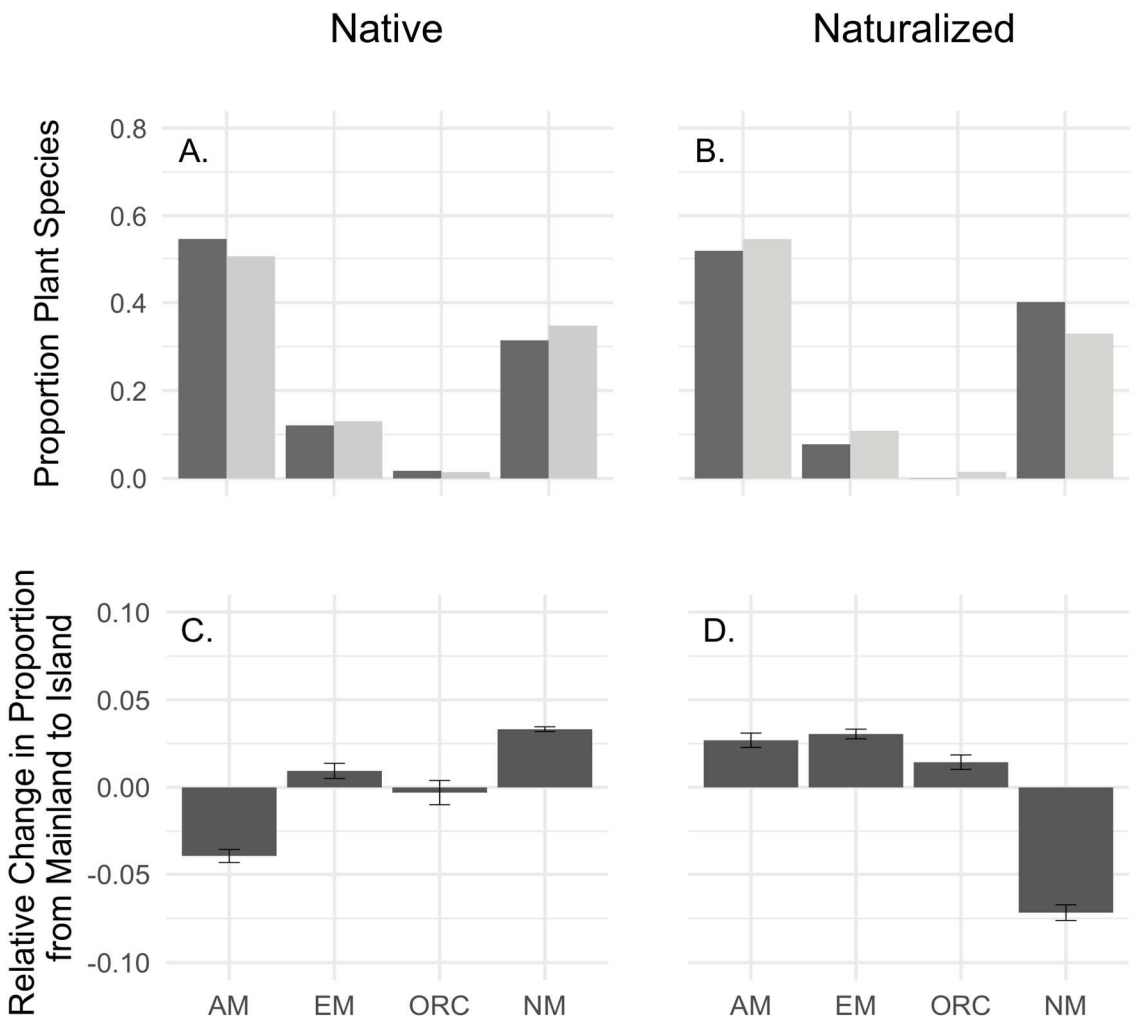


Fig. 2. Proportion of plant species of each mycorrhizal type in native and naturalized flora. Arbuscular mycorrhizal (AM) plant species represent a lower proportion of species in the native floras on oceanic islands (light grey) than on mainlands (dark grey), while all mycorrhizal types represent a higher proportion of species on islands than mainlands in the naturalized flora. Proportion of plant species within each mycorrhizal type: arbuscular mycorrhizal (AM), ectomycorrhizal (EM; includes ecto- and ericoid mycorrhizal plants), orchid mycorrhizal (ORC) and non-mycorrhizal (NM). (A) and (B) show these proportions for native (A) and naturalized

(B) plant species; (C) and (D) show the difference between oceanic island and mainland in each type for native (C) and naturalized (D) species. Error bars represent standard errors of the means. All relevant statistics and sample sizes can be found in tables S2-S6.

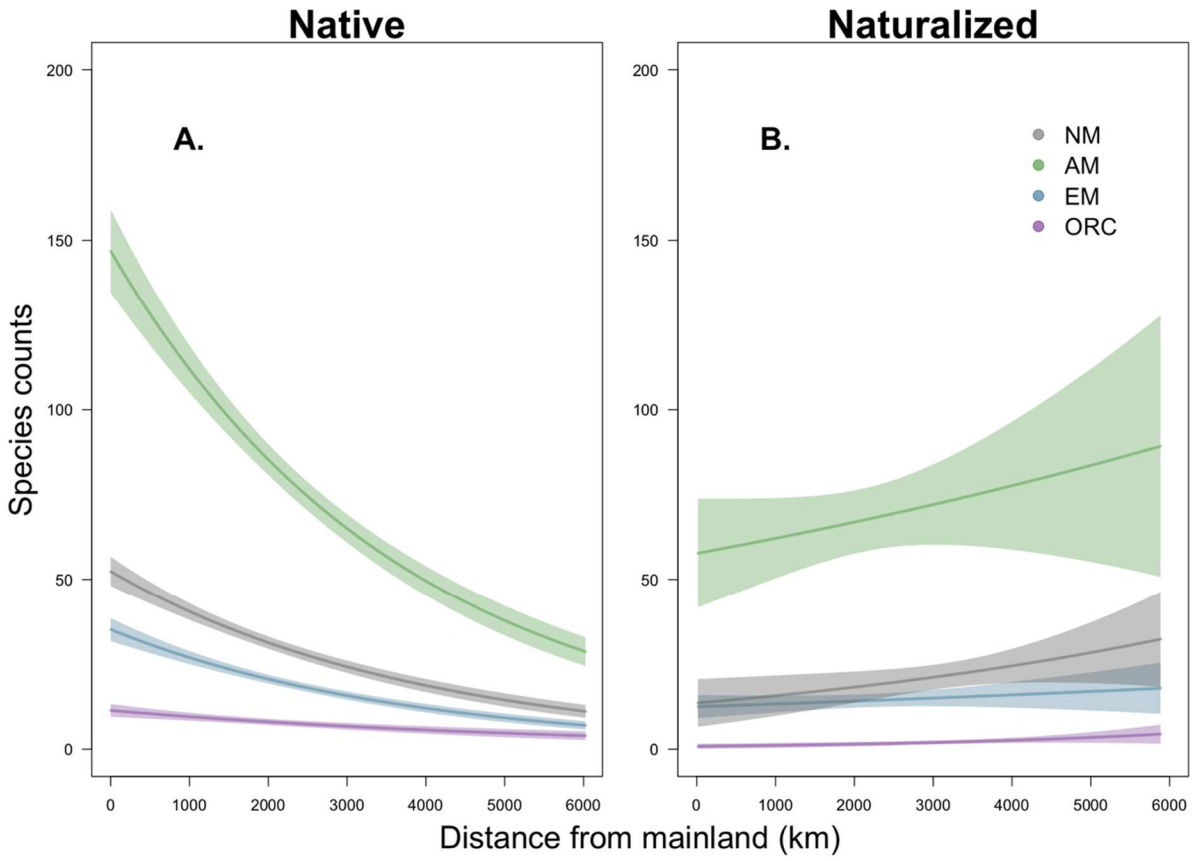


Fig. 3. Distance patterns across mycorrhizal types in native and naturalized oceanic island floras.

The proportion of AM:NM plants in the native island flora decreases with oceanic island distance from the mainland (A, distance estimate = -0.034 ± -0.006, $p < 0.01$, n = 325; GLM), consistent with AM plants being differentially limited in colonization of far islands. In contrast, no patterns with distance are detectable in naturalized oceanic island floras (B, distance estimate = 0.034 ± 0.005, $p = 0.25$, n = 105; GLM).

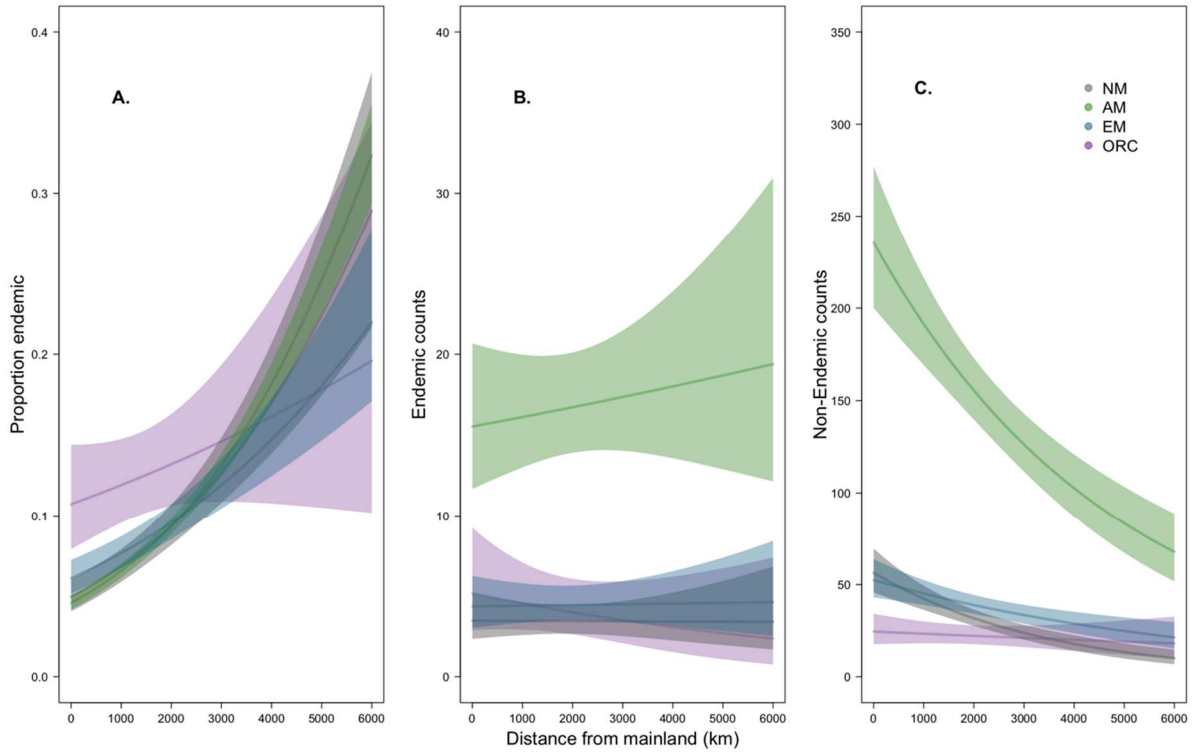


Fig 4. Variation in mycorrhizal types in oceanic island floras with distance from mainland source regions.

The proportion of plant species endemic to non-endemic increases most rapidly with distance for AM plant species (A, estimate = 0.432 ± 0.063, $p < 0.001$, n = 254; GLM). The number of endemic AM species does not change with distance (B, estimate 0.048 ± 0.147, $p = 0.74$, n = 254; GLM). The non-endemic species for AM decreases most strongly compared to other mycorrhizal types and to NM plants (C, estimate = -0.265 ± 0.067, $p < 0.001$, n = 254; GLM).

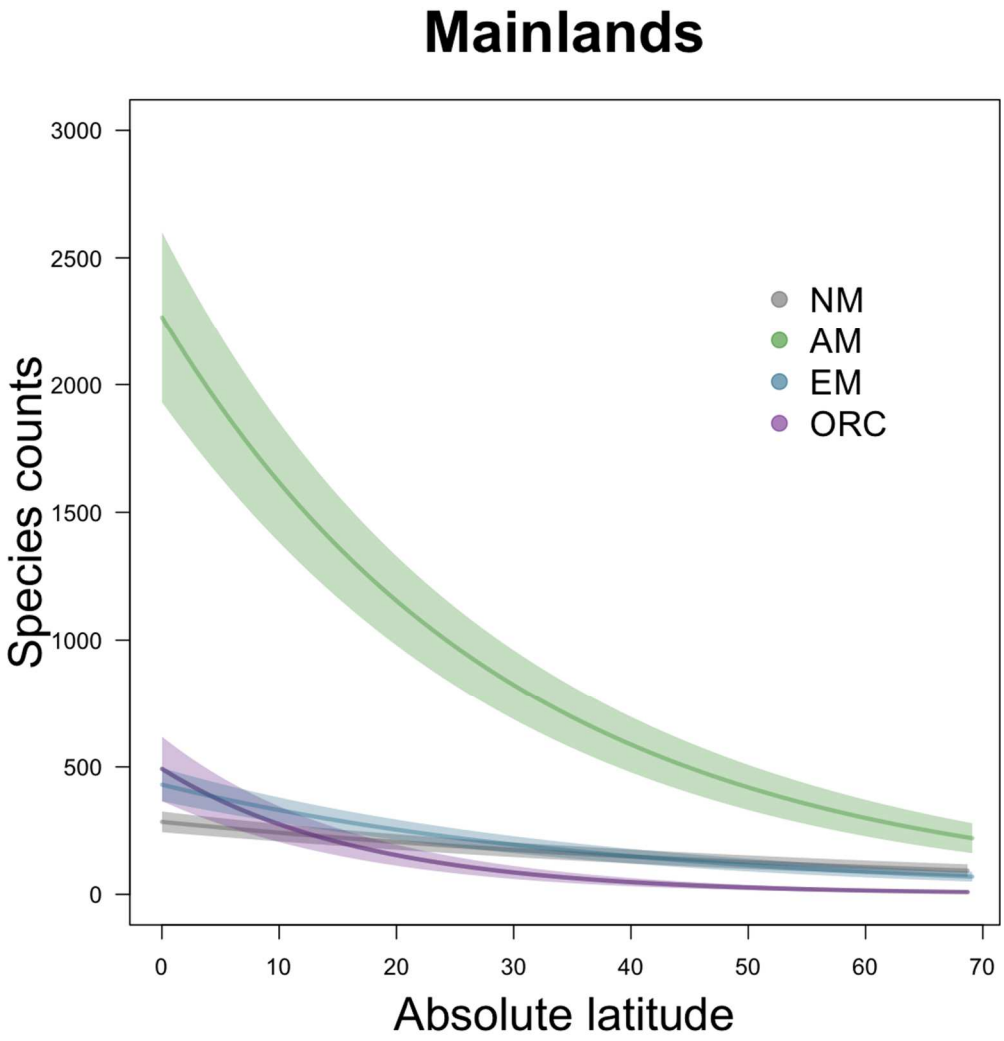


Fig. 5. Latitudinal patterns across mycorrhizal types in native mainland plant species.

The latitudinal plant species gradient is strongly influenced by arbuscular and orchid mycorrhizal plant species. In mainland regions, the proportion of mycorrhizal plant species decreases with absolute latitude (º from equator) more strongly for arbuscular mycorrhizal (green line: absolute latitude estimate = -0.042 ± 0.080, $p = 0.60$, n = 515; squared latitude estimate -0.414 ± 0.080, $p < 0.001$, n = 515; GLM), than for ectomycorrhizal plant species (blue line: absolute latitude estimate = 0.051 ± 0.082, $p = 0.54$, n = 515; squared latitude estimate = -0.400 ± 0.082, $p < 0.001$, n = 515 ; GLM) and orchid mycorrhizal plant species (purple line: absolute latitude

estimate = -1.216 ± 0.144, $p < 0.001$, n = 486; squared latitude estimate = 0.283 ± 0.144, $p = 0.05$, n = 486). Non-mycorrhizal species counts plotted for reference (grey line: absolute latitude estimate = 0.132 ± 0.132, $p = 0.1$, n = 486; squared latitude estimate = -0.330 ± 0.080, $p < 0.001$, n = 486; GLM).

Chapter 3: Root pathogen diversity and composition varies with climate in undisturbed grasslands, but less so in anthropogenically-disturbed grasslands

Material from: Delavaux, C.S., Schemanski, J.L., House, G.L. *et al.* (2021). Root pathogen diversity and composition varies with climate in undisturbed grasslands, but less so in anthropogenically disturbed grasslands. *ISME J*ournal. DOI: https://doi.org/10.1038/s41396-020-00783-z

Abstract

Soil-borne pathogens structure plant communities, shaping their diversity, and through these effects may mediate plant responses to climate change and disturbance. Little is known, however, about the environmental determinants of plant pathogen communities. Therefore, we explored the impact of climate gradients and anthropogenic disturbance on root-associated pathogens in grasslands. We examined the community structure of two pathogenic groups – fungal pathogens and oomycetes – in undisturbed and anthropogenically-disturbed grasslands across a natural precipitation and temperature gradient in the Midwestern USA. In undisturbed grasslands, precipitation and temperature gradients were important predictors of pathogen community richness and composition. Oomycete richness increased with precipitation, while fungal pathogen richness depended on an interaction of precipitation and temperature, with precipitation increasing richness most with higher temperatures. Disturbance altered plant pathogen composition and precipitation and temperature had a reduced effect on pathogen richness and composition in disturbed grasslands. Because pathogens can mediate plant

community diversity and structure, the sensitivity of pathogens to disturbance and climate suggests that degradation of the pathogen community may mediate loss, or limit restoration of, native plant diversity in disturbed grasslands, and may modify plant community response to climate change.

Introduction

Experimental and theoretical evidence show that plant pathogens play an important role in structuring plant communities, especially in maintaining plant community diversity (van der Heijden et al. 2008, Mordecai 2011, Bever et al. 2015). For example, soil pathogen accumulation near mature trees is a likely driver of poor performance by seedlings of the same species (Janzen 1970, Connell 1971, Augspurger 1984, Comita et al. 2010, Mangan et al. 2010). This pathogen suppression of conspecific seedlings can give heterospecific species the opportunity to succeed in these patches, resulting in a more diverse plant community. Soil pathogens are a major cause for the negative feedback commonly observed between plants and their soil communities, a mechanism which maintains large-scale patterns of plant diversity (Van der Putten et al. 2013, Eppinga et al. 2018, Crawford et al. 2019). Similarly, when plants move out of their native range, release from pathogens may help drive their successful invasion of new regions (Callaway et al. 2004, Mitchell et al. 2006), further evidence for the critical role pathogens play in structuring plant communities. Given the important role of pathogens in plant community structure and diversity, responses of plant communities to perturbations may be mediated by the sensitivities of their pathogen communities.

Fungi and fungus-like organisms are major soil-borne plant pathogens. While we know how fungi generally respond to both edaphic properties (Fierer et al. 2009, Rousk et al. 2010, Thomson et al. 2015, Van Agtmaal et al. 2017) and climate (Talley et al. 2002, McGuire et al. 2012, Tedersoo et al. 2014, Rincón et al. 2015, Newsham et al. 2016, Zhou et al. 2016, Oliverio et al. 2020), it is unclear if plant pathogens mirror these broader responses to environmental factors. Individual studies have shown that pathogen composition in root-infecting fungi is driven primarily by soil pH (Van Agtmaal et al. 2017), while diversity has been shown to respond to precipitation (Spear 2017) and richness to respond to vapor pressure deficit (Talley et al. 2002). The composition of oomycetes, common, fungus-like pathogens, depends on a combination of edaphic traits and environmental conditions, including soil pH, nitrogen, phosphorus, latitude, air temperature, and precipitation (Geml et al. 2014, Rojas et al. 2017, Oliverio et al. 2020). Water availability is likely to be particularly important for oomycete distribution, as wet conditions are required for most oomycete zoospore release and flagellar movement (van West et al. 2003). Plant pathogens are also likely to be affected by anthropogenic disturbance, given the large effect of disturbance on plant communities. Land use disturbances such as tillage, fertilizer additions, heavy grazing, and row crop monocultures have been shown to alter mycorrhizal fungal communities (Oehl et al. 2003, House and Bever 2018b), but the sensitivity of plant pathogens to disturbance are not well understood, particularly if their responses interact with climate. Understanding plant pathogen responses to environmental drivers is particular important given contemporary and future pressure from anthropogenic change, including changes in land use, temperature, and the intensity and frequency of precipitation events (IPCC 2014, Trenberth et al. 2014). Because root-associated plant pathogens

have large effects on their plant hosts, their responses to climate and land-use may mediate plant responses to these anthropogenic impacts.

Here, we use a naturally occurring climate gradient across United States grasslands to investigate root-associated plant pathogen response to climate gradients and anthropogenic disturbance. Specifically, we compare root-associated plant pathogen community diversity and composition across remnant, native grasslands (those without anthropogenic disturbances), and disturbed grasslands (those with a history of anthropogenic disturbances) across a Midwestern US precipitation and temperature gradient from Illinois to Oklahoma. We focus on two groups of root-associated pathogens: fungal pathogens and oomycetes. We hypothesize that root-associated pathogen community structure will be strongly impacted by anthropogenic disturbance, and drive differences in community responses to climate. Undisturbed native grasslands should show the greatest sensitivities to climate variables, such as precipitation and temperature, because the long co-evolutionary history of plants and pathogens there should allow differentiation with respect to climate. Disturbed grasslands are likely dominated by fewer pathogen species, many of which are disturbance-adapted and therefore less sensitive to climate variables. In addition, these disturbed sites likely harbor more homogenous plant communities to serve as pathogen hosts, possibly acting as a filter for establishment of plant pathogens and reducing the range of pathogen response to climate across these sites. Both temperature and precipitation should limit pathogen diversity in undisturbed, native grasslands (Zhang et al. 2015), such that increasing precipitation or temperature will increase pathogen diversity and shift community composition. Precipitation and temperature effects may interact, as has been shown for bacteria (Sheik et al. 2011), overall microbial communities (Zhang et al. 2015), and soil respiration (Wu et al. 2011). Finally, because fungal pathogens are phylogenetically distributed throughout the fungal

kingdom, we compare fungal pathogen results to those of fungal saprotrophs (decomposers) to determine if responses are pathogen-specific or in line with broader variation in the fungal community.

Materials and Methods

Field Sampling

Samples were collected from paired remnant and disturbed grassland sites across the Midwestern United States (Fig. 1), from Illinois to Oklahoma. We paired remnants or clusters of remnants with nearby disturbed grasslands, totaling 19 remnant and 16 disturbed sites. Remnant grassland sites were defined by the absence of tilling or intensive grazing and were dominated by late successional native tallgrass prairie plant species, including *Andropogon gerardii, Schizachyrium scoparium, Sorghastrum nutans, Amorpha canescens, Echinachea pallida, and Silphium lacinatum*. Disturbed grassland sites had known histories of soil disturbance such as tillage (sites ranged from ~20 to 50 years since disturbance), and clear signs of anthropogenic disturbance, including overgrazing and dominance of non-native plant species, including *Festuca arundinaceae, Bromus inermis, Bromus tectorum, Poa pratensis,* and *Bothriochloa ischaemum*. Remnant grasslands were generally more diverse than disturbed grasslands ($\bar{x}$ = 18.8 versus 7.5 plant species per plot, respectively). We sampled four plots arbitrarily located within each site. Four soil cores (width 2 cm, depth 15 cm) were collected arbitrarily within each of the four quadrants of each 1 m^2 plot and composited into one sample for sequence analysis. Fine roots were collected from each sample, soil removed by hand, and frozen until DNA extraction. Soil

chemical analyses including pH, Bray 1 phosphorus, and micronutrients (Melich 3) as well as Bray 2 phosphorus and C/N (Dumas method), were also conducted for most soil samples (A & L Great Lakes Labs, Fort Wayne, Indiana). Climate variables including mean annual temperature and mean annual precipitation were extracted from National Weather locations closest to each site (House and Bever 2018b). Soil chemical analyses results and climate variables can be found in Table S1.

Library Preparation and Sequencing

DNA was extracted from 35 mg of each root sample using the PowerSoil Kit (Qiagen, Hilden Germany). PCR amplification targeted the internal transcribed spacer section (ITS) of ribosome encoding genes for both fungi and oomycetes. Forward primer fITS7 (Ihrmark et al. 2012) and reverse primer ITS4 (White et al. 1990) were used to amplify the ITS2 region. This region is a universal barcode for fungi (Schoch et al. 2012) and is particularly suited for short Illumina MiSeq sequencing (Oliver et al. 2015). PCR amplification for oomycetes was done using recently developed oomycete-specific primers in the ITS2 region (Riit et al. 2016): ITS3oo (Forward, AGTATGYYTGTATCAGTG) and ITS4 (Reverse, TCCTCCGCTTATTGATATGC). PCR products were visually checked on agarose gels to ensure successful amplification and cleaned using Agencourt AMPure XP magnetic beads (Beckman Coulter, Indianapolis, USA).

The fungal PCR reaction was performed using the following reactants per sample: 0.5 µl of each primer, 1 µl of extracted DNA template, 12.5 µl Phusion mastermix with HF buffer (New England Biolabs, Ipswich, MA) and 10.5 µl ddH_2O. We used the following thermocycler program for fungal PCR: 5 min at 94°C, 35 cycles of (30s at 94°C, 30s at 57c, 30s at 72°C), and

a final 7 min extension step at 72°C. Fungal PCR resulted in amplicons of ~700 bases including primers and Illumina adapters. Oomycete PCR was performed using the following reactants in each sample: 0.5 µl of both primers, 1.0 µl of DNA template, 5.0 µl HOT FIREpol (Solis Biodyne, Tartu, Estonia) and 18 µl of ddH_2O. For oomycete PCR, we used the following thermocycler program: 5 min at 95°C, 35x (30s at 95°C, 30s at 55C°, 60s at 72°C), and 10 min at 72°C. The oomycete reaction created amplicons of variable length between 400 - 700 bases.

DNA libraries for each sample and target group were created using a Nextera protocol, pooled, then sequenced using Illumina Mi-Seq (Illumina, San Diego, USA). Following the first cleanup, an indexing PCR was carried out to ligate unique 8 base-pair long sequences (molecular barcodes; Illumina, San Diego, CA, USA) to each sample. The PCR was run under similar conditions as initial PCR, except 5 µl of the primary PCR amplicon was used instead of the original DNA template, and the number of cycles was reduced to 8. Secondary PCR amplicons were purified with Agencourt AMPure XP magnetic beads and DNA concentrations were assessed by Qubit 2.0 (LifeTechnologies, Carlsbad, USA). Samples were pooled in equimolar concentration to a single library for each target group (fungi and oomycetes). Fungal and oomycete sequences were generated using an Illumina Mi-Seq (Illumina, San Diego, USA) at the KU Sequencing Core (Lawrence, KS). Raw sequencing data (fastq files) are available at Sequence Read Archive, BIOPROJECT #PRJNA532765.

Bioinformatics

Bioinformatic analysis of sequencing data used an operational taxonomic unit (OTU) approach through the Qiime pipeline, followed by taxonomic, ecological group and phylogenetic

assignment. Sequencing data were analyzed following Caporaso *et al.* (Caporaso et al. 2010) using Qiime v.1.9.1. Quality and barcode filtering resulted in 11 951 250 reads with an average phred score ≥ 20 and median length of 278.69 bases for fungal sequencing and 20 752 280 reads with an average phred score ≥ 20 and median length of 287.24 bases for oomycete sequencing. Open-reference OTU picking using sortmerna_sumaclust (pick_open_reference_otus.py) and the UNITE fungal ITS reference database v7 (Kõljalg et al. 2013) or a custom curated oomycete reference database (available upon request) were used to cluster OTUs at 97% similarity. All OTUs with < 5 reads overall were removed to eliminate potential PCR/sequencing artefacts, as recommended by Lindahl *et al*. (Lindahl et al. 2013). All data were normalized using DESeq2 implemented in Qiime (Love et al. 2014), using the normalize_table.py script before analysis. In total, there were 866 fungal pathogen, 3595 oomycete and 3414 fungal saprotroph OTUs we could identify in this study. Saturation curves for each analyzed group show that more diversity is present in our system than identified here (Fig. S2). The entire bioinformatics pipeline and OTU tables are available upon request.

To identify putative fungal plant root-associated pathogens from the broader fungal OTUs, we assigned taxonomy from UNITE using RDP (Wang et al. 2007). Then, because pathogenicity arose independently in multiple fungal lineages (James et al. 2006) and therefore pathogens are often closely related to non-pathogenic species, we contrasted the resulting taxonomic identities against the FUNGuild database (Nguyen et al. 2016). Overall, 15.4% of fungal taxa were assignable to functional guild using FUNGuild (Table S4). We identified putative fungal pathogens within this group based on a FUNGuild assignment that contained "pathotroph" and were categorized with confidence of either highly probable or probable (17.8 %, Table S4b). In this way, the fungal pathogen assignment was liberal to ensure that fungi

which can be pathogens in certain environments were not excluded. Although FUNGuild and other existing databases are incomplete, our analyses that use these databases to identify taxa and putative fungal pathogens are robust to assess our hypotheses on climate and land use. One might expect pathogens from disturbed sites to be overrepresented in these databases, as the majority of plant-pathogen work has historically been agricultural, but we find little evidence for this bias in identification between remnant (11.4 %) and disturbed (13.6 %) sites. In addition, fungal saprotrophs were identified using FUNGuild as described above for fungal pathogens to assess whether fungal pathogen responses match those of other fungi identified through this process (Table S4). For oomycetes, we checked the identity of resulting OTUs either against a database containing all NCBI oomycote ITS2 sequence results using the Basic Local Alignment Search Tool, BLAST v. 2.6.0 (Altschul et al. 1997), using default parameters, or through placing OTUs in the oomycete clade, as the Oomycota are thought to have arisen from a common ancestor forming a conserved clade (Rujirawat et al. 2018) and generally function as pathogens (van West et al. 2003, Rojas et al. 2017).

Statistical Analysis

All statistical analyses were carried out on two plant pathogen groups: fungal pathogens and oomycetes. In addition, we analyzed fungal OTUs identified as saprotrophs (decomposers) to compare with fungal pathogen results. We ran all analyses for phylogenetically and BLAST determined oomycete OTUs, but because oomycete OTUs were not as effectively identified by BLAST, we report phylogenetic oomycete results here (BLAST results can be found in Supplementary Information for both GLM (Table S2) and PERMANOVA analyses (Table S3)).

We tested the impact of disturbance, temperature and precipitation (alongside other edaphic variables) on phylogenetic species richness (PSR-see below; GLM), and community composition (PERMANOVA). We then assessed differential presence (Venn diagrams) and abundance (DESeq2) of each OTU between undisturbed and disturbed grasslands. All statistical analyses were carried out in R version 3.4.1 (Team 2017).

Estimating Phylogenetic Richness

Phylogenetic species richness (PSR, (Helmus et al. 2007)) accounts for phylogenetic distance among taxa by using branch lengths extracted from a phylogenetic tree. We used RAxML to create our phylogenetic trees (Stamatakis 2006). However, the evolution rate of the ITS region is relatively fast (Nilsson et al. 2008) and thus is not suitable to build a global tree to assess the PSR of fungal pathogens or saprotrophs. Instead, we built a family-level tree from the small ribosomal subunit (SSU) using the kingdom-level fungal tree based on six genes as a backbone constraint (James et al. 2006). We then manually edited the phylogenetic matrix to include the number of ITS2 identified OTUs per family, setting the distance between OTUs in the same family at 0.05, a small number relative to the distance between neighboring families. While this assumption limits the information on relationships within family, this approach represents the major advantage of PSR, which is sensitive to the distribution of OTUs across the deeper nodes of the tree. With both trees constructed, we used the pez package (Pearse et al. 2015) in R to extract PSR values. The fungal outgroup used to root our phylogenetic tree was *Rhizopus oryzae* (Fitzpatrick et al. 2006). For the oomycetes, no reference tree is available, so we constructed a tree from the ITS sequences using two outgroups: *Phaeodactylum tricornutum* and *Thalassiosira*

pseudonana (Rujirawat et al. 2018).

Analysis of Phylogenetic Species Richness Differences

We used generalized linear mixed effect models (GLMs) to test whether disturbance (remnant or disturbed) and environmental variables explained differences in fungal pathogen and oomycete phylogenetic species richness across (1) all sites, then separately across (2) remnant sites and (3) disturbed sites. We ran these separate analyses for remnant and disturbed sites to further explore significant disturbance by environmental variable interactions present in the all sites model. For the "all-sites" models, we ran linear models testing mean annual precipitation, mean annual temperature and their individual interactions with land use. Within the separate remnant and disturbed sites only data, we ran separate linear regressions testing mean annual precipitation, mean annual temperature and their individual interactions. For the "all-sites" models, we nested disturbance within site (random effect, intercept) and for models for remnant or disturbed sites included site as a random effect to properly account for non-independence of replicate samples within site and allow generalization across the sampled area. Mean annual precipitation and temperature were mean-centered and scaled prior to analysis. Our variable selection was informed by literature investigating environmental predictors of soil microbial diversity (Lauber et al. 2008, Geml et al. 2014, Zhou et al. 2016) and function (Chaudhary et al. 2014, Newsham et al. 2016).

Analysis of Differences in Community Composition

We used a permutational multivariate analysis of variance (PERMANOVA) to test whether disturbance (remnant or disturbed) and environmental variables explained differences in fungal pathogen and oomycete community composition, respectively, across all sites. Our environmental predictor variables included mean annual precipitation, mean annual temperature, phosphorus, calcium, potassium and soil pH (as well as each in an interaction with land use for analysis across all sites). Because some disturbance by environmental variable interactions were significant in our model for all sites (see results), we also used a PERMANOVA to assess how environmental variables impacted pathogen community composition in remnant and disturbed sites separately. Finally, we reran these PERMANOVAs to test for an interaction between temperature and precipitation as these were our two major climate change gradients and did not covary (see Fig. 1). We stratified the PERMANOVA by each combination of disturbance and site to account for random effects due to spatial proximity of paired disturbed and remnant plots within any one site. These PERMANOVA tests were performed using Morisita's dissimilarity index, which is robust to unequal sampling (Morisita 1959), and the *adonis2* function in vegan Version 2.4-6 (Oksanen et al. 2013).

Analysis of Differential Abundance and Occurrence

Finally, we analyzed the data to understand differential presence (Venn diagrams) and abundance (DESeq2; (Love et al. 2014)) of OTUs between remnant and disturbed grasslands. We constructed Venn diagrams using VennDiagram (version 1.6.19) to determine shared and unique OTUs between disturbed and remnant grasslands. We then analyzed the data using DESeq2, which allows comparison of individual OTU's differential abundance between two

groups of sites while correcting for both variation in sequence number across samples and variance in sequence number for each OTU (McMurdie and Holmes 2014). We binned sites into low (< 800 mm annual precipitation) and high (< 800 mm) levels of precipitation, with Western sites representing low precipitation, and Eastern sites representing high precipitation. We then used DESeq2 to examine turnover between these Western and Eastern sites within remnant and disturbed sites separately. Because remnant grasslands had greater turnover across the East-West precipitation gradient (see results, Supplementary Information Fig. S3), we then reran DESeq2 analysis within Western sites only and within Eastern sites only to determine variation between disturbed and remnant grasslands in these two specific regions.

Results

In both groups of root-associated plant pathogens studied here – fungal pathogens and oomycetes – richness and community composition responded to environmental variables, in remnant, undisturbed grasslands, but showed a reduced sensitivity to environmental variation in disturbed grasslands.

Phylogenetic Richness

For fungal pathogens, phylogenetic species richness (PSR) was predicted by environmental variables, particularly precipitation, in remnant (Table 1b; $F_{1,13.55} = 4.26$, $p = 0.06$), but not in disturbed grasslands (Fig. 2, Table 1b). In remnant grasslands only, precipitation and temperature interacted to determine PSR, with precipitation associated with greater fungal pathogen PSR when temperature was high, but not when temperature was low (Fig. 3; Table 1b; $F_{1,36} = 6.22$, p

= 0.02). Oomycetes showed similar responses, with oomycete PSR in remnant grasslands increased with precipitation (Table 1; $F_{1,17.75} = 4.62$, $p = 0.05$), but in disturbed grasslands oomycete PSR was unrelated to environmental variables.

Differences in Community Composition

Anthropogenic disturbance of grasslands, as well as precipitation and temperature, influenced fungal pathogen and oomycete composition (Table 2; disturbance, precipitation and temperature $p < 0.001$). As with richness, environmental factors predicted soil pathogen community composition in remnant grasslands, but this sensitivity was reduced in disturbed sites (Table 2). We found a significant temperature by precipitation interaction in fungal pathogens in both remnant and disturbed sites (Table 2b, remnant: $p = 0.04$, $R^2 = 0.049$; disturbed: $p = 0.01$, $R^2 = 0.099$). In remnant sites, the significant environmental factors explain a total of 39 percent of variation, while in disturbed sites they explain 10 percent of variation; although the precipitation by temperature interaction is significant in both disturbance groups, edaphic responses were absent, leading to a much lower impact of environmental variables on community composition in disturbed sites. Mean annual temperature and calcium were significant predictors of remnant community composition in both fungal pathogens and oomycetes (Table 2; *fungal pathogens*: temperature: $p = 0.007$, $R^2 = 0.074$; calcium: $p = 0.01$, $R^2 = 0.067$; *oomycetes*: temperature: $p = 0.03$, $R^2 = 0.056$, calcium: $p = 0.04$, $R^2 = 0.05$). Fungal pathogen remnant community composition was also significantly predicted by phosphorous, soil pH and potassium (Table 2; phosphorus: $p = 0.009$, $R^2 = 0.074$; soil pH: $p = 0.01$, $R^2 = 0.07$; potassium: $p = 0.03$, $R^2 = 0.056$).

Differential Abundance and Occurrence

There were a greater number of unique pathogen OTUs present in remnant versus disturbed grasslands as found in the Venn diagrams (Supplementary Information S1); this was especially striking for oomycetes (BLAST) with over double the unique OTUs (1555) compared to disturbed grasslands (628). Comparison of the relative abundance of OTUs via DESeq2 confirms greater turnover in remnant than in disturbed sites across the precipitation gradient (West versus East; Supplementary Information Fig. S3). Because of the divergent composition across remnant grasslands, we compared differential abundance of OTUs in remnant versus disturbed grasslands in eastern and western sites separately. Remnant sites tended to have fewer differentially abundant OTUs (between E and W sites) than disturbed sites when analyzing fungal pathogens and oomycetes (Supplementary Fig. S4). Although there are fewer OTUs in disturbed grasslands, a greater proportion of these OTUs are differentially abundant in disturbed grasslands compared to remnant grasslands, suggesting that they could be disturbance specialists.

Fungal saprotrophs

Fungal pathogens and saprotrophs differed in diversity responses to climate and land use, but had similar community composition responses. While climate factors only predicted pathogen PSR in remnant sites, climate predicted saprotroph PSR in both remnant and disturbed sites (Table 1c; remnant: $F_{1,11.7} = 5.67$, $p = 0.04$; disturbed: $F_{1,25} = 13.46$, $p = 0.001$). Similar to pathogens, the interaction of precipitation and temperature predicted saprotroph PSR (Table 1c; $F_{1,36} = 13.73$, $p = 0.001$; Fig S5) in remnant grasslands. Precipitation was positively correlated to PSR when temperature was high, but not when temperature was low. Saprotroph community composition responses mirrored those found for fungal pathogens, with several significant climate predictors

for remnant, but none for disturbed (Table 2c). Therefore, disturbance had distinct effects on climate relationships for pathogen richness as compared to saprotrophs, but showed similar results in terms of community composition.

Discussion

In a comprehensive test of root-associated pathogen sensitivity to environmental factors, we find that the community structure of fungal pathogens and oomycetes changes with anthropogenic disturbance. Moreover, we find that root-associated fungal and oomycete pathogen communities are sensitive to climate gradients, particularly precipitation and temperature, in undisturbed grasslands, but that disturbance disrupts the responses of these root-associated plant pathogens to environmental factors. As with other recent work, edaphic factors play an important role in structuring these grassland fungal communities (Lauber et al. 2008, Rousk et al. 2010, Rincón et al. 2015, Thomson et al. 2015, Newsham et al. 2016, Van Agtmaal et al. 2017). Together, these results identify interactive effects of climate and disturbance on plant pathogen communities, with implications for understanding potential patterns of the impact of pathogens on plant community composition and diversity.

Climatic determinants of root-associated plant pathogen communities in remnant grasslands

In the absence of disturbance, the structure of root-associated plant pathogen communities in remnant grasslands changes with climatic factors, including both precipitation and temperature. In contrast, most existing literature on fungal communities find either that climatic variables are not important to community structure (Thomson et al. 2015, Van Agtmaal

et al. 2017) or these variables are not explored (Lauber et al. 2008, Rousk et al. 2010). Our work adds to the growing evidence that soil fungi in general respond to climatic factors in addition to edaphic properties (Rincón et al. 2015, Zhou et al. 2016, Spear 2017). For example, Zhou et al. (2016) investigated six forests across northern and central America, representing a 30ºC temperature gradient and found that fungal diversity was better predicted by variation in temperature than edaphic properties. Likewise, Rincón et al. (2015) showed that fungal community composition responded to temperature and precipitation across a set of scots pine forests in France and Spain. Recent work by Spear (2017) showed that diversity of putative fungal pathogens from leaf stem and root tissue isolated on media responds positively to precipitation across a natural rainfall gradient in Panama. Our study is the first to show similar patterns for root-assocated pathogens in undisturbed grasslands.

Oomycetes also respond to precipitation in remnant grasslands, perhaps due to their life history. For example, oomycete zoospore release and subsequent flagellar movement to find a host explicitly depend on wet conditions (van West et al. 2003). Precipitation has previously been shown to be an important driver of oomycete community composition, although most studies showing this were conducted in agricultural settings (Rojas et al. 2017). In agreement with a recent global analysis of oomycete environmental drivers showing the positive relationship between precipitation and oomycete abundance (Oliverio et al. 2020), oomycete richness in our study responded positively to precipitation in remnant, undisturbed grasslands.

Temperature modifies the response of fungal pathogen diversity to precipitation (i.e. temperature and precipitation interact, Fig. 3). This has important implications for predicting the impact of these two major climate variables on remaining grassland systems. In sites with especially high average temperatures, increasing precipitation corresponds to an increase in OTU

richness, but this effect is absent across sites with colder average temperatures. Zhang et al. (2015) also found a similar temperature by precipitation interaction using PLFAs to assess soil fungi: precipitation and temperature interacted to promote stimulation of functional groups, while under drought, this relationship disappeared. In addition, Talley et al. (2002) found that vapor pressure deficit, a metric combing temperature and relative humidity, explained fungal richness better than temperature alone. In contrast, Ochoa-Hueso et al. (2018) found soil fungal diversity *decreases* with precipitation, although these results may be a product of different temperature regimes. While this interaction has been shown for bacteria (Sheik et al. 2011) and fungi in general (Zhang et al. 2015), our results are, to our knowledge, the first to show it for root-associated fungal pathogens. Given predicted shifts in both temperature and precipitation due to climate change, experimental assessment of the interactions between these factors is sorely needed.

Anthropogenic disturbance shifts root-associated plant pathogen composition and alters dependence on climate

Anthropogenic disturbance impacted pathogen community composition. The community composition of both oomycetes and fungal pathogens differed in disturbed compared to remnant grasslands. Our results are consistent with other studies showing strong effects of land use in non-pathogenic microbes (Dequiedt et al. 2011, House and Bever 2018b). We note that these differences in pathogen composition persisted at some sites decades after disturbance ended. One might ask why this difference has persisted so long. It is quite possible that there were few opportunities for dispersal of native pathogens to disturbed grasslands, as the vast majority of remnant grassland has been destroyed by tillage over the last hundred of years of agriculture.

With remnant grasslands occurring in less than four percent of original extent (Samson et al. 2004), there are very few remaining sources of native microbes, including pathogens, and these sources could be many miles from the disturbed grasslands we sampled. Alternatively, the successful colonization of disturbed grasslands by native plant pathogens could be limited by other persistent legacies of anthropogenic disturbance. As anthropogenic disturbance includes tillage and fertilization effects, its impact on the microbial composition could be mediated by changes in soil structure or fertility. Edaphic mediation of disturbance effects has been observed on non-pathogenic microbial groups (Lauber et al. 2008, Thomson et al. 2015). However, our data show a consistent differentiation of root-associated pathogen community structure between remnant and disturbed sites independent of measured edaphic properties. It is also possible, and perhaps, likely that the persistent change in plant composition following disturbance could contribute to these shifts in pathogen community composition. The disturbed grasslands sampled here had a markedly different plant composition, including dominance by non-native plant species, compared to remnant grasslands. Because plant community composition overlapped so little between remnant and disturbed sites (with many disturbed sites having zero overlap in plant species composition with remnant sites), linking pathogen shifts to individual plant species differences was not possible. However, given host-specificity of plant pathogens (Gilbert and Webb 2007, Bever et al. 2015), it is likely that the loss of the native prairie plant species in disturbed grasslands would limit establishment success of pathogens from remnant grasslands.

We also find that plant pathogens in anthropogenically disturbed grasslands are less responsive to variation in climate than in remnant grasslands, compared to other functional groups. Our results indicate a disturbance-induced reduction in climate sensitivity of root-associated pathogens. Increased homogenization of both soil properties and plant communities in

disturbed grasslands likely leads to a prevalence of shared, disturbance-adapted pathogens across the sites. Fungal saprotroph community composition was also linked to climate in remnant, but not disturbed grasslands. Unlike fungal pathogens and oomycetes, however, climate factors predicted PSR of fungal saprotrophs in both remnant and disturbed systems. In previous work, arbuscular mycorrhizal fungi (AMF) communities in remnant sites also differed in response to precipitation, similar to patterns we find in pathogens, but AMF richness was not affected by climate (House and Bever 2018b). Despite some similarities across functional groups, our data support distinct OTU richness responses of pathogenic fungi, saprotrophic fungi, and AMF to disturbance and climate. Given their different functional roles, these data support expectations that different groups within the microbial community can react differently to climatic and land use drivers.

Our results support the hypothesis that pathogen communities in undisturbed native grasslands are more responsive to precipitation and temperature than disturbed grasslands, yet we urge careful interpretation of these results. Taxonomic and functional group assignment rely on well-informed reference databases, which may be lacking particularly in remnant, undisturbed systems. The common practice of removing all OTUs that do not match a database (e.g. BLAST) may skew results toward cultured, heavily studied, or economically important organisms, such as those found in agricultural settings. For example, 509 oomycete taxa were excluded from our initial OTU table based on matching BLAST sequences because of the limited reference database available. Phylogenetic taxa delineation (used here) rather than a BLAST approach is more appropriate for the poorly described pathogens of native communities and generated a larger pool of resident oomycetes. Functional variation may also impact our conclusions. For example, the assumption that all Oomycota are pathogens is widely supported. Certain

oomycetes, however, have been shown to be saprophytic instead of pathogenic (van West et al. 2003) and may have a spectrum of pathogen and saprotrophic potential (Robideau et al. 2011). Likewise, our liberal inclusion of fungi designated by FUNGuild as pathogens likely masks a spectrum of functional variation. Inclusion of only high confidence designations, similar designations among site types, and comparison to FUNGuild-designated saprotrophs supports that these results are not products of database bias alone. Ideally, a more complete, experimental assessment of pathogenicity among oomycetes and fungal pathogens might allow more accurate ecological inferences about these groups across grasslands.

Study Implications

Our findings have implications for restorations of disturbed grasslands as well as remnant grasslands that have undergone the effects of climate change. To the extent that pathogens contribute to the maintenance of plant diversity (Bever et al. 2015), degradation of pathogen diversity and composition could contribute to the reduced plant diversity often observed following anthropogenic disturbance (Leach and Givnish 1996, Samson et al. 2004, Martin et al. 2005). Successful restoration of native plant diversity in grasslands may depend on reintroduction of these lost pathogens. In undisturbed systems, greater precipitation increases pathogen diversity, both for oomycetes and fungal pathogens, potentially contributing to increased native plant diversity. Within a changing climate, however, focusing solely on precipitation may not effectively predict these microbial communities, since precipitation effects here depended on temperature. While we cannot separate the direct effects of climate on pathogen composition from those effects mediated through plant responses, our results suggest that incorporation of environmental sensitivities of pathogens may be important to long-term

predictions of plant community response to climate. Further work is necessary to understand the causes and consequences of the precipitation and temperature interaction in pathogen groups to enact effective management strategies.

Conclusion

In conclusion, our study shows that different groups of root-associated plant pathogenic microbes are sensitive to land use disturbance and environmental gradients. Environmental gradients are important in driving pathogen community responses in undisturbed remnant, but less so in disturbed, grasslands. By clarifying root-associated plant pathogen response to temperature and precipitation gradients, we highlight the indirect consequences that climate shifts may have on plants through their microbiome. The root-associated plant pathogens studied here represent an often-overlooked mediator of plant community composition and diversity. Therefore, a clear understanding of how the plant microbiome responds to climate change will help us secure the future of remaining native plant communities and improve restoration of degraded ones.

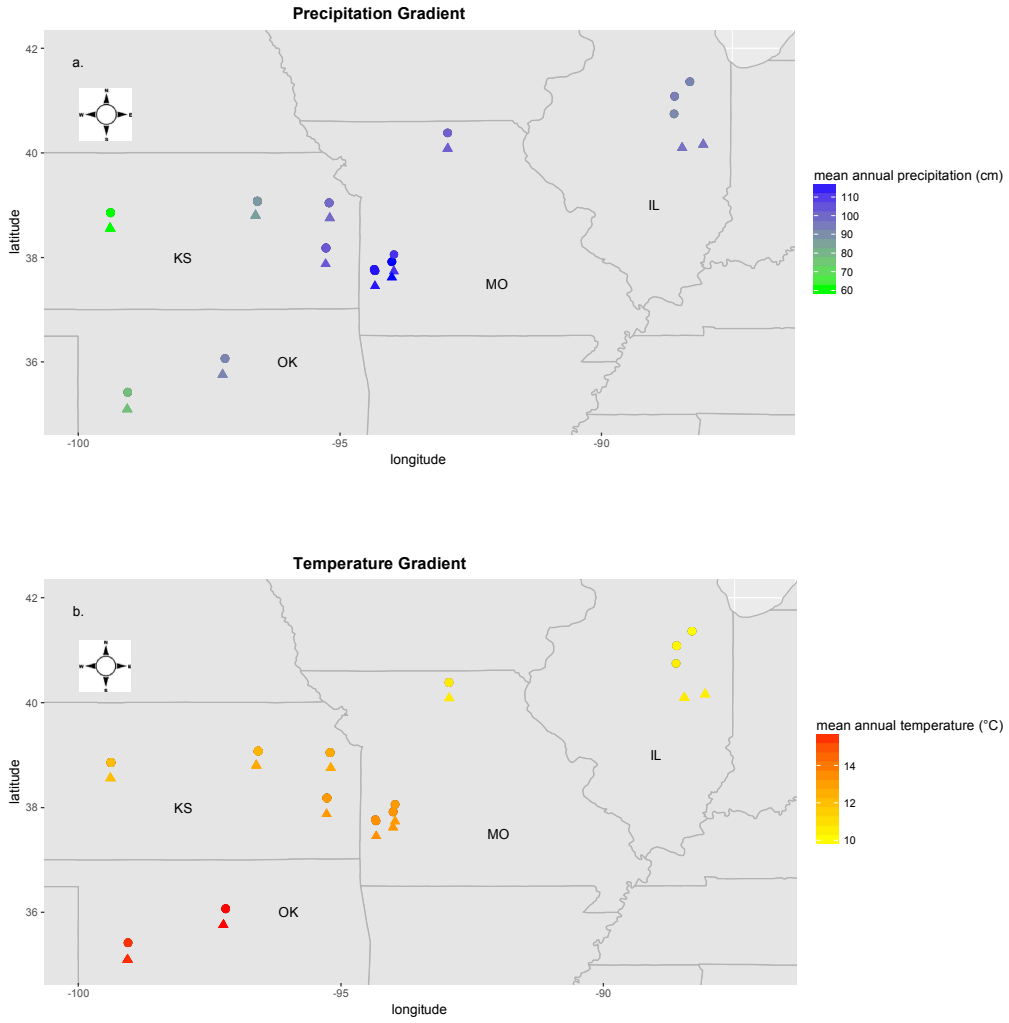


Figure 1. The precipitation and temperature gradient across our sampling sites.

Remnant sites are indicated by filled circles, while disturbed sites are indicated by filled triangles. Sites are skewed vertically to avoid overlap to clarify where different sites are located. Color intensity represents rainfall (a) and temperature (b) intensity.

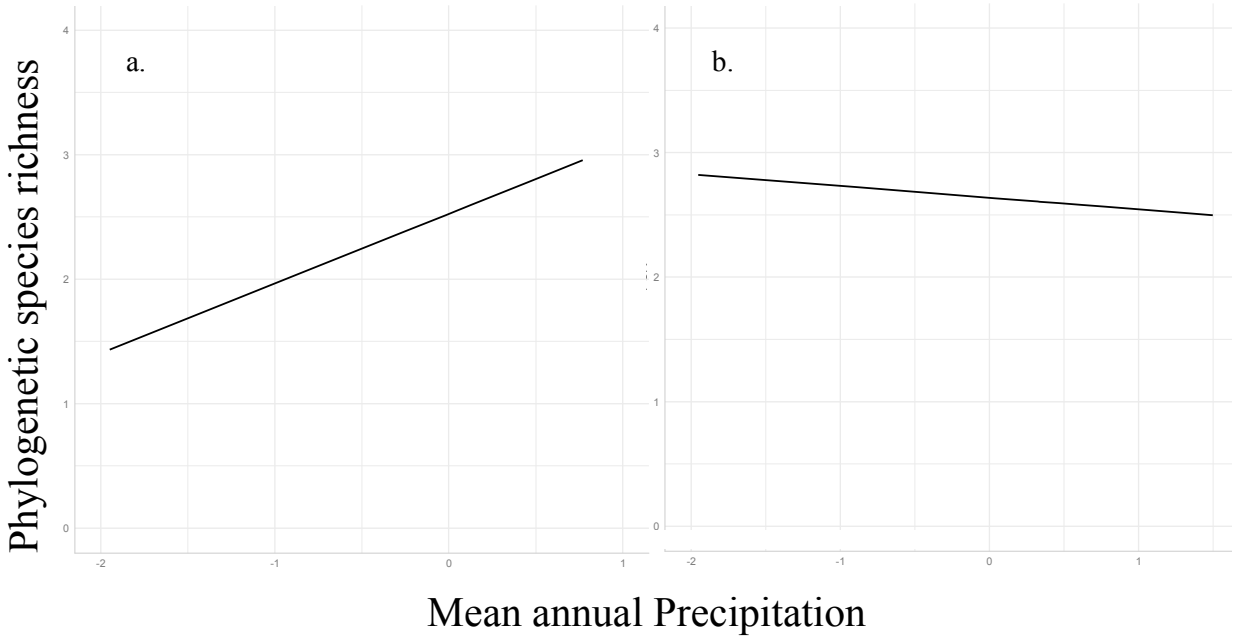


Figure 2. Phylogenetic species richness of fungal pathogens increases with temperature in remnant, but not disturbed sites.

GLM results showing mean annual precipitation prediction of phylogenetic species richness in fungal pathogens (a., remnant p = 0.06, b., disturbed p = 0.62). Points represent the raw data; the trendline is the predicted probability from the GLM.

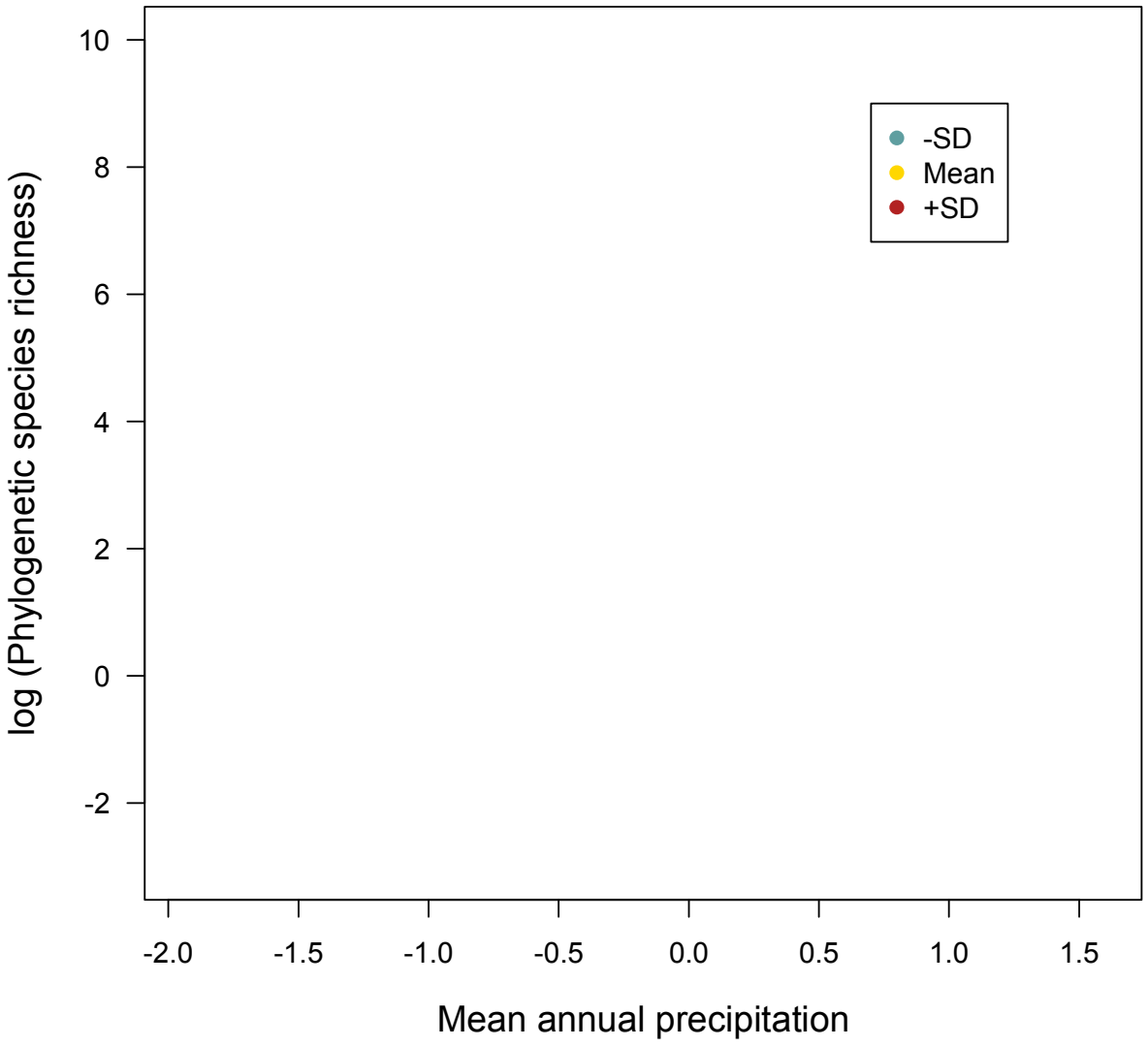


Figure 3. Soil fungal pathogen richness depends on the interaction between precipitation and temperature in remnant grasslands.

Pathogen phylogenetic species richness increases with precipitation at higher temperature (+ SD), but decreases with precipitation at low temperature (- SD; Table 1b, p = 0.02).

Table 1. PSR richness GLM results.

PSR richness GLM results for oomycetes (a), fungal pathogens (b) and fungal saprotrophs (c). These tests are univariate tests including either the interaction of the predictor variable and disturbance (if across all sites), or only the predictor variable in determining PSR richness. Tests of all samples include a random effect of disturbance nested within site; tests of remnant or disturbed samples include a random effect of plot. The model distribution is poisson. All significant predictors are in bold.

TABLE 1			
Subset of samples	Predictor variables	Estimate	*p* value
a. Oomycetes			
All samples	Disturbance	-0.022	0.389
	Disturbance × Mean Annual Precipitation	0.004	0.863
	Disturbance × Mean Annual Temperature	-0.036	0.183
Remnant samples only	Mean Annual Precipitation	0.033	**0.046**
	Mean Annual Temperature	-0.012	0.494
	Precipitation × Temperature	-0.03	0.233
Disturbed samples only	Mean Annual Precipitation	0.029	0.123
	Mean Annual Temperature	0.024	0.257
	Precipitation × Temperature	-0.002	0.951
b. Fungal Pathogens			
All samples	Disturbance	-0.005	0.990
	Disturbance × Mean Annual Precipitation	0.645	0.080
	Disturbance × Mean Annual Temperature	-0.369	0.326
Remnant samples only	Mean Annual Precipitation	0.559	**0.059**
	Mean Annual Temperature	-0.386	0.199
	Precipitation × Temperature	0.990	**0.017**
Disturbed samples only	Mean Annual Precipitation	-0.094	0.620
	Mean Annual Temperature	-0.018	0.921
	Precipitation × Temperature	-0.282	0.412
c. Fungal Saprotrophs			
All samples	Disturbance	-0.353	0.314

	Disturbance × Mean Annual Precipitation	0.366	0.291
	Disturbance × Mean Annual Temperature	-0.090	0.748
Remnant samples only	Mean Annual Precipitation	0.415	0.126
	Mean Annual Temperature	-0.524	**0.035**
	Precipitation × Temperature	1.033	**0.001**
Disturbed samples only	Mean Annual Precipitation	0.050	0.817
	Mean Annual Temperature	-0.444	**0.001**
	Precipitation × Temperature	-0.041	0.840

Table 2. PERMANOVA results.

PERMANOVA results for fungal pathogens (a) and phylogenetic oomycetes (b). *P < 0.05, **P < 0.01, ***P < 0.001. Separate tests were run within each (a) and (b) for all, remnant and disturbed sites; a second set of analyses was run for remnant and disturbed sites to test for the interaction between temperature and precipitation.

Table 2			
Subset of samples	Predictor variables	R^2 value	*p* value
a. Oomycetes			
All samples	Disturbance	0.06095	***
	Mean Annual Precipitation	0.09154	***
	Mean Annual Temperature	0.04369	**
	Bray 2 Phosphorus	0.05375	***
	Potassium	0.03497	**
	Calcium	0.02882	*
	Soil pH	0.01182	
	Disturbance × Mean Annual Precipitation	0.03847	**
	Disturbance × Mean Annual Temperature	0.01909	
	Disturbance × Bray 2 Phosphorus	0.02392	
	Disturbance × Potassium	0.02246	
	Disturbance × Calcium	0.02535	*
	Disturbance × Soil pH	0.02013	
	Sequence number	0.05307	***
Remnant samples only	Mean Annual Precipitation	0.03048	
	Mean Annual Temperature	0.05564	*

	Bray 2 Phosphorus	0.04834	
	Potassium	0.03747	
	Calcium	0.05045	*
	Soil pH	0.04273	
	Sequence number	0.05245	*
	Mean Annual Precipitation × Temperature	0.02258	
Disturbed samples only	Mean Annual Precipitation	0.06063	
	Mean Annual Temperature	0.06397	
	Bray 2 Phosphorus	0.06231	
	Potassium	0.05985	
	Calcium	0.06649	
	Soil pH	0.05359	
	Sequence number	0.04380	
	Mean Annual Precipitation × Temperature	0.04835	
b. Fungal Pathogens			
All samples	Disturbance	0.06506	***
	Mean Annual Precipitation	0.09626	***
	Mean Annual Temperature	0.05531	***
	Bray 2 Phosphorus	0.04962	**
	Potassium	0.05579	**
	Calcium	0.01072	
	Soil pH	0.01828	
	Disturbance × Mean Annual Precipitation	0.02817	
	Disturbance × Mean Annual Temperature	0.01612	
	Disturbance × Bray 2 Phosphorus	0.01375	
	Disturbance × Potassium	0.01763	
	Disturbance × Calcium	0.01577	
	Disturbance × Soil pH	0.03520	*
	Sequence number	0.02710	
Remnant samples only	Mean Annual Precipitation	0.04967	
	Mean Annual Temperature	0.07432	**
	Bray 2 Phosphorus	0.07250	**
	Potassium	0.05635	*
	Calcium	0.06655	*
	Soil pH	0.07011	*
	Sequence number	0.02311	
	Mean Annual Precipitation × Temperature	0.04921	*
Disturbed samples only	Mean Annual Precipitation	0.04435	
	Mean Annual Temperature	0.04988	

	Bray 2 Phosphorus	0.04943	
	Potassium	0.04619	
	Calcium	0.05155	
	Soil pH	0.04459	
	Sequence number	0.05557	
	Mean Annual Precipitation × Temperature	0.09856	*
c. Fungal Saprotrophs			
All samples	Disturbance	0.09910	***
	Mean Annual Precipitation	0.16866	***
	Mean Annual Temperature	0.05275	***
	Bray 2 Phosphorus	0.08111	***
	Potassium	0.03255	**
	Calcium	0.03513	**
	Soil pH	0.02150	
	Disturbance × Mean Annual Precipitation	0.01594	
	Disturbance × Mean Annual Temperature	0.01392	
	Disturbance × Bray 2 Phosphorus	0.02141	
	Disturbance × Potassium	0.01040	
	Disturbance × Calcium	0.01498	
	Disturbance × Soil pH	0.01962	
	Sequence number	0.01155	
Remnant samples only	Mean Annual Precipitation	0.02287	
	Mean Annual Temperature	0.05175	*
	Bray 2 Phosphorus	0.05037	*
	Potassium	0.03346	
	Calcium	0.05243	*
	Soil pH	0.04690	*
	Sequence number	0.02615	
	Mean Annual Precipitation × Temperature	0.02532	
Disturbed samples only	Mean Annual Precipitation	0.03934	
	Mean Annual Temperature	0.03678	
	Bray 2 Phosphorus	0.03710	
	Potassium	0.03976	
	Calcium	0.03093	
	Soil pH	0.04844	
	Sequence number	0.03609	
	Mean Annual Precipitation × Temperature	0.03933	

Chapter 4: Evidence for the Evolution of Mycorrhizal Response in Post-Agricultural Grasslands

Abstract

Plant-microbe interactions play an essential role in structuring plant communities. Arbuscular mycorrhizal fungi (AMF) are particularly important, especially in native systems. Nonetheless, increasing anthropogenic disturbance will lead to novel plant-AMF interactions, altering a longstanding co-evolutionary trajectory between plants and their associated AMF. Although emerging work shows that plant-AMF response can evolve over short time scales due to anthropogenic change, little work has evaluated how plant AMF response specificity may evolve due to novel interactions. Therefore, we examine changes in plant-AMF interactions in novel grassland systems by comparing the mycorrhizal response of plant populations from unplowed native prairies with populations from post-agricultural grasslands to inoculation with both native prairie AMF and non-native AMF. Across four plant species, we find support for evolution of mycorrhizal response specificity consistent with expectations of local adaptation, with plants from native populations responding most to native AMF and plants from post-agricultural populations responding most to non-native AMF. We also find evidence of evolution of mycorrhizal response in two of the four plant species, as overall responsiveness to AMF changed from native to post-agricultural populations. Finally, across all four plant species, roots from native prairie populations had lower levels of mycorrhizal colonization than those of post-agricultural populations. While further work is necessary to confirm the genetic basis of these traits, our results highlight that widespread anthropogenic disturbance can have unintended

impacts on the genetic propensities of native plant species' association with AMF, causing rapid evolutionary change in the benefit native plant species gain from native symbioses.

Introduction

There has been a growing understanding of the vital links between plant communities and their soil microbiome (Bever et al. 2010, Barberán et al. 2015, Delgado-Baquerizo et al. 2016), with both mutualistic and pathogenic microbes implicated in the maintenance of plant diversity. Evidence to date shows that plant pathogens structure plant communities and maintain plant community diversity (van der Heijden et al. 2008, Mordecai 2011, Van der Putten et al. 2013, Bever et al. 2015, Eppinga et al. 2018, Crawford et al. 2019). A major group of mutualists, mycorrhizal fungi, are also important in determining plant community structure (van der Heijden et al. 1998, Bever 2002, Vogelsang et al. 2006, Mangan et al. 2010). These symbionts play an essential role in mediating plant succession (Janos 1980, Koziol and Bever 2015, Koziol and Bever 2019) and in the establishment and distribution of plant species worldwide (Delavaux et al 2019).

Plant interactions with arbuscular mycorrhizal fungi (AMF) are particularly important in native systems, with both partners coevolving over time. There is strong evidence of coevolution of the relationship between plants and their mycorrhizal fungi (Brundrett 2002). Indeed, AMF are hypothesized to have played a major role in land colonization by plants, serving as root-like structures to aquatic plants (Redecker et al. 2000). Evidence shows that the arbuscular mycorrhizal state is the ancestral state, with later plant groups evolving ectomycorrhizal or non-mycorrhizal relationships (Brundrett 2002, Maherali et al. 2016). Further, this evolutionary

history has been shown to be a dominant driver of mycorrhizal response. A recent meta-analysis (Hoeksema et al. 2018) showed that evolutionary history is a strong driver of plant response to mycorrhizal fungi, and is more important in determining the interaction outcome than other traditionally studied environmental moderators. Selection and breeding in agriculture also offer evidence for more recent evolutionary influence on the plant-AMF interaction, often leading to lower responsiveness to AMF (Koziol et al. 2012, Turrini et al. 2016, Martín-Robles et al. 2018).

Increasingly, anthropogenic disturbance, including species introductions and range shifts, will lead to novel plant-microbe interactions, interfering with this longstanding co-evolutionary trajectory (Pringle et al. 2009, Dickie et al. 2017). Evidence suggests that these changes may result in rapid evolution, with AMF responsiveness evolving over a few decades. Invasion offers an important perspective into formation of novel plant-mycorrhizal relationships, with invasive plants often showing reduced response to AMF (Pringle et al. 2009, Seifert et al. 2009, Cheeke et al. 2019), or directly reducing AMF abundance (Stinson et al. 2006, Callaway et al. 2008, Vogelsang and Bever 2009, Crawford et al. 2019), but see (Bunn et al. 2015). Seifert et al. (2009) found that St. John's wort showed reduced responsiveness to AMF during invasion of North America compared to native St. John's wort source populations. As St. John's wort is abundant in anthropogenically-disturbed areas of North America, this study suggests that rapid evolution of mycorrhizal response may occur due to anthropogenic disturbance. Nonetheless, it is unknown whether the specificity of plant response to AM fungal composition may also evolve rapidly in general and specifically in response to anthropogenic disturbance.

The tallgrass prairie system in the Midwestern US is an ideal system in which to ask questions related to evolution of mycorrhizal response and specificity, with a long history of research into the ecology of plant-mycorrhizal relationships. Work in tallgrass prairies has shown

that plant-mycorrhizal interactions sustain native plant diversity, with late successional prairie dominants showing high responsiveness to AMF and fungal composition (Wilson and Hartnett 1998, Vogelsang et al. 2006, Koziol and Bever 2015, Koziol and Bever 2016, Cheeke et al. 2019, Koziol and Bever 2019). Further, as most prairie systems have been altered by human activity (Samson et al. 2004), novel plant-microbial interactions are dominant in these heavily degraded systems. Specifically, anthropogenic disturbance of prairies has been shown to degrade AMF communities (House and Bever 2018a), and reintroduction of native AMF into these settings increases the establishment success and growth of late successional prairie plant species (Middleton et al. 2015, Koziol and Bever 2017, Koziol et al. 2018a, Lubin et al. 2019). However, a subset of native prairie plant species has been more successful in colonizing in post-agricultural sites, often becoming more abundant than they were in native prairies. These early successional native prairie plant species have been shown to be less dependent on AMF than late successional prairie plant species (Koziol and Bever 2015, Bauer et al. 2018) and their response is less sensitive to AMF species identity (Koziol and Bever 2016, Cheeke et al. 2019). However, whether these species differentially respond to native AMF and whether they have evolved in their relationship with AMF during colonization of post-agricultural sites has not been explored.

Here, we investigate the evolution of plant mycorrhizal response and the specificity of this response across tallgrass prairies in Eastern Kansas. We first test for differences in mycorrhizal responsiveness between two plant population types, native and post-agricultural, across four native plant species. We then test for differences in specificity of mycorrhizal response by comparing the response of these population types to native prairie AMF inocula with the response to novel non-native AMF inocula. Local adaptation predicts that populations from undisturbed prairies will benefit most from native prairie AMF, while populations from disturbed

prairies will benefit most from novel AMF. This work clarifies how populations of the same native plant species in native and post-agricultural prairies differ in their growth response to mycorrhizal fungi generally (mycorrhizal response) and to inocula origin specifically (mycorrhizal response specificity), informing potential evolutionary consequences of novel interactions resulting from anthropogenic change.

Methods

Site description

To test differences in mycorrhizal response between native and post-agricultural plant populations, we selected eight prairie sites across Kansas, USA. Four sites were classified as native, representing native prairies sites without human disturbance. The four remaining sites were post-agricultural sites which were abandoned agricultural fields (between 20-50 years) and represent novel, disturbed sites. The native sites included two sites at the University of Kansas Field Station (Rockefeller Prairie and Dogleg Prairie), Prairie Nature Park Prairie, and Kill Creek Prairie. The post-agricultural sites included a site at The Land Institute in Lawrence, KS, two sites of the University of Kansas Field station (Welda Prairie and Plot 4010) and the Rock Chalk Park walking trails.

Seed collections

Seeds were hand collected during September and October 2018. The following species were collected from each site when possible for this study: *Apocynum cannabinum* (Dogbane),

Vernonia fasciculate (Ironweed), *Asclepias syriaca* (Milkweed), and *Solidago canadensis* (Solidago). Seeds were air dried, hand separated and stored at 4ºC in a walk-in fridge.

Mycorrhizal inocula

Two types of mycorrhizal inocula were used in this study to test differential response of each plant population type to inocula representing mycorrhizal fungi that would be found in association with each of these plant population types. To represent mycorrhizal fungal species found in native prairie sites, we used a mixture of 11 AMF species cultured from tallgrass prairie remnants. To represent novel mycorrhizal fungal species as might be found in disturbed post-agricultural sites (House and Bever 2018), we used 10 AMF species across the AMF phylogeny from the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM, West Virginia University, West Virginia, USA). Species from both inocula types were grown as single species cultures in the same greenhouse, soil and with the same plant hosts during the previous summer. Each of these two inocula mixes were homogenized before use in the experiment (Table S1).

Greenhouse Assay

In Spring 2019, seeds were cleaned and planted in sterile potting soil and cold stratified for six weeks. The planted seeds were then placed in a greenhouse to germinate for two weeks. Three seedlings from a given individual were taken and planted in each of three soil treatments: sterile, native prairie fungi, or non-native fungi, with five replicates (individuals) for each treatment per species per site (Table S2). Plants were then grown for 13 weeks in the greenhouse. Once growth reached an asymptote, representing peak growth, plants were harvested. At harvest, we measured

plant height and weighed aboveground and belowground biomass. All roots (belowground biomass) were hand cleaned to remove soil and small pebbles.

Root architecture and colonization

We conducted root architecture (root length) analysis on root systems of plants in the sterile treatment. Root architecture in the sterile treatment has been found to correlate with mycorrhizal response (Seifert et al. 2009, Koziol and Bever 2015); smaller root systems tend to be more responsive to mycorrhizal fungi, whereas larger ones tend to be less responsive. Therefore, we analyzed roots *without* mycorrhizal fungal inoculation (sterile treatment) to explore this relationship. Entire root systems were scanned at harvesting before being dried for biomass. Root systems were quantified using WinRhizo (Regent Instruments Inc., Quebec, Canada).

We also inspected root colonization in a subset of 36 study plants to confirm that our sterile controls did not have AMF colonization and that the AMF treatments showed AMF colonization. Root colonization was assayed using Trypan Blue staining and subsequent microscope inspection (Giovanetti and Mosse 1980). Stained roots were mounted on slides and were analyzed using 25 vertical transects for colonization as well as for presence of arbuscules, vesicles, coils and hyphae (Table S3).

Statistical Analyses

We tested for differences in colonization between AMF inoculation treatments and differences in colonization across the population types and species by using a generalized linear mixed model (GLMM) predicting colonized to non-colonized counts of mycorrhizal occurrence as a combined response variable, specifying the binomial family with a logit link. We tested for consistent

differences between sterile and inoculated treatments treating AMF inocula as a fixed effect and the interaction between species and population type as a random effect. Because the AMF colonization level in sterile was near zero, in a second analysis, we omitted the sterile treatment and analyzed the inoculated plants to test whether the population types differed in overall infection. We tested for consistent differences between population types across the four plant species by treating population type, species, AMF inocula and the interaction of population type by AMF inocula as fixed effects and the interaction of population type within species and of this term with AMF inocula as random effects. To analyze the difference in measured root length between species, we used a linear model predicting specific root length (total root length ÷ root biomass) based on species, and included the random effect of plant species interacting with plant population type. We reran this model to test for the interaction between species and plant population type. These statistical analyses were carried out using R v. 3.6.0 (Team 2019).

We used mixed models to test whether the two population types differed consistently in response to inoculation across the four replicate populations of each of the four plant species. Specifically, we identified population type, species, AMF inocula and their interactions as fixed effects, and population identity within population type within plant species and the interaction of this term with AMF inocula as random effects. AMF inocula effects and all interactions with AMF inocula were decomposed into two orthogonal *a priori* contrasts that separately tested the average response to AMF (AMF vs sterile) and the differences in response to the two sources of AMF (native vs non-native). Consistent differences between populations from native versus disturbed locations across all populations of all plant species were detected as significant population type effects in the mixed model. Consistent differences in population type interactions with mycorrhizal fungi were detected within the population type by AMF inocula

interaction, which was broken into the overall mycorrhizal response (population type by sterile vs AMF) and specificity of mycorrhizal response (population type by AMF type, native vs non-native origin). Plant height of seedlings after transplanting was included as a covariate in all models, which can partly account for potential maternal effects such as seed size differences.

To test mycorrhizal response (biomass), we used generalized linear mixed effects (GLMM) models to predict either aboveground or belowground biomass. We first conducted these analyses on all data across the four species, and when the interactions with plant species were significant, we tested each species individually. For these analyses, no single transformation adequately homogenized the variation between treatments. We therefore rank transformed the above- or below-ground biomass within plant species as the best available option. Our analyses thus corresponded to tests of medians of the distributions of biomass rather than means (Conover and Iman 1981). To predict this rank transformed biomass, we used treatment, species, population type and initial height at planting, as our independent predictor variables. We also included the random effects of treatment nested within species nested within population replicate nested within population type, species nested within population replicate nested within population type and species replicate (in greenhouse experiment) nested within species nested within population replicate nested within population type (native or post-agricultural) to account for non-independence of samples. We then reran these analyses for each species, with a model structure analogous to that used for all species described above. These GLMMs were run in SAS (Institute 2012).

As the rank transformation did not perfectly satisfy parametric assumptions, we used permutation approaches to assess the robustness of inference from the analyses of ranks. We conducted 1,000 permutations to construct a p-value based on the distribution of the resulting

estimates; when estimates were not available we used p-values instead. To do this, we resampled the data, grouping the data by species, reassigning the joint value of mycorrhizal treatment, population type and population replicate in R v. 3.6.0 (Team 2019) using the tidyverse package (Wickham et al. 2019). We then reran the previously described GLMMs with these 1,000 datasets in SAS. We then calculated the new p-value based on the actual distribution of permutations in R. The plots presented here used the output from these models to calculate mycorrhizal (growth) response (MGR), using the following formula: $(Biomass_{AMF}\text{-}Biomass_{Sterile}) \div Biomass_{Sterile}$.

Finally, we investigated correlations between MGR and specific root length (SRL) as well as between MGR and colonization using linear models. To investigate correlations between MGR and SRL, we used the interaction of SRL and plant species to predict MGR, with the random effect of plant species interacting with plant population type. To investigate correlations between MGR and colonization, we used MGR as the response variable predicted by the interactions of logit colonization proportion (colonization) and plant population type and of colonization and species, with the random effect of plant species interacting with plant population type.

Results

Mycorrhizal Colonization

We found that the treatments were successful, with mycorrhizal treatments showing significantly higher colonization than sterile treatments ($F_{1,34} = 68.09$, $p < 0.001$; mean of 12.38 occurences per 24 root intersections), with very few instances of mycorrhizas encountered in the sterile

treatment (Fig S1; mean of 0.25 occurrences per 25 root intersections). Across all plant species, we found that post-agricultural populations showed greater colonization than native populations (Fig. 1, $F_{1,3} = 16.24$, $p = 0.002$).

Specific root length

We did not find that specific root length (SRL) varied consistently with plant population type across the four plant species ($F_{3,84} = 0.077$, $p = 0.97$). Comparing species, we found a marginally significant species effect with Dogbane showing longer SRL compared Ironweed (Fig S2; $F_{3,4.56} = 3.935$, $p = 0.1$).

Mycorrhizal growth response

Overall, plants grew significantly larger with AMF than in the sterile treatment, both in terms of aboveground (rank: $p < 0.0001$, $F_{1,175} = 312.98$; permutation: $p < 0.0001$) and belowground (rank: $p < 0.0001$, $F_{1,178} = 201.16$; permutation: $p < 0.0001$) biomass. When analyzing individual plant species, each species was mycorrhizally responsive and showed greater growth in AMF versus sterile treatments, for aboveground biomass (rank: Dogbane: $F_{1,31.6} = 6.30$, $p = 0.018$; Ironweed: $F_{1,9.28} = 129.5$, $p < 0.0001$; Milkweed: $F_{1,34.9} = 133.53$, $p < 0.0001$; Solidago: $F_{1,9.8} = 192.59$, $p < 0.0001$; permutation: Dogbane: $p = 0.019$; Ironweed: $p < 0.0001$; Milkweed: $p < 0.0001$; Solidago: $p < 0.0001$), while almost all, with the one exception of Dogbane, was mycorrhizally responsive for belowground biomass (rank: Ironweed: $F_{1,83} = 61.58$, $p < 0.0001$; Milkweed: $F_{1,5.23} = 129.88$, $p < 0.0001$; Solidago: $F_{1,9.8} = 126.36$, $p < 0.0001$; permutation: Dogbane: $p = 0.085$; Ironweed: $p < 0.0001$; Milkweed: $p < 0.0001$; Solidago: $p < 0.0001$). We found a significant difference between the two types of AMF inocula in

belowground biomass, with post-agricultural AMF resulting in a higher growth increase than native AMF (rank: $F_{1,180} = 5.15$, $p = 0.024$; permutation: $p = 0.056$).

Population differences in mycorrhizal growth response

We found consistent differences between the two population types in specificity of response to the two types of AMF inocula. Across all four plant species, we found post-agricultural populations generally grew best with non-native AMF, while native plants grew best with native AMF inocula, with a significant interaction between origin of AMF inocula and plant population for aboveground biomass (Fig 2A; rank: $F_{1,175} = 4.87$, $p = 0.03$; permutation: $p = 0.08$).

We also found differences between population types in overall mycorrhizal responsiveness (regardless of AMF type), but this effect varied significantly with plant species, with a significant three-way interaction between AMF versus sterile by plant population type by plant species for belowground biomass (Fig 2B, rank: $F_{3,177} = 4.46$, $p = 0.0048$; permutation: $p < 0.0001$). Specifically, for Dogbane, native plant populations were more mycorrhizally responsive than post-agricultural plant populations (rank: $F_{1,31.6} = 3.53$, $p = 0.07$, permutation $p = 0.058$), while for Ironweed, post-agricultural populations were more responsive than native plant populations (rank: $F_{1,83} = 6.56$, $p = 0.01$; permutation: $p = 0.036$). This pattern was found aboveground as well, but was weaker (rank: $F_{3,174} = 2.56$, $p = 0.06$; permutation: $p < 0.0001$).

Mycorrhizal growth response, specific root length and mycorrhizal colonization

We did not find an overall correlation between specific root length (SRL) and mycorrhizal growth response (MGR; $F_{1,17} = 0.535$, $p = 0.47$). We found that this relationship varied depending on species, and found an interaction between plant species and SRL in predicting

mycorrhizal growth response ($F_{3,17} = 5.123$, $p = 0.01$). Specifically, Solidago and Ironweed showed a positive correlation between MGR and SRL, while Milkweed showed a negative correlation. Further, we found a significant interaction between colonization and plant population type in predicting MGR (Fig. 3, $F_{1, 5.94} = 14.395$, $p = 0.01$), with a negative correlation in native and no correlation in post-agricultural plant populations. We also found a significant interaction between colonization and plant species (Fig. S3, $F_{3,4.68} = 8.98$, $p = 0.02$), with Ironweed showing a positive correlation, while other species showed a negative correlation.

Discussion

Here, we found evidence of rapid evolution of plant-mycorrhizal interactions in anthropogenically-disturbed, post-agricultural grasslands. Across four native plant species, we found that post-agricultural plant populations had higher mycorrhizal colonization than plant populations from undisturbed grasslands. We also found that populations from undisturbed grasslands generally showed greater mycorrhizal response to native AMF, while populations from post-agricultural grasslands showed greater response to non-native AMF. These two results were consistent across all four plant species tested in our study, suggesting that the plant-AMF interaction evolved in response to anthropogenic disturbance in predictable directions. We also found evidence that overall AMF responsiveness evolved in post-agricultural populations in two of the plant species. These findings suggest that overall mycorrhizal response and mycorrhizal response to specific AMF can evolve within a rapid timeframe, highlighting potential evolutionary consequences of anthropogenic disturbance on plant-mycorrhizal interactions.

We found novel evidence for the evolution of mycorrhizal response specificity. Plants from native populations responded more positively to native AMF, while plants from populations colonizing disturbed, post-agricultural, areas responded more positively to non-native AMF. Although this result was only marginally statistically significant, it was in the direction predicted from *a priori* expectations of plant-AMF co-adaptation. Moreover, this shift in responsiveness was accompanied by a statistically robust shift in levels of mycorrhizal colonization. Specifically, we find populations colonizing disturbed land (post-agricultural populations) consistently exhibited greater mycorrhizal colonization rates regardless of the inocula source compared to populations from unplowed, native prairies. In addition, we found that native plant populations showed a negative relationship between AMF colonization and growth response to AMF, while post-agricultural populations did not. That native plants were less colonized overall and showed greatest mycorrhizal response with lower colonization suggests that native populations are more selective in their mycorrhizal associations, perhaps because they benefit most from native AMF. In contrast, post-agricultural plant populations had higher colonization rates and showed no relationship between mycorrhizal colonization and response, suggesting that these plants are more permissive of AMF infection.

Our results provide evidence for the evolution of overall AMF response during colonization of disturbed land in eastern Kansas, though the direction of this effect varied between two species. We found that Dogbane mycorrhizal responsiveness decreased, while Ironweed mycorrhizal responsiveness increased in post-agricultural plant populations. Anthropogenic disturbance results in strong degradation of the AMF community (House and Bever 2018a) and in benefit to native prairie plant species (Koziol and Bever 2018). Evolution of decreased mycorrhizal response in anthropogenically disturbed land, as we observed in Dogbane,

is consistent with expectations based on this loss of AMF function. This is also consistent with previous work showing evolution of reduced response to AMF of invasive plants which dominate in disturbed lands of North America (Seifert et al. 2009), and loss of response during selection for yield in disturbed agricultural systems (Koziol et al. 2012, Turrini et al. 2016, Martín-Robles et al. 2018).

The increased response in Ironweed post-agricultural populations is counter to our *a priori* expectation. The variation in response between these plant species suggests that factors other than the degradation of AMF may be important in determining the direction of evolution of mycorrhizal response. For example, the absence of competition from later successional species in post-agricultural lands may generate selection for more late successional traits, such as high responsiveness to AMF (Bauer et al. 2018), in mid-successional Ironweed. Alternatively, tradeoffs with pathogen defense, a non-nutritional benefit of AMF (Delavaux et al. 2017) could alter simple expectations. There is evidence for the importance of mycorrhizal induced resistance by native AMF in grassland systems through reduced effects of herbivory (Middleton et al. 2015) and fungal pathogens (Sikes et al. 2009). Further research will be needed to experimentally test relative pathogen impacts on these plant populations and respective AMF communities within these species.

Although mycorrhizal fungi are known to be important in tallgrass prairie systems, the early successional plant species such as those used in this study have generally been shown to be less responsive (Koziol and Bever 2015, Bauer et al. 2018) and less sensitive to AMF identity (Koziol and Bever 2016, Cheeke et al. 2019) than late successional species. However, we found that these early successional species were responsive to AMF, consistent with Reynolds et al. (2020) and that responsiveness can vary with the population sources and with AMF identity.

While our study provides the first evidence of rapid evolution of mycorrhizal response specificity in response to anthropogenic disturbance, we must qualify our confidence in attribution of the observed shifts in the mycorrhizal interactions from native to post-agricultural populations as an evolutionary shift. As we used field collected seeds, it is possible that environmental differences in seed origin contributed to maternal differences, such as seed provisioning, that influenced our observed responses. We note that we attempted to minimize this possibility, by including collections from independent mother plants as our replicates within each of multiple independent populations within each plant species, by germinating all seed in a common environment, and by including initial height of seedlings at planting as a partial control of potential differences in maternal provisioning. Nevertheless, further work reducing the potential for confounding maternal effects and identifying the genetic basis of the shifts in plant responses to mycorrhizal fungi is needed to confirm the evolutionary nature of the observed phenotypic shifts. While we observed these phenotypic shifts within sites that have been abandoned for between one to a few decades, we note that we cannot be definitive on the timescale of the potential evolutionary response. Because we do not know the precise origin and evolutionary trajectory of these plant populations, we acknowledge that they may have been evolving in disturbed locations before colonizing the post-agricultural sites sampled in this study.

We show for the first time that evolution of mycorrhizal response specificity is possible over relatively short timescales following disturbance. The native plant species targeted in this study showed a shift in overall growth response based on both plant population type and AMF inocula type, with native plant populations more responsive to native AMF inocula and post-agricultural populations more responsive to non-native AMF inocula. Our results highlight the sensitivity of native plant-mycorrhizal associations to human disturbance. On a practical level,

our work supports the importance of plant source (population type) and mycorrhizal inocula origin for reestablishing grasslands. More generally, continuing anthropogenic disturbance has the potential to alter the longstanding co-evolution trajectory of plants and their associated mycorrhizal fungi. Here, we find that this anthropogenic change leads to shifts in the functionality of this plant-mycorrhizal relationship, with consequences for our understanding of plant-AMF co-evolution and strategies to recreate native ecosystems.

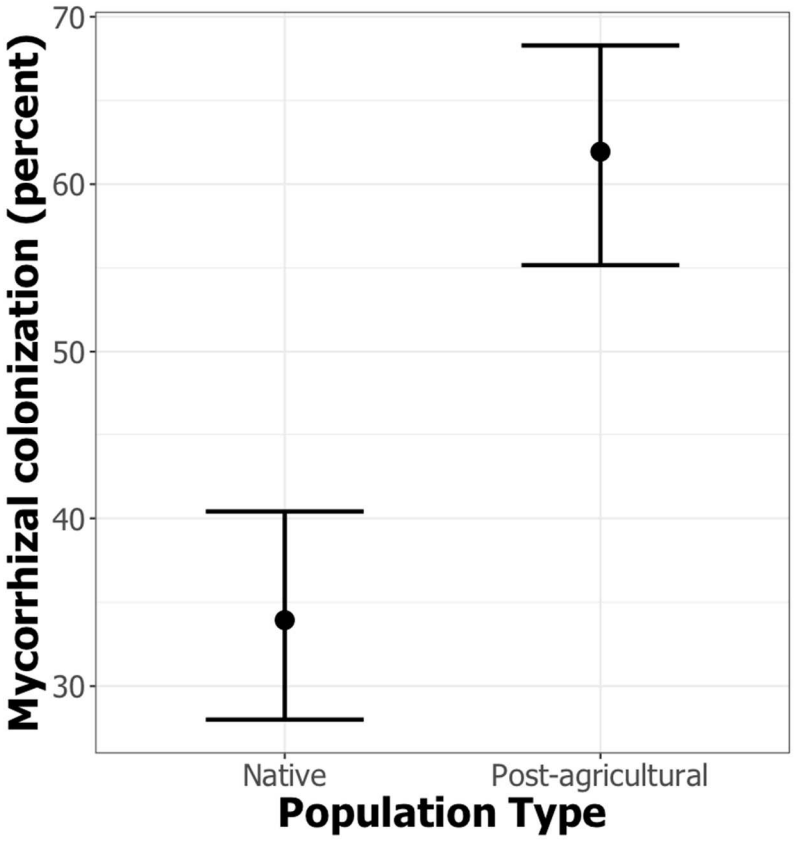


Figure 1. Mycorrhizal colonization differences across study species.

GLM shows that there is a significantly greater arbuscular mycorrhizal colonization in post-agricultural compared to native populations ($F_{1,3} = 16.24$, $p = 0.002$).

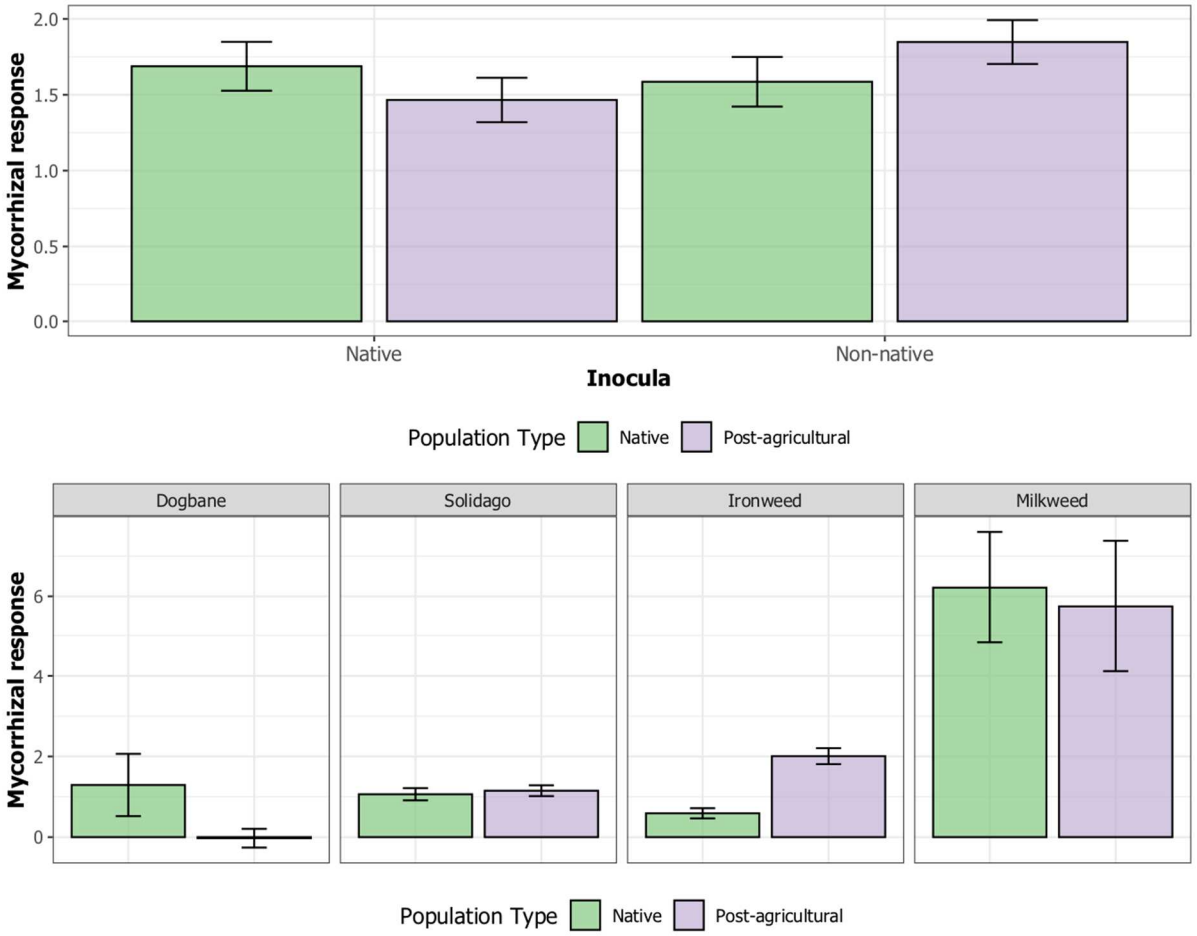


Figure 2. Evolution of mycorrhizal response.

Abovergorund, across the four species included in the study, native plant populations showed the greatest mycorrhizal response to native AMF inocula, while post-agricultural plant populations showed the greatest mycorrhizal response to non-native AMF inocula (A, rank: $F_{1,175} = 4.87$, $p = 0.03$; permutation: $p = 0.08$). Belowground, within species, for dogbane, native plant populations are more mycorrhizally responsive than post-agricultural plant populations (B, rank: $F_{1,31.6} = 3.53$, $p = 0.07$, permutation $p = 0.058$), while for ironweed, post-agricultural populations are more responsive than native plant populations (rank: $F_{1,83} = 6.56$, $p = 0.01$; permutation: $p = 0.036$).

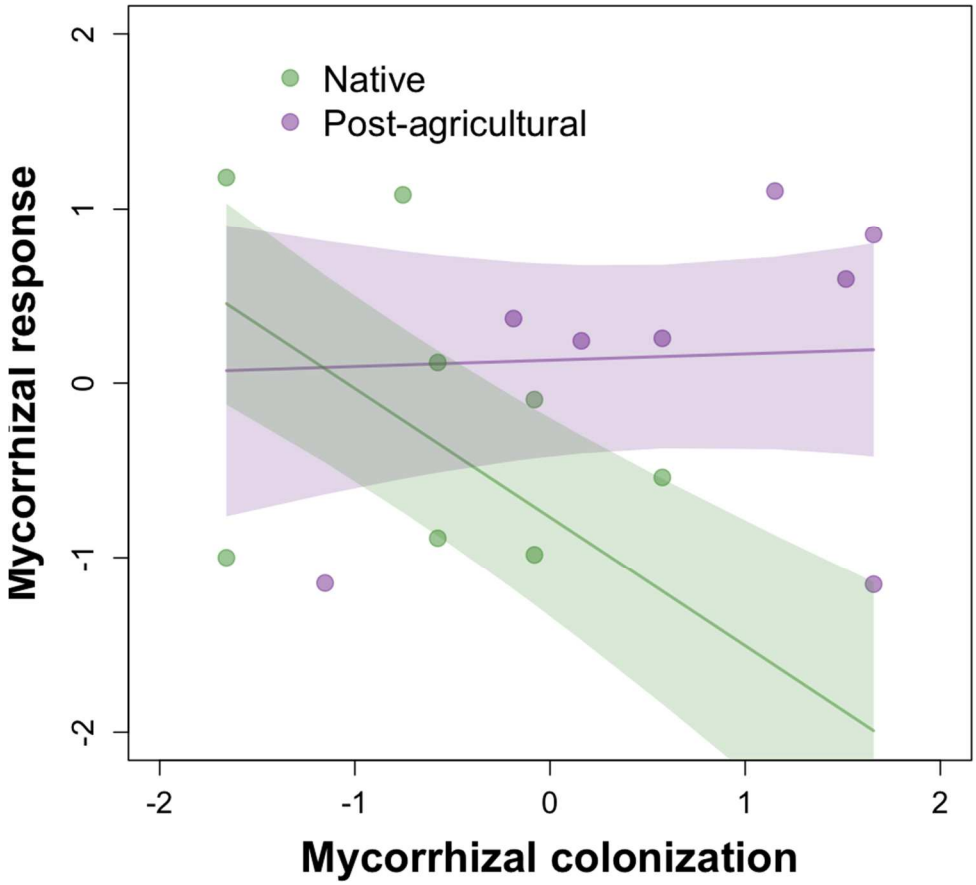


Figure 3. Population type differences in relationship between mycorrhizal colonization and response.

The relationship between logit colonization proportion and mycorrhizal growth response depends on population type, with native populations showing a more negative relationship ($p = 0.01$).

Conclusion

Here, I have presented work clarifying the roles of two major groups of plant-associated microbes – mutualists and pathogens - in structuring plant communities in co-evolved native as well as in anthropogenically-altered systems. The work in this thesis demonstrates that global island biogeography is shaped by co-limitation of plants *and* their mycorrhizal partners, that plant-associated pathogens respond to climate and land use, and that there is evidence for local evolution of mycorrhizal response in disturbed post-agricultural sites representing novel plant-mycorrhizal relationships. In Chapter 1, mycorrhizal fungi were found to shape island biogeography at a global scale by impacting which types of plants establish on islands and contribute to the latitudinal species diversity gradient. In Chapter 2, mycorrhizal type was found to be important in mediating this mycorrhizal filter of plant colonization to oceanic islands. Plants associating with more dispersal limited AM fungi were underrepresented on islands and this pattern intensifyed with distance, especially within non-endemic plant species. Across the Midwestern United States (Chapter 3), pathogen communities, long implicated in driving plant diversity, were shown for the first time to respond to climate variables and land use change, clarifying their response to anthropogenic forces and their potential to indirectly mediate plant response to global change. Finally, at the local scale (Chapter 4), the plant-mycorrhizal relationship was found to evolve in native plant species in Kansas in response to a disturbed novel environment. Overall, this work has important implications for our understanding of island biogeography, plant invasion risk, and the consequences of human impact to plant-microbe interactions. This deepened understanding of patterns and processes governing plant-microbiome

relationships will improve our ability to predict the continuing effects of anthropogenic change and equip us with knowledge for better management and restoration of degraded sites.

Appendix A: Keeping it cool: Soil sample cold pack storage and DNA shipment up to one month does not impact metabarcoding results

Material from: Delavaux, C. S., J. D. Bever, E. M. Karppinen, and L. D. Bainard. (2020). Keeping it cool: Soil sample cold pack storage and DNA shipment up to 1 month does not impact metabarcoding results. *Ecology and Evolution.* DOI: https://doi.org/10.1002/ece3.6219

Abstract

With the advances of sequencing tools, the fields of environmental microbiology and soil ecology have been transformed. Today, the unculturable majority of soil microbes can be sequenced. Although these tools give us tremendous power and open many doors to answer important questions, we must understand how sample processing may impact our results and interpretations. Here, we test the impacts of four soil storage methods on downstream amplicon metabarcoding and qPCR analyses for fungi and bacteria. We further investigate the impact of thaw time on extracted DNA to determine a safe length of time during which this can occur with minimal impact on study results. Overall, we find that storage using standard cold packs with subsequent storage at -20º C is little different than immediate storage in liquid nitrogen, suggesting that the historical and current method is adequate. We further find evidence that storage at room temperature or with aid of RNAlater can lead to changes in community composition and in the case of RNAlater, lower gene copies. We therefore advise against these storage methods for metabarcoding analyses. Finally, we show that over one month, DNA extract thaw time does not impact diversity or qPCR metrics. We hope that this work will help

researchers working with soil bacteria and fungi make informed decisions about soil storage and transport to ensure repeatability and accuracy of results and interpretations.

Introduction

In the last few decades, metagenomic tools have dramatically transformed the fields of environmental microbiology and soil ecology (Nesme et al. 2016). The advent of sequencing environmental DNA, the entire (sub) community in a field sample, represents an important advance in these fields. These tools have allowed researchers studying soil microbes directly from soil to analyze an increasing proportion of the previously unculturable microbiome. Although these tools have enabled an unprecedented view into soil microbial diversity, we must use caution when interpreting results. To wield these powerful tools responsibly, we must understand how robust environmental sequencing results are to methods of soil storage and processing. This will allow us to make decisions we know do not alter results, or at the very least, understand how they do so.

Two important sample processing choices that may alter experimental results are (1) the type of soil storage method and (2) DNA extract thaw time, the length of time for which extracted DNA is transported and left to thaw. To date, only a handful of studies have examined consequences of soil storage methods on study results (i.e. temperature, absolute ethanol, freeze-drying, RNAlater, PLFA) on targets such as DNA, RNA, bacteria, fungi, and arbuscular mycorrhizal fungi (Harry et al. 2000, Klammer et al. 2005, Tzeneva et al. 2009, Lauber et al. 2010, Rissanen et al. 2010, Rubin et al. 2013, Brandt et al. 2014, Cui et al. 2014, Weißbecker et al. 2017). These studies have broadly found little impact of storage method, but they do not

thoroughly explore common storage practices used in the field and focus overwhelmingly on bacteria. This leaves researchers with an unclear understanding of how their choice of soil storage will impact study conclusions and interpretations. Most studies find no impact of soil sample storage over short periods of time under 1 month at temperatures from 4 to - 80 ºC (Harry et al. 2000, Klammer et al. 2005, Lauber et al. 2010, Schnecker et al. 2012, Brandt et al. 2014, Tatangelo et al. 2014, Weißbecker et al. 2017). Some studies compare freeze-drying in addition to storage at different temperatures (Klammer et al. 2005, Cui et al. 2014, Tatangelo et al. 2014, Weißbecker et al. 2017). Nonetheless, most of these studies fail to investigate several practical and commonly used storage methods besides different temperatures (Lauber et al. 2010, Rubin et al. 2013, Brandt et al. 2014, Tatangelo et al. 2014). For example, liquid nitrogen (N_2), thought to be the most effective storage method and the relevant 'control' comparison, is not analyzed in any of these papers. RNAlater, another potential method to store soil samples without refrigeration has received only moderate attention, with mixed results (Rissanen et al. 2010, Schnecker et al. 2012). Rissanen et al. (2010) found that storage in RNAlater decreased nucleic acid yields drastically at all temperatures (-80 ºC to 4 ºC), while Schnecker et al. (2012) found no significant difference between RNAlater storage and other study treatments (-20 ºC, 4 ºC, direct extraction from fresh soil), although these authors were looking at total phospholipid fatty acid (PLFA) content and not nucleic acids. In addition, Nilsson et al. (2019) caution against using RNAlater for high-throughput analyses, as it fails for complex substrates, although this is for use with RNA. Finally, an extremely low proportion of these studies investigate storage impacts on fungi (Schnecker et al. 2012, Cui et al. 2014, Weißbecker et al. 2017), with the majority focusing on bacteria (Harry et al. 2000, Tzeneva et al. 2009, Lauber et al. 2010, Rissanen et al. 2010, Rubin et al. 2013, Brandt et al. 2014, Tatangelo et al. 2014). The few that include fungi do not

test the impact on common amplicon and qPCR metabarcoding analyses (Schnecker et al. 2012, Cui et al. 2014). Nonetheless, these kinds of analyses are still the predominant approach in most soil microbial studies.

In addition to soil storage method, DNA extract thaw time may be important in determining study outcomes due to DNA degradation. In our experience, isolated field locations often ship samples of extracted DNA for library preparation to equipped laboratories. There is often much less regulation surrounding the shipment of DNA as compared to live soil, making the option of sending extracted DNA much more practical. For example, the United States Department of Agriculture (USDA) currently has no regulations for extracted DNA. To our knowledge, the impact of DNA extract thaw time on samples (DNA degradation) has not been studied before. Nonetheless, this is an important issue that may have consequences for study results and interpretation. Researcher decisions involving shipment speed or the length of time extracted DNA will travel should be made based on an accurate understanding of the impact of alternatives on work and conclusions.

Here, we assess the impact of four different soil storage methods and extracted thaw time on soil sample DNA extracts for both bacterial and fungal communities. The storage methods range from the bare minimum (room temperature), to the assumed best method (liquid nitrogen), but also includes the most common method in the field (cooler with cold packs) as well as an additional potentially useful method in situations lacking facilities with fridges or freezers (RNAlater). In addition, we aim to get a better understanding of the impact of DNA extract thaw time to ultimately suggest a 'safe time' in which this can occur: a time up until which thawing DNA extract will not degrade and impact results. We assess soil storage method and DNA extract thaw time in terms of commonly used community composition, diversity and gene

abundance metrics for microbial metabarcoding, specifically amplicon sequencing, including both OTU based analyses and qPCR gene copy measurements. We hope that this study will help researchers make more informed decisions about soil storage methods and transportation of extracted DNA, ultimately resulting in reliable and reproducible results.

Materials and Methods

Here, we used amplicon sequencing to assess the consequences of soil storage method and thaw time on DNA extracts. We included viable and common options of sample storage in soil ecology, including room temperature, cold packs (referred to herein as 'cooler'), liquid nitrogen, and RNAlater. We then examined these different soil storage methods to determine the impact this would have on subsequently extracted DNA composition (community composition and diversity metrics) and quantity (qPCR). We further investigated the impacts of DNA extract thaw time, testing the same community and quantity metrics. All amplicon metabarcoding analyses were conducted for bacteria and fungi, as these are two major microbial groups of interest in soil.

Sample Collection

We collected soil from two remnant prairie locations in North America, with one in Kansas (KS), USA, and the other in Saskatchewan (SK), Canada in September 2018. Here, we defined remnant as having experienced minimal anthropogenic disturbance and therefore representing mostly intact native prairie ecosystems. We chose remnant prairie to represent soils with high plant and microbial diversity. The Kansas prairie, Welda, is in Anderson County Prairie Preserve, which is part of the University of Kansas Field Station sites. This site has a mean

annual temperature of 13.14 ºC and a mean annual precipitation of 104.08 cm. The Saskatchewan prairie is part of the Swift Current Research and Development Centre research farm. This site has a mean annual temperature of 9.7 ºC and mean annual precipitation of 36.56 cm.

We collected a total of 12, 2 ml soil samples per site to be stored in cryogenic tubes, for a total of 24 samples. These samples were from a large homogenized soil sample from each site (total of 2 large samples). At each site, this homogenized soil sample was formed by sampling a central core and four cores at 90º (corners of a square) 3 meters apart from each other around the central core. All cores were taken at a depth of 10 cm, which included soil horizons A and B. We stored soil using four methods, room temperature, cooler, liquid nitrogen, and RNAlater (Ambion, Austin, TX), in replicates of three per location. For RNAlater samples, we added 6 ml of RNAlater to each tube. For the liquid nitrogen storage method, we used a vapor shipper (CBS transport SC4/2V series; Horsham, PA). After 24 hours in each respective soil storage method, we stored all samples in their permanent storage method, or post-transport storage method in the laboratory, that most closely aligned with protocols used in practice (Table 1).

Library Preparation

We extracted DNA from 0.25 g of soil in triplicate for each sample using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) ten days after sample collection. DNA was extracted in batches of 12 samples using an automated system (QIAcube, Qiagen) for all samples in Swift Current, Saskatchewan, and in one batch of 36 samples using the standard (manual) protocol for all Kansas samples. Kansas sample DNA extracts were sent to Saskatchewan for all downstream amplicon and qPCR preparation and analysis in a liquid nitrogen (CBS transport SC4/2V series;

Horsham, PA). Extracted DNA was quantified using a Qubit dsDNA BR Assay Kit (Thermo Fisher, Waltham, MA) and NanoDrop 1000 spectrophotometer (ThermoFisher, Waltham, MA). We conducted bacterial and fungal 1) amplicon sequencing and 2) qPCR measurements on all storage methods. For amplicon sequencing, DNA extracts were shipped on dry ice to the Genome Quebec Innovation Center (Montreal, Canada) for amplicon library preparation and Illumina MiSeq sequencing (see Supporting Information for detailed methods) of the bacterial 16S rRNA gene using primers 515-F and 806-R (Caporaso et al. 2011) and fungal ITS1 region using primers ITS1F and 58A2R (Martin and Rygiewicz 2005). The raw amplicon sequencing dataset is available in the NCBI Sequence Read Archive under BioProject ID: PRJNA575860. Full descriptions of amplicon library preparation and Illumina Miseq sequencing are available in Supplementary Methods 1 and 2.

For the qPCR assays, we used the same primers as used in amplicon sequencing to quantify the abundance of 16S (515-F and 806-R) and ITS1 (ITS1F and 58A2R) copies in the sample DNA extracts. The 25 µl reactions consisted of 5 µl of standardized DNA (5 ng/µl), 2 X Rotor-gene SYBR Green PCR master mix (Qiagen), 0.2 µM of each primer, 0.5 µM BSA (Invitrogen), and nuclease free water. Reactions were run on a Rotor-Gene Q real-time PCR cycler (Qiagen) using the Rotor-Disc 100 (Qiagen) format. The qPCR amplification conditions for bacteria (16S) consisted of an initial denaturing for 3 min at 95 °C, then 40 cycles of 45 s denaturing at 95 °C and 1 min annealing at 60 °C, and extension at 72 °C, followed by a melt curve analysis. The qPCR amplification conditions for fungi (ITS1) consisted of an initial denaturing for 3 min at 95 °C, then 40 cycles of 15 s denaturing at 95 °C and 30 s annealing/extension at 59°C, followed by a melt curve analysis. A standard curve prepared in triplicate, ranging in concentration from 10^1 to 10^7 gene copies μL^{-1} was used to quantify gene

copies in each sample DNA extract. Sample DNA extracts were amplified in duplicate and each run included controls lacking template.

To investigate the impact of thaw time on extracted DNA, we used only cooler samples due to the high sample number this test involves. We chose this method because cooler transport is the most common and cost-effective method used in soil ecology, especially at remote field sites. For the DNA extract thaw time test, we took cooler sample DNA extracts stored at -20 ºC for ten days and conducted sequencing and qPCR analyses at five different time intervals of thawing at room temperature (21 ºC) over 2 months (60 days) at 0, 3, 15, 30, and 60 days. All DNA yield (Qubit and Nanodrop) and qPCR values are reported in SI Table SI1. From this table, we conclude that all sample DNA extracts are within a reasonable range of 260/280, with optimal ratio at 1.8 indicating pure DNA; the 260/230 values are relatively low compared to the optimal value of 2.0, but should be interpreted as a secondary measure of purity, with these low values likely an indicator of some contaminants in the 230 nm range.

Bioinformatics

Raw paired reads were processed using the UPARSE pipeline and USEARCH v.9 (Edgar 2013). Paired reads were merged using the fastq_mergepairs command with a maximum of five (i.e., default) mismatches in the alignment. Merged reads were quality filtered using the command fastq_filter that discarded all reads that were less than 200 bp and those with expected errors > 1. Sequences were dereplicated and the command cluster_otus was used to perform operational taxonomic unit (OTU) clustering (based on 97% similarity) and chimera filtering. Taxonomic identity was assigned using the RDP classifier (Wang et al. 2007) and 16S rRNA training set (version 16) for bacteria/archaea and ITS UNITE database for fungi (Kõljalg et al. 2013). Before

all analyses, we filtered out all unmatching domains (including only archaea and bacteria in bacteria analyses; only fungi in fungal analyses). OTU tables for each analysis were filtered to include OTUs with a minimum of 5 sequences. Finally, OTU tables for each analysis were normalized to the lowest number of sequences in a sample within the subset of data being analyzed using rrarefy from the R package vegan (Oksanen et al. 2013). Rarefaction curves are provided for each set of analyses in Figure SI1 and indicate sufficient sequencing.

Statistical Analyses

We conducted analyses to understand the impact of (1) soil storage method and (2) DNA extract thaw time on the composition (sequencing) and quantity (qPCR) of bacteria and fungi from our soil samples. We first analyzed soil storage method and DNA extract thaw time impact on bacterial and fungal community composition with permutational analysis of variance (PERMANOVA) tests. As part of this set of analyses, we also analyzed soil storage method and DNA extract thaw time on bacterial and fungal diversity metrics (OTU number, Simpson diversity, and Evenness) through general linearized mixed effects models (GLMMs). We also reran these models for each bacterial and fungal phylum for which we had enough available data (at least 40 observations). Finally, we analyzed soil storage method and DNA extract thaw time impact on bacterial and fungal gene copy quantity (qPCR) using GLMMs.

In our PERMANOVAs, we used either storage or time to predict the community composition of bacteria or fungi, as well as location and either storage or time's interaction with location. In the storage tests, we included the strata argument to account for non-independency of sampling as replicate of storage. The strata argument (Oksanen et al. 2013) constrains permutations to a group and is the only option to account for this non-independency of sampling

in this package. In the DNA extract thaw time tests, we included only replication in the strata argument. We ran PERMANOVAs on all the data, and then within location (Kansas or Saskatchewan). When storage significantly impacted community composition in either location, we ran pairwise comparisons comparing liquid nitrogen storage to each other storage to obtain a better understanding of which storage methods alter community compositions. All PERMANOVAs were implemented in *adonis2* in vegan (Oksanen et al. 2013) using the morisita dissimilarity matrix, as it is robust to differences in sample size (Morisita 1959). In our GLMMs to analyze diversity metrics, we used either storage or time to predict each diversity metric. Our metrics, used as response variables, included OTU number, Simpson diversity, or community Evenness. Simpson diversity was calculated using the diversity function within vegan; Evenness was calculated manually following the vignette for vegan by dividing Shannon diversity by the log of species number (Oksanen 2013). Each GLMM model included the interaction of location and storage or location and time, with the random effect of storage replicate nested within storage type nested with location. We repeated PERMANOVAs and GLMMs to analyze diversity metrics for each bacterial and fungal phylum for which we had enough available data. We were able to run these analyses for the bacterial phyla of Actinobacteria, Planctomycetes and Proteobacteria and for the fungal phyla of Ascomycetes, Basidiomycetes and Zygomycetes.

In our GLMMs to assess qPCR results, we used 16S copies per gram of dry soil for bacteria and ITS1 copies per gram of dry soil for fungi as the response variables. Each model included the interaction of location and storage or location and time, with the random effect of storage replicate nested within storage type nested with location. All GLMM models were run using the *lme4* package (Bates et al. 2015) and all statistical analyses were carried out in R

version 3.4.1 (Team 2019). We report only significant results in the Results section, but all results can be found in Supplementary Tables (Table SI1-5)

After processing our data, we noticed that the qPCR results showed a strong trend in the sample DNA extracts from Kansas. Gene copies resulting from qPCR were higher in samples that were extracted later in time. This is because in Kansas, the DNA extractions for 36 samples were conducted in one batch DNA extraction. To test this effect statistically, we reran each GLMM model (OTU richness, Simpson diversity, community Evenness, or qPCR gene copy quantity) as well as PERMANOVA model for the entire bacterial and fungal datasets using order as a covariate instead of time or storage; the random effect structures and use of the strata argument remained the same as in other analyses.

Results

Storage

For bacterial community composition, we found an impact of storage method in both locations (Table 2A, KS p = 0.04, SK p = 2.00E-04; Figure 1A and B), as well as a significant interaction between storage and location (Table 2A, p = 4.00E-03). When comparing each storage method to liquid nitrogen, the assumed best method, we found a significant impact of storage in room temperature (Table 2A, p = 4.00E-3) and in RNAlater (Table 2A, p = 2.00E-4) in Saskatchewan samples; we found no impact of storage between liquid nitrogen and each other method in Kansas samples (Table 2A). Nonetheless, we found that in both Kansas and Saskatchewan, storage between cooler and liquid nitrogen does not impact community composition (Table 2A, KS p = 0.19, SK = 0.14). We found that fungal community composition was not affected by

storage method in Saskatchewan samples, but was in KS samples (Table 2, All Samples p = 0.46, KS p = 1.40E-03, SK p = 0.30; Figure 1C and D). Within Kansas samples, we found a significant impact of storage in room temperature (p = 0.04) and in RNAlater (Table 2A, p = 0.03) as compared to liquid nitrogen; again, we did not find a significant impact of storage on community composition when comparing cooler and liquid nitrogen storage (Table 2A, p = 0.44). When subsetting the data to specific bacterial and fungal phyla, community compositions across all samples were not impacted by storage, although we did find that within each location, storage is significant in certain phyla, particularly in bacterial phyla (Table SI2A). When analyzing each phylum at each location with a significant storage effect comparing liquid nitrogen to each other storage method, we found that cooler is no different than liquid nitrogen, with the exception of Saskatchewan Planctomycetes (p = 0.01) and Basiodiomycota (p = 4.00E-3).

We found that diversity metrics (OTU richness, Simpson diversity and Evenness; Figure 2) were sensitive to storage, but responses varied mostly by location; diversity metrics were not impacted differentially by cooler compared to liquid nitrogen storage. For bacteria, Simpson diversity and Evenness were greater in Saskatchewan than Kansas (Table SI3A; Simpson diversity p =2.42E-09; Evenness p = 1.52E-09) and greater in RNAlater than liquid nitrogen storage (Table SI3a; Simpson diversity p = 2.52E-02; Evenness p = 4.37E-03; Figure 2C, 2E). We also saw an interaction between location and RNAlater, with RNAlater storage resulting in lower Evenness (Table SI3A; p = 3.84E-02) in Kansas, but not Saskatchewan samples (Figure 2C, 2E). For fungi, we only found significant results when looking at OTU number, with OTU number being lower in Saskatchewan in RNAlater (Table SI3A; p = 1.88E-02) and room

temperature (Table SI3A; p = 1.60E-02) as compared to Kansas (Figure 2B). All diversity metrics are depicted in Figure 2 (A-E) and Table SI3A.

For diversity results into specific phyla, we again found no evidence of differential effects of cooler storage versus liquid nitrogen. We found that bacteria are most impacted by other storage methods, while fungi are almost not (Table SI4A). RNAlater resulted in both lower (Actinobacteria OTU richness, p = 2.85E-04; Planctomycetes OTU richness, p = 1.19E-02) and higher (Proteobacteria Simpson diversity, p = 5.38E-06; Proteobacteria Evenness, p = 4.50E-05) diversity metrics as compared to cooler storage for bacteria. For fungi, RNAlater was only a significant predictor of Evenness within the Ascomycota (p = 2.22E-02). Moreover, there was an RNAlater by location interaction in both bacteria and fungi, with RNAlater reducing most diversity metrics (Proteobacteria Simpson diversity, p = 4.98E-04; Ascomycota OTU richness, p = 3.25E-02; Basidiomycota OTU number, p = 7.18E-03) compared to cooler storage in Saskatchewan, with the exception of Actinobacteria, which increased diversity metrics (OTU richness, p = 3.09E-02). Room temperature significantly predicted an increase in the Simpson diversity of Proteobacteria (p = 3.37E-04). In addition, there was a room temperature by location interaction in two phyla of fungi, with both Ascomycota (OTU richness, p = 1.55E-02) and Basidiomycota (OTU richness, p = 4.40E-02) predicting a decrease in diversity metrics. Finally, we found that location was important in determining these results, much like in the broader analysis (see Table SI4a for detailed results).

Storage in cold packs versus liquid nitrogen did not impact qPCR (quantity) results. However, storage in RNAlater reduced qPCR estimates of gene copy number for both fungi and bacteria in Saskatchewan (Table 3; bacterial p = 1.62E-08, fungal p = 2.66E-04; Figure 3A and 3B).

DNA Extract Thaw Time

Overall, entire community composition was only impacted by DNA extract thaw time in Saskatchewan bacteria (Table 2B, p = 0.05). Within phyla, community composition was impacted by time in two bacterial phyla. Specifically, the community composition of Actinobacteria (All samples p = 3.00E-03) and Proteobacteria (All samples p = 2.00E-04) were significantly impacted by time (Table SI2B). In contrast, diversity metrics including OTU number, Simpson diversity, and Evenness were not impacted by time overall or within phyla.

However, time impacted both bacterial and fungal gene copies (quantity) in Saskatchewan only. In both bacteria (p = 1.00E-02) and fungi (p = 3.00E-03) in Saskatchewan, quantity decreased with time (Table 3B). To determine at what time point this decrease in quantity occurs, we reran the analyses progressively removing the oldest time point. After removing the oldest time point (60 days) we determined that this impact of time in Saskatchewan disappears (Figure 4, bacterial p = 1.12E-01, fungal p = 1.34E-1), suggesting that qPCR results are unimpacted by DNA extract thawing over one month, or 30 days.

Order of DNA extraction batch

We found that community composition of fungi (Table SI5A, p = 1.20E-03) was impacted by DNA extraction order in a batch for Kansas samples (36 samples in one batch). In contrast, none of our diversity metrics were significantly impacted by this order for fungi or bacteria (Table SI5b). When looking at qPCR results, we found a positive correlation between qPCR gene copy quantity and order for bacteria (Table SI5C, p = 5.87E-08) and fungi (Table SI5C, p = 2.99E-05)

for Kansas samples, with samples prepared later in the batch having a higher number of gene copies.

Discussion

Here, we find broad support for the common use of cold packs in coolers for soil storage prior to DNA extraction, as there was little difference with immediate immersion in liquid nitrogen for downstream amplicon and qPCR analyses for fungi and bacteria. We further find that DNA extract thaw time can alter community composition and qPCR results, but found that 30 days of thaw time does not alter qPCR results or diversity metrics (bacteria, fungi and phyla within). Overall, the finding of little difference between liquid nitrogen and the conventional cooler method is positive for soil ecology, as most studies transport soils in a cooler and do not have the capacity to store them in liquid nitrogen. Further, our finding that 30 days of DNA extract thaw time does not impact qPCR results is helpful for many studies that ship extracted DNA and may not be able to do so with temperature control.

Liquid nitrogen soil storage for transport to the lab is the standard method to preserve nucleic acids (Weißbecker et al. 2017, Nilsson et al. 2019). Therefore, we expected this method to yield the best results. Nonetheless, we show that in terms of the most commonly used analyses conducted with molecular data in the field of microbial ecology, namely community composition, diversity, and qPCR analyses, liquid nitrogen is little different than cooler storage. Storage does not impact overall community composition, in agreement with studies using both amplicon sequencing (Lauber et al. 2010, Brandt et al. 2014, Weißbecker et al. 2017) and community fingerprinting methods (Klammer et al. 2005, Tatangelo et al. 2014), but we find that

storage may matter in finer grain analyses of phyla, in agreement with Rubin (2013) and Risannen (2010). This is in contrast to some community fingerprinting studies, that find storage does impact community composition (Tzeneva et al. 2009, Cui et al. 2014). Storage may impact diversity metrics, but understanding which storage method is better may be location dependent. Temperature, precipitation and pH have been shown to impact microbial structure in both bacteria and fungi (Lauber et al. 2008, Fierer et al. 2009, Rincón et al. 2015, Newsham et al. 2016, Zhou et al. 2016) and may alter storage effectiveness. Therefore, future work could incorporate soil chemical properties and environmental data to help understand location or soil physicochemical differences. Previous studies have also found storage method to impact diversity metrics in bacteria (Rubin et al. 2013, Weißbecker et al. 2017) and fungi (Cui et al. 2014). Finally, we see no difference of impact of storage on qPCR results between liquid nitrogen and cooler storage in our study which aligns with previous work (Brandt et al. 2014). This lack of a result is again positive for field ecology because there are no differences in broad fungal or bacterial results between liquid nitrogen and cooler storage; the traditional and much more cost-effective method of cooler storage is likely sufficient for molecular studies for both bacteria and fungi.

Here, we find evidence that soil storage in RNAlater leads to sample degradation. When comparing samples stored in RNAlater to those stored in liquid nitrogen, we find several instances of community composition shifts. In addition, we find several differences with RNAlater in terms of diversity metrics, with conflicting directions (increasing or decreasing). We also show that RNAlater may lead to lower gene copy quantity from qPCR results, as seen here in the Saskatchewan results. This is indicative of degrading material and is not desirable. This is consistent with results from Rissanen et al. (2010) showing reduced yields with RNAlater and

ethanol preservation. Tatangelo et al. (2014) also find reduced number of terminal restriction fragments in soil stored using LifeGuard (Qiagen, Hilden, Germany), another solution-based preservation method. Combining these inconsistent and negative results, we suggest future studies focused on DNA avoid using RNAlater.

Finally, to answer our second question looking at DNA extract thaw time, we can conclude that keeping extracted DNA at room temperature (e.g. for shipping purposes) is likely acceptable for up to one month. Nonetheless, leaving extracted DNA at room temperature can impact community composition and gene copy quantity. The impact on gene copy quantity only occurred in the Saskatchewan samples, possibly due to a difference in microbial community composition between sites. Time is not important in the Saskatchewan samples in predicting gene copy quantity when we remove the last time-point (60 days), which suggests that keeping DNA at room temperature for up to 30 days has no impact on gene copy quantity results. We also find that commonly used diversity metrics are unaffected by DNA extract thaw time. Overall, we find that extracted DNA is not impacted by a month of room temperature storage in terms of gene copy quantity (qPCR) or diversity metrics; this result is positive for those scientists who work in remote locations and may worry about their sample DNA extracts thawing and degrading during travel.

Our incidental finding related to DNA extraction order in a batch may be useful to researchers extracting DNA broadly. We find that when working with large numbers of samples (here, 36), extraction in one batch is not advisable for consistency across samples. We find a strong relationship between order number in the batch and community composition as well as with gene copies. For gene copies, we find that later samples - those left in solutions longer - are those with greater gene copies, or yield. This suggests that at least for qPCR studies, a longer

incubation time in solutions using the PowerSoil Pro kit may be optimal. However, we did not explicitly test this, and our results are from one site only (Kansas), so we cannot report an optimal time past recommended time for which to leave samples in kit solution.

Understanding sample processing is important for soil microbial molecular studies. Several steps may impact results and must be evaluated to make informed decisions. Here, we investigated impacts of soil sample storage method as well as DNA extract thaw time (DNA degradation). We urge further research into each step of soil sampling and processing to get a more complete understanding of how these decisions impact study results and interpretations. For example, several papers look at the impact of different bioinformatical pipelines on results (Bokulich et al. 2013, Cline et al. 2017, Egan et al. 2018). We are hopeful that this work will help scientists evaluate the costs and benefits of experimental approaches when studying soil bacteria and fungi. This work shows that in terms of community composition, diversity metrics and gene copy quantity, liquid nitrogen shows no clear difference from traditional cooler storage. In addition, researchers working abroad or in remote areas can be confident that for at least one month, thawing extracted DNA will not impact qPCR or commonly used diversity metric results.

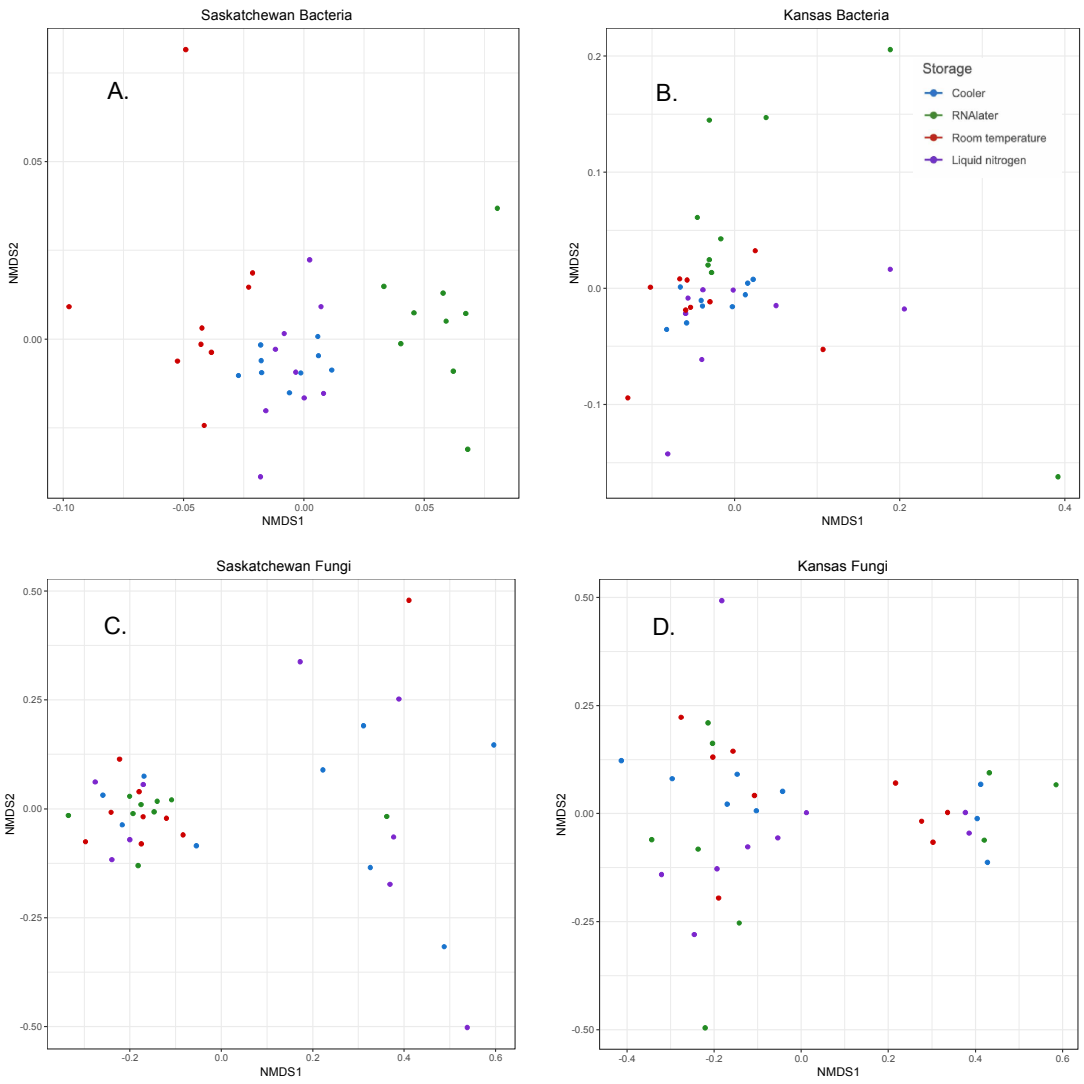


Figure 1. NMDS plots show composition differentiation based on storage method.

NMDS of bacterial (A, B) and fungal (C, D) communities coded by storage (color) for each location. These plots show that there is some differentiation of communities based on storage, particularly in Saskatchewan bacteria.

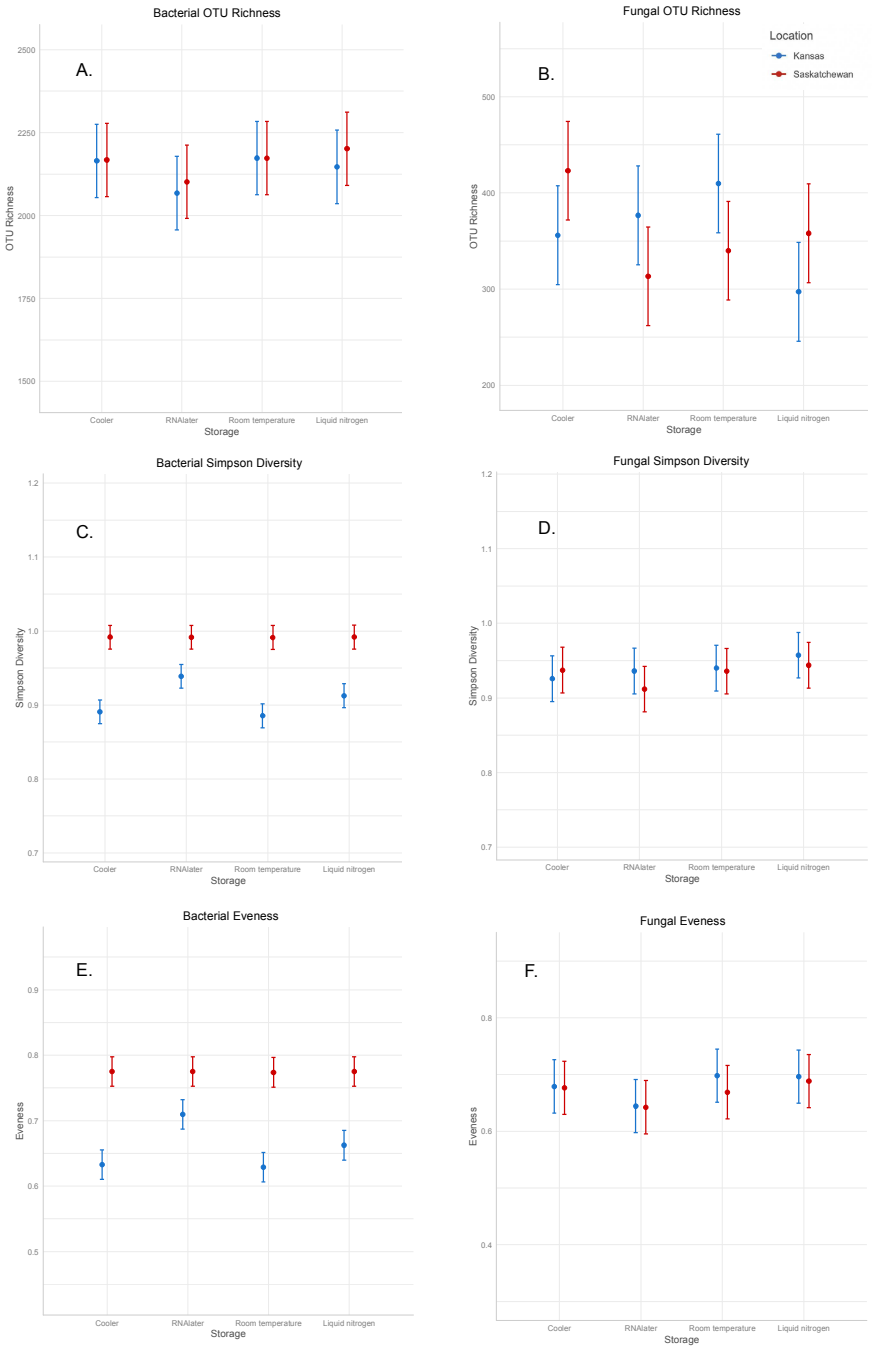


Figure 2. Diversity metrics of bacteria and fungi across storage methods.

Diversity metrics of bacteria and fungi predicted by storage, with 95% confidence intervals: (A) bacterial OTU richness, (C) bacterial Simpson diversity, (E) bacterial evenness, (B) fungal OTU richness, (D) fungal Simpson diversity and (F) fungal evenness. For bacteria, Simpson diversity

(C) and Evenness (E) were greater in Saskatchewan than Kansas and greater for RNAlater than cooler. For fungi, OTU richness (B) is lower in Saskatchewan in RNAlater than room temperature as compared to Kansas.

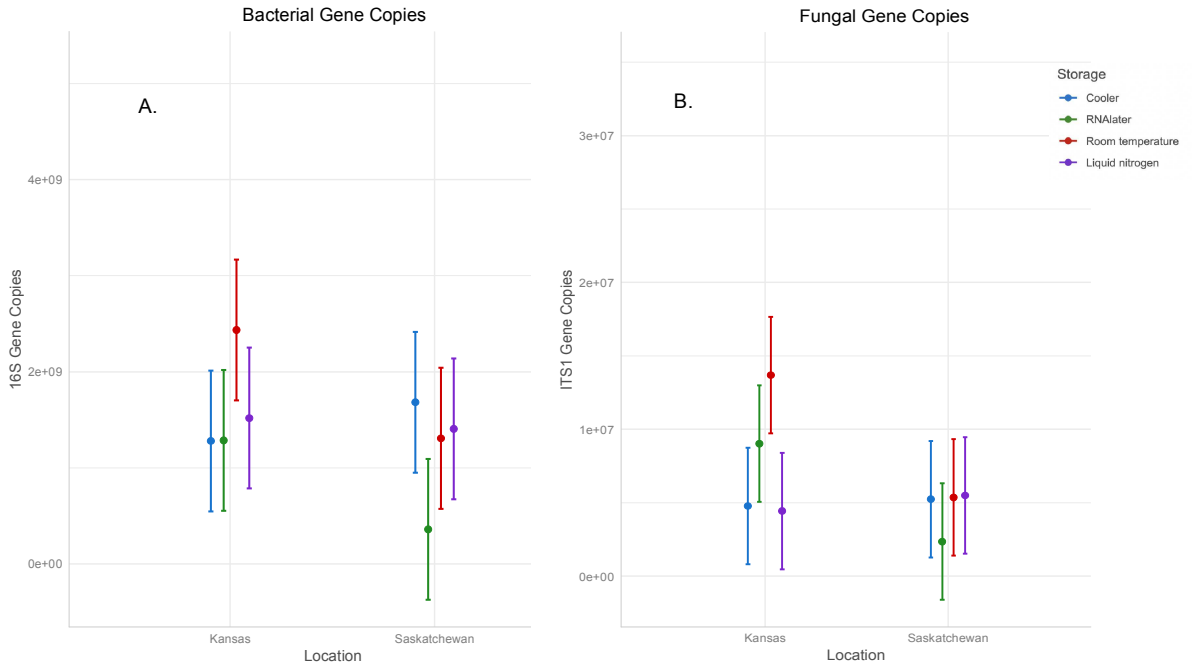


Figure 3. Gene copy number for bacteria and fungi across two locations and storage method.

Gene copy number for 16S (bacteria; A) and ITS (fungi; B) based on storage and location, with 95% confidence intervals. Lower gene copies of both bacteria (A) and fungi (B) were found in Saskatchewan compared to the cooler storage method.

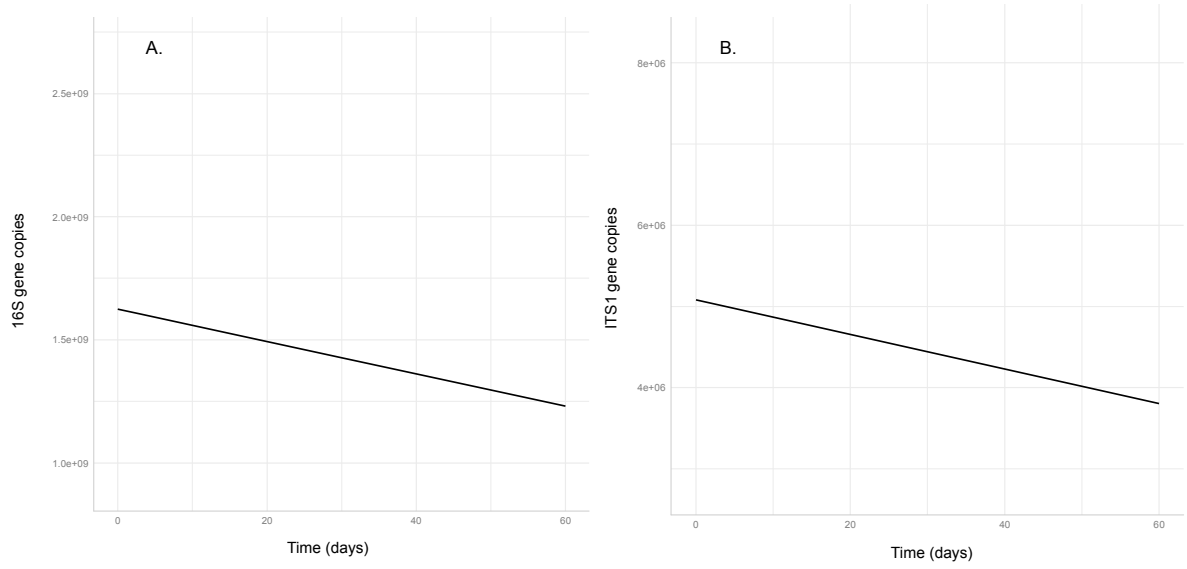


Figure 4. Gene copy number decreases with increased thaw time.

Gene copy quantity, as measured by qPCR, decreases with longer thaw time in both bacterial (a) and fungal (b) samples in Saskatchewan (bacteria p = 0.01; fungi, p = 3e-3). This is no longer true when removing the last time point of 60 days (bacteria p = 0.11; fungi, p = 0.13).

Table 1. Sampling and storage for each storage method.

Sampling was repeated at Anderson County Prairie Preserve in Kansas, USA and at Conway pasture in Swift Current, Saskatchewan, Canada.

Storage Method	**Permanent Storage Method**
liquid nitrogen	-80
liquid nitrogen	-80
liquid nitrogen	-80
cooler	-20
cooler	-20
cooler	-20
RNAlater	room temp
RNAlater	room temp

RNAlater	room temp
room temp	room temp
room temp	room temp
room temp	room temp

Table 2. PERMANOVA results across storage method and sites.

PERMANOVA results for fungal and bacterial community composition across a. storage methods, with analyses either including room temperature, cooler, liquid nitrogen, and RNAlater or each method compared to liquid nitrogen within location where storage across all storage methods was significant and b. degradation over time using cooler storage. Each analysis is run for all samples and then for Kansas (KS) and Saskatchewan (SK) samples. Results significant at a level of $p < 0.05$ are in bold.

a. Storage Methods (all time 1)			
	Parameter	R^2	*p* value
Bacteria all storage methods			
All Samples	storage:Loc	0.00559	**0.03**
KS	storage	0.23452	**0.04**
SK	storage	0.75249	**2.00E-4**
Bacteria liquid nitrogen versus cooler			
KS	storage	0.14257	0.19
SK	storage	0.12182	0.14
Bacteria liquid nitrogen versus room temperature			
KS	storage	0.16891	0.14
SK	storage	0.38047	**4.00E-03**
Bacteria liquid nitrogen versus RNA later			
KS	storage	0.0164	0.54
SK	storage	0.74225	**2.00E-04**
Fungi all storage methods			
All Samples	storage:Loc	0.03635	0.46
KS	storage	0.15843	**1.4E-03**
SK	storage	0.10428	0.30
Bacteria liquid nitrogen versus cooler			

KS		0.05861	0.44
Bacteria liquid nitrogen versus room temperature			
KS		0.09516	**0.04**
acteria liquid nitrogen versus RNA later			
KS		0.12074	**0.03**
b. Over Time (all cooler)			
Bacteria			
All Samples	time:Loc	-0.00001	1
KS	time	-0.00108	0.65
SK	time	0.06844	**0.05**
Fungi			
All Samples	time:Loc	0.01330	0.29
KS	time	0.00532	1
SK	time	0.00487	0.86

Table 3. qPCR GLM results across storage method and sites.

GLM results for fungal and bacterial gene copies resulting from qPCR measurements across a. storage methods, including room temperature, cooler, liquid nitrogen, and RNAlater (with liquid nitrogen as the storage reference) and b. degradation over time using cooler storage. Each analysis is run for all samples and then for Kansas (KS) and Saskatchewan (SK) samples. Results significant at a level of $p < 0.05$ are in bold.

a. Storage Methods (all time 1)				
Parameter	Estimate (coefficient)	Std. Error	t-value	*p* value
Bacteria (16S)				
All Samples				
Intercept	1.52E+09	3.67E+08	4.133	**3.58E-05**
Location (SK)	-1.12E+08	5.19E+08	-0.217	8.29E-01
cooler	-2.37E+08	5.19E+08	-0.457	6.48E-01
RNAlater	-2.31E+08	5.19E+08	-0.445	6.56E-01

room temperature	9.18E+08	5.19E+08	1.769	7.69E-02
Loc*cooler	5.16E+08	7.34E+08	0.703	4.82E-01
Loc*RNAlater	-8.15E+08	7.34E+08	-1.11	2.67E-01
Loc*room temperature	-1.02E+09	7.34E+08	-1.384	1.66E-01
KS				
Intercept	1.52E+09	5.02E+08	3.02	**2.53E-03**
cooler	-2.37E+08	7.10E+08	-0.334	7.38E-01
RNAlater	-2.31E+08	7.10E+08	-0.325	7.45E-01
room temperature	9.18E+08	7.10E+08	1.293	1.96E-01
SK				
Intercept	1.41E+09	1.31E+08	10.724	**< 2.00E-16**
cooler	2.79E+08	1.85E+08	1.504	1.33E-01
RNAlater	-1.05E+09	1.85E+08	-5.648	**1.62E-08**
room temperature	-9.80E+07	1.85E+08	-0.529	5.97E-01
Fungi (ITS)				
All Samples				
Intercept	4.44E+06	1.99E+06	2.234	**2.55E-02**
Location (SK)	1.07E+06	2.81E+06	0.38	7.04E-01
cooler	3.42E+05	2.81E+06	0.122	9.03E-01
RNAlater	4.59E+06	2.81E+06	1.635	1.02E-01
room temperature	9.25E+06	2.81E+06	3.295	**9.85E-04**
Loc*cooler	-6.02E+05	3.97E+06	-0.152	8.80E-01
Loc*RNAlater	-7.74E+06	3.97E+06	-1.95	5.12E-02
Loc*room temperature	-9.39E+06	3.97E+06	-2.365	**1.80E-02**
KS				
Intercept	4.44E+06	2.79E+06	1.589	1.12E-01
cooler	3.42E+05	3.95E+06	0.087	9.31E-01
RNAlater	4.59E+06	3.95E+06	1.163	2.45E-01

room temperature	9.25E+06	3.95E+06	2.344	**1.91E-02**
SK				
Intercept	5502222	361006	15.241	**3.40E-07**
cooler	-260000	510539	-0.509	6.24E-01
RNAlater	-3153333	510539	-6.176	**2.66E-04**
room temperature	-141111	510539	-0.276	7.89E-01
b. Over Time (all cooler)				
Bacteria (16S)				
All Samples				
Intercept	1.20E+09	1.51E+08	7.926	**2.00E-15**
Location (SK)	4.27E+08	2.14E+08	2.001	**5.00E-02**
time	-4.27E+06	4.91E+06	-0.869	3.80E-01
Loc*time	-2.28E+06	6.94E+06	-0.328	7.40E-01
KS				
Intercept	1.20E+09	1.98E+08	6.045	**1.00E-09**
time	-4.27E+06	6.43E+06	-0.663	5.10E-01
SK				
Intercept	1.62E+09	8.01E+07	20.286	**< 2.00E-16**
time	-6.54E+06	2.60E+06	-2.515	**1.00E-02**
Fungi (ITS)				
All Samples				
Intercept	4.72E+06	7.50E+05	6.291	**4.00E-05**
Location (SK)	3.65E+05	1.06E+06	0.344	7.00E-01
time	2.06E+04	2.25E+04	-0.917	3.60E-01
Loc*time	7.10E+02	3.17E+04	-0.022	9.80E-01
KS				
Intercept	4.72E+06	1.04E+06	4.532	**4.00E-03**
time	-2.06E+04	3.09E+04	-0.666	5.10E-01
SK				
Intercept	5.08E+06	2.19E+05	23.171	**< 2.00E-16**
time	-2.13E+04	7.13E+03	-2.986	**3.00E-03**

Supplementary Information

Table SI1. DNA yield and qPCR results for each study sample.

DNA yield (Quibit; nanodrop) and qPCR results for each sample, alongside sample structure.

ID	storage	Loc	DNA_conc	Nanodrop	Bact_qPCR	Fun_qPCR
K1	liquid nitrogen	KS	58.0	127.0	1.43E+08	1.25E+05
K2	liquid nitrogen	KS	45.3	122.3	1.41E+08	8.86E+04
K3	liquid nitrogen	KS	44.3	119.7	1.60E+08	1.74E+05
K4	cooler	KS	105.0	171.7	3.47E+08	5.54E+05
K5	cooler	KS	86.3	151.3	2.89E+08	1.09E+06
K6	cooler	KS	53.0	151.3	3.73E+08	7.71E+05
K7	RNAlater	KS	28.8	54.7	3.41E+06	3.42E+04
K8	RNAlater	KS	20.9	54.3	6.50E+06	9.50E+04
K9	RNAlater	KS	93.0	157.0	2.21E+07	1.76E+05
K10	room temperature	KS	142.0	134.7	1.57E+09	5.89E+06
K11	room temperature	KS	161.7	140.3	1.37E+09	1.11E+07
K12	room temperature	KS	181.3	139.0	3.03E+09	1.69E+07
K13	liquid nitrogen	KS	153.0	140.3	1.05E+09	3.63E+06
K14	liquid nitrogen	KS	154.0	132.7	7.03E+08	2.99E+06
K15	liquid nitrogen	KS	101.7	143.0	5.08E+08	1.19E+06
K16	cooler	KS	94.7	131.7	4.40E+08	1.12E+06
K17	cooler	KS	123.3	121.3	1.16E+09	4.60E+06
K18	cooler	KS	124.0	114.0	1.23E+09	5.29E+06
K19	RNAlater	KS	192.7	235.0	1.60E+09	1.11E+07
K20	RNAlater	KS	234.7	286.3	1.26E+09	1.00E+07
K21	RNAlater	KS	161.3	220.7	1.22E+08	6.21E+05
K22	room temperature	KS	92.7	144.3	3.10E+08	1.06E+06
K23	room temperature	KS	248.7	311.0	1.21E+09	3.63E+06
K24	room temperature	KS	460.0	388.0	4.92E+09	3.19E+07
K25	liquid nitrogen	KS	453.3	394.3	3.94E+09	1.70E+07
K26	liquid nitrogen	KS	363.3	327.0	3.01E+09	5.27E+06
K27	liquid nitrogen	KS	423.3	376.7	4.00E+09	9.45E+06
K28	cooler	KS	423.3	349.7	1.97E+09	4.37E+06
K29	cooler	KS	373.3	324.3	2.74E+09	1.11E+07

K30	cooler	KS	356.7	311.3	2.97E+09	1.41E+07
K31	RNAlater	KS	206.3	258.7	5.26E+09	3.52E+07
K32	RNAlater	KS	209.3	266.7	1.63E+09	1.33E+07
K33	RNAlater	KS	215.0	259.7	1.67E+09	1.07E+07
K34	room temperature	KS	486.7	429.0	4.06E+09	2.25E+07
K35	room temperature	KS	430.0	383.3	3.82E+09	1.90E+07
K36	room temperature	KS	201.7	179.7	1.63E+09	1.12E+07
S1	liquid nitrogen	SK	282.3	276.0	1.70E+09	6.59E+06
S2	liquid nitrogen	SK	272.7	268.3	1.23E+09	6.19E+06
S3	liquid nitrogen	SK	294.3	250.0	2.13E+09	5.95E+06
S4	cooler	SK	323.3	290.7	1.92E+09	6.95E+06
S5	cooler	SK	253.7	241.0	1.70E+09	4.67E+06
S6	cooler	SK	294.7	265.0	2.46E+09	6.45E+06
S7	RNAlater	SK	112.7	115.3	3.52E+08	1.88E+06
S8	RNAlater	SK	111.0	99.3	3.59E+08	2.70E+06
S9	RNAlater	SK	125.3	104.7	4.30E+08	3.66E+06
S10	room temperature	SK	316.7	270.3	1.15E+09	4.38E+06
S11	room temperature	SK	276.3	243.3	1.08E+09	4.94E+06
S12	room temperature	SK	260.0	231.0	1.45E+09	6.82E+06
S13	liquid nitrogen	SK	265.7	278.7	2.16E+09	6.89E+06
S14	liquid nitrogen	SK	265.0	292.3	1.48E+09	5.01E+06
S15	liquid nitrogen	SK	252.3	233.3	1.01E+09	3.91E+06
S16	cooler	SK	267.7	265.7	1.31E+09	5.41E+06
S17	cooler	SK	256.3	248.0	1.52E+09	4.46E+06
S18	cooler	SK	273.3	243.3	1.86E+09	5.89E+06
S19	RNAlater	SK	104.7	109.7	2.14E+08	1.35E+06
S20	RNAlater	SK	129.7	114.7	4.02E+08	2.72E+06
S21	RNAlater	SK	112.7	94.3	2.48E+08	1.96E+06
S22	room temperature	SK	278.7	247.3	1.19E+09	5.58E+06
S23	room temperature	SK	315.7	278.0	7.21E+08	4.89E+06
S24	room temperature	SK	281.0	252.0	1.09E+09	6.34E+06
S25	liquid nitrogen	SK	281.7	277.7	9.40E+08	5.39E+06
S26	liquid nitrogen	SK	294.0	295.3	1.01E+09	5.51E+06
S27	liquid nitrogen	SK	247.7	221.7	9.83E+08	4.08E+06
S28	cooler	SK	266.0	263.7	1.37E+09	4.26E+06

S29	cooler	SK	253.7	237.0	1.59E+09	5.44E+06
S30	cooler	SK	296.0	234.3	1.42E+09	3.65E+06
S31	RNAlater	SK	112.0	113.3	3.60E+08	2.20E+06
S32	RNAlater	SK	121.7	114.7	3.77E+08	2.06E+06
S33	RNAlater	SK	139.3	124.3	4.84E+08	2.61E+06
S34	room temperature	SK	279.3	248.3	1.33E+09	5.29E+06
S35	room temperature	SK	262.7	262.0	1.25E+09	4.61E+06
S36	room temperature	SK	336.7	293.3	2.50E+09	5.40E+06

Table SI2. Phylum-level PERMANOVA results across storage method and sites.

PERMANOVA results for fungal and bacterial community composition for each phylum across a. storage methods, including room temperature, cooler, liquid nitrogen, and RNAlater, and b. degradation over time using cooler storage. Each analysis is run for all samples and then for Kansas (KS) and Saskatchewan (SK) samples. Results significant at a level of $p < 0.05$ are in bold.

a. Storage Methods (all time 1)			
	Parameter	R^2	*p* value
Bacteria (Actinobacteria)			
All Samples	storage:Loc	-0.0001	1
KS	storage	0.26775	**9.80E-06**
SK	storage	0.21848	**0.05**
Bacteria (Planctomycetes)			
All Samples	storage:Loc	-0.00095	1
KS	storage	0.05587	0.71
SK	storage	0.33733	**2.00E-04**
Bacteria (Proteobacteria)			
All Samples	storage:Loc	-0.00825	1
KS	storage	0.63049	**2.00E-04**
SK	storage	0.53557	**2.00E-04**
Fungi (Ascomycota)			

All Samples	storage:Loc	0.02691	0.19
KS	storage	0.11742	**0.01**
SK	storage	0.06194	0.67
Fungi (Basidiomycota)			
All Samples	storage:Loc	0.03788	0.08
KS	storage	0.13404	**0.04**
SK	storage	0.18744	**2.20E-03**
Fungi (Zygomycota)			
All Samples	storage:Loc	0.02356	0.57
KS	storage	0.11111	0.91
SK	storage	0.02604	0.72
b. Over Time (all cooler)			
Bacteria (Actinobacteria)			
All Samples	time:Loc	0.00013	**3.40E-03**
KS	time	0.04207	0.17
SK	time	0.00711	0.5
Bacteria (Planctomycetes)			
All Samples	time:Loc	0.00008	0.16
KS	time	0.01921	0.49
SK	time	0.05845	0.12
Bacteria (Proteobacteria)			
All Samples	time:Loc	0.00041	**2.00E-04**
KS	time	0.00711	50
SK	time	0.05407	0.22
Fungi (Ascomycota)			
All Samples	time:Loc	0.00173	0.96
KS	time	0.00852	0.97
SK	time	0.00855	0.67
Fungi (Basidiomycota)			
All Samples	time:Loc	0	1
KS	time	0.00009	1
SK	time	0.00115	1
Fungi (Zygomycota)			
All Samples	time:Loc	-0.00062	0.89

KS	time	-0.08827	0.83
SK	time	-0.01755	0.93

Table SI3. GLM results for diversity metrics across storage method and sites.

GLM results for fungal and bacterial diversity metrics (OTU numbers, Simpson diversity, and community Evenness) across a. storage methods, including room temperature, cooler, liquid nitrogen, and RNAlater (with liquid nitrogen as the storage reference) and b. degradation over time using cooler storage. Each analysis is run for all samples and then for Kansas (KS) and Saskatchewan (SK) samples. Results significant at a level of $p < 0.05$ are in bold.

a. Storage Methods (all time 1)				
parameter	Estimate (coefficient)	Std. Error	t-value	*p* value
Bacteria (OTU number)				
Intercept	2139.78	55.98	38.222	**< 2.00E-16**
Location (SK)	64.78	79.17	0.818	4.16E-01
cooler	20.44	79.17	0.258	7.97E-01
RNAlater	-82.89	79.17	-1.047	2.99E-01
room temperature	41.56	79.17	0.525	6.01E-01
Loc*cooler	-57.67	111.96	-0.515	6.08E-01
Loc*RNAlater	-21	111.96	-0.188	8.52E-01
Loc*room temperature	-76.56	111.96	-0.684	4.97E-01
Bacteria (Simpson Diversity)				
Intercept	0.912431	0.008104	112.592	**< 2.00E-16**
Location (SK)	0.079466	0.011461	6.934	**2.43E-09**
cooler	-0.021596	0.011461	-1.884	6.41E-02
RNAlater	0.026263	0.011461	2.292	**2.52E-02**

room temperature	-0.026866	0.011461	-2.344	**2.22E-02**
Loc*cooler	0.021529	0.016208	1.328	1.89E-01
Loc*RNAlater	-0.026668	0.016208	-1.645	1.05E-01
Loc*room temperature	0.026359	0.016208	1.626	1.09E-01
Bacteria (Evenness)				
Intercept	0.66267	0.01133	58.503	**< 2.00E-16**
Location (SK)	0.11293	0.01602	7.05	**1.52E-09**
cooler	-0.02867	0.01602	-1.79	7.82E-02
RNAlater	0.04733	0.01602	2.955	**4.37E-03**
room temperature	-0.03409	0.01602	-2.128	**3.72E-02**
Loc*cooler	0.02822	0.02265	1.246	2.17E-01
Loc*RNAlater	-0.04791	0.02265	-2.115	**3.84E-02**
Loc*room temperature	0.03265	0.02265	1.441	1.54E-01
Fungi (OTU Number)				
Intercept	296.556	25.76	11.512	**< 2.00E-16**
Location (SK)	58.556	36.431	1.607	1.13E-01
cooler	61.444	36.431	1.687	9.66E-02
RNAlater	83.667	36.431	2.297	**2.49E-02**
room temperature	113.667	36.431	3.12	**2.71E-03**
Loc*cooler	8.556	51.521	0.166	8.69E-01
Loc*RNAlater	-124.222	51.521	-2.411	**1.88E-02**
Loc*room temperature	-127.556	51.521	-2.476	**1.60E-02**
Fungi (Simpson Diversity)				
Intercept	0.957456	0.015302	62.569	**< 2.00E-16**
Location (SK)	-0.013514	0.021641	-0.624	5.35E-01
cooler	-0.031537	0.021641	-1.457	1.50E-01

RNAlater	-0.0213	0.021641	-0.984	3.29E-01
room temperature	-0.01776	0.021641	-0.821	4.15E-01
Loc*cooler	0.024624	0.030605	0.805	4.24E-01
Loc*RNAlater	-0.010879	0.030605	-0.355	7.23E-01
Loc*room temperature	0.009706	0.030605	0.317	7.52E-01
Fungi (Evenness)				
Intercept	0.696316	0.023432	29.717	**< 2.00E-16**
Location (SK)	-0.009109	0.033137	-0.275	7.84E-01
cooler	-0.017604	0.033137	-0.531	5.97E-01
RNAlater	-0.052489	0.033137	-1.584	1.18E-01
room temperature	0.001907	0.033137	0.058	9.54E-01
Loc*cooler	0.006722	0.046863	0.143	8.86E-01
Loc*RNAlater	0.007601	0.046863	0.162	8.72E-01
Loc*room temperature	-0.018998	0.046863	-0.405	6.87E-01
b. Over Time (all cooler)				
Bacteria (OTU Number)				
Intercept	1945.3632	21.1731	91.879	**3.00E-11**
Location (SK)	8.5199	29.9432	0.285	7.90E-01
time	-0.6176	0.4544	-1.359	1.80E-01
Loc*time	0.3082	0.6427	0.48	6.30E-01
Bacteria (Simpson Diversity)				
Intercept	8.97E-01	6.43E-03	139.42	**5.00E-11**
Location (SK)	9.54E-02	9.10E-03	10.488	**7.00E-05**
time	2.52E-05	1.16E-04	0.217	8.30E-01
Loc*time	-3.12E-05	1.64E-04	-0.189	8.50E-01
Bacteria (Evenness)				
Intercept	6.48E-01	7.51E-03	86.192	**2.00E-10**
Location (SK)	1.38E-01	1.06E-02	13.022	**1.00E-05**

time	2.82E-05	1.48E-04	0.19	8.50E-01
Loc*time	-8.34E-05	2.10E-04	-0.398	6.90E-01
Fungi (OTU Number)				
Intercept	344.21273	19.41618	17.728	**< 2.00E-16**
Location (SK)	24.50325	27.45862	0.892	3.80E-01
time	-0.53351	0.63101	-0.845	4.00E-01
Loc*time	0.07238	0.89238	0.081	9.40E-01
Fungi (Simpson Diversity)				
Intercept	9.19E-01	2.06E-02	44.577	**3.00E-10**
Location (SK)	2.75E-02	2.92E-02	0.942	3.80E-01
time	-2.29E-04	4.90E-04	-0.466	6.40E-01
Loc*time	4.03E-05	6.94E-04	0.058	9.50E-01
Fungi (Evenness)				
Intercept	0.6662125	0.020545	32.427	**< 2.00E-16**
Location (SK)	0.0257326	0.029055	0.886	3.80E-01
time	-0.0004139	0.0006677	-0.62	5.40E-01
Loc*time	0.0001309	0.0009443	0.139	8.90E-01

Table SI4. Phylum-level GLM results for diversity metrics across storage method and sites. GLM results for fungal and bacterial diversity metrics (OTU numbers, Simpson diversity, and community Evenness) for each phylum across a. storage methods, including room temperature, cooler, liquid nitrogen, and RNAlater (with liquid nitrogen as the storage reference) and b. degradation over time using cooler storage. Each analysis is run for all samples and then for Kansas (KS) and Saskatchewan (SK) samples. Results significant at a level of $p < 0.05$ are in bold.

a. Storage Methods (all time 1)

parameter	Estimate (coefficient)	Std. Error	t-value	*p* value
Bacteria (Actinobacteria)				
Bacteria (OTU number)				
Intercept	321.889	4.682	68.754	**< 2e-16**
Location (SK)	-3.778	6.621	-0.571	5.70E-01
cooler	5.444	6.621	0.822	4.14E-01
RNAlater	-20.556	6.621	-3.105	**2.84E-03**
room temperature	5.556	6.621	0.839	4.05E-01
Loc*cooler	-2.556	9.363	-0.273	7.86E-01
Loc*RNAlater	20.667	9.363	2.207	**3.09E-02**
Loc*room temperature	-1.444	9.363	-0.154	8.78E-01
Bacteria (Simpson Diversity)				
Intercept	9.87E-01	4.63E-04	2130.429	**<2e-16**
Location (SK)	-1.30E-02	6.55E-04	-19.827	**<2e-16**
cooler	4.52E-04	6.55E-04	0.69	4.93E-01
RNAlater	-8.02E-05	6.55E-04	-0.122	9.03E-01
room temperature	8.00E-04	6.55E-04	1.222	2.26E-01
Loc*cooler	5.51E-06	9.27E-04	0.006	9.95E-01
Loc*RNAlater	5.03E-04	9.27E-04	0.543	5.89E-01
Loc*room temperature	2.71E-04	9.27E-04	0.293	7.71E-01
Bacteria (Evenness)				
Intercept	0.8448468	0.0024267	348.144	**< 2e-16**
Location (SK)	-0.0576602	0.0034319	-16.801	**1.38E-11**
cooler	0.0019698	0.0034319	0.574	5.74E-01
RNAlater	0.0041636	0.0034319	1.213	2.43E-01
room temperature	0.0053274	0.0034319	1.552	1.40E-01
Loc*cooler	-0.0041749	0.0048534	-0.86	4.02E-01

Loc*RNAlater	-0.0034507	0.0048534	-0.711	4.87E-01
Loc*room temperature	-0.0007612	0.0048534	-0.157	8.77E-01
Bacteria (Planctomycetes)				
Bacteria (OTU number)				
Intercept	347.556	10.374	33.503	**<2e-16**
Location (SK)	9.111	14.671	0.621	5.37E-01
cooler	9.556	14.671	0.651	5.17E-01
RNAlater	-38	14.671	-2.59	**1.19E-02**
room temperature	11.111	14.671	0.757	4.52E-01
Loc*cooler	-6.111	20.748	-0.295	7.69E-01
Loc*RNAlater	23.667	20.748	1.141	2.58E-01
Loc*room temperature	-12.333	20.748	-0.594	5.54E-01
Bacteria (Simpson Diversity)				
Intercept	0.9801027	0.0014166	691.852	**< 2e-16**
Location (SK)	0.0085689	0.0020034	4.277	**6.44E-05**
cooler	0.0013011	0.0020034	0.649	5.18E-01
RNAlater	-0.0011364	0.0020034	-0.567	5.73E-01
room temperature	0.001626	0.0020034	0.812	4.20E-01
Loc*cooler	-0.0020187	0.0028333	-0.712	4.79E-01
Loc*RNAlater	0.0006248	0.0028333	0.221	8.26E-01
Loc*room temperature	-0.0015185	0.0028333	-0.536	5.94E-01
Bacteria (Evenness)				
Intercept	0.858927	0.005294	162.234	**< 2e-16**
Location (SK)	0.02591	0.007487	3.46	**9.65E-04**
cooler	0.004254	0.007487	0.568	5.72E-01
RNAlater	0.003829	0.007487	0.511	6.11E-01
room temperature	0.004428	0.007487	0.591	5.56E-01

Loc*cooler	-0.007858	0.010589	-0.742	4.61E-01
Loc*RNAlater	-0.00352	0.010589	-0.332	7.41E-01
Loc*room temperature	-0.003955	0.010589	-0.374	7.10E-01
Bacteria (Proteobacteria)				
Bacteria (OTU number)				
Intercept	258.111	7.081	36.453	**< 2e-16**
Location (SK)	34.889	10.014	3.484	**3.06E-03**
cooler	15.222	10.014	1.52	1.48E-01
RNAlater	4.667	10.014	0.466	6.47E-01
room temperature	16.222	10.014	1.62	1.25E-01
Loc*cooler	-24.556	14.161	-1.734	1.02E-01
Loc*RNAlater	-5.778	14.161	-0.408	6.89E-01
Loc*room temperature	-19.111	14.161	-1.35	1.96E-01
Bacteria (Simpson Diversity)				
Intercept	0.859711	0.00461	186.478	**< 2e-16**
Location (SK)	0.103852	0.00652	15.928	**3.09E-11**
cooler	0.011806	0.00652	1.811	8.90E-02
RNAlater	0.043487	0.00652	6.67	**5.38E-06**
room temperature	0.029577	0.00652	4.536	**3.37E-04**
Loc*cooler	-0.014571	0.009221	-1.58	1.34E-01
Loc*RNAlater	-0.040098	0.009221	-4.349	**4.98E-04**
Loc*room temperature	-0.024287	0.009221	-2.634	**1.80E-02**
Bacteria (Evenness)				
Intercept	0.657488	0.005897	111.493	**< 2e-16**
Location (SK)	0.127708	0.00834	15.313	**5.60E-11**
cooler	0.013735	0.00834	1.647	1.19E-01
RNAlater	0.046186	0.00834	5.538	**4.50E-05**

room temperature	0.028313	0.00834	3.395	**3.70E-03**
Loc*cooler	-0.017899	0.011794	-1.518	1.49E-01
Loc*RNAlater	-0.033329	0.011794	-2.826	**1.22E-02**
Loc*room temperature	-0.014681	0.011794	-1.245	2.31E-01
Fungi (Ascomycota)				
Fungi (OTU Number)				
Intercept	80.444	13.396	6.005	**9.93E-08**
Location (SK)	44.444	18.945	2.346	**2.21E-02**
cooler	32.778	18.945	1.73	8.84E-02
RNAlater	40.889	18.945	2.158	**3.47E-02**
room temperature	64	18.945	3.378	**1.25E-03**
Loc*cooler	-3.444	26.792	-0.129	8.98E-01
Loc*RNAlater	-58.556	26.792	-2.186	**3.25E-02**
Loc*room temperature	-66.667	26.792	-2.488	**1.55E-02**
Fungi (Simpson Diversity)				
Intercept	0.942211	0.012065	78.095	**<2e-16**
Location (SK)	-0.04073	0.017062	-2.387	**1.99E-02**
cooler	0.015279	0.017062	0.895	3.74E-01
RNAlater	-0.015182	0.017062	-0.89	3.77E-01
room temperature	0.009398	0.017062	0.551	5.84E-01
Loc*cooler	-0.024972	0.02413	-1.035	3.05E-01
Loc*RNAlater	-0.016565	0.02413	-0.686	4.95E-01
Loc*room temperature	-0.010101	0.02413	-0.419	6.77E-01
Fungi (Evenness)				
Intercept	0.801765	0.01899	42.22	**< 2e-16**
Location (SK)	-0.101903	0.026856	-3.794	**3.31E-04**

cooler	0.003514	0.026856	0.131	8.96E-01
RNAlater	-0.062924	0.026856	-2.343	**2.22E-02**
room temperature	-0.021809	0.026856	-0.812	4.20E-01
Loc*cooler	-0.035289	0.03798	-0.929	3.56E-01
Loc*RNAlater	0.034682	0.03798	0.913	3.65E-01
Loc*room temperature	0.017276	0.03798	0.455	6.51E-01
Fungi (Basidiomycota)				
Fungi (OTU Number)				
Intercept	15	2.0581	7.288	**6.80E-10**
Location (SK)	8.4444	2.9105	2.901	**5.14E-03**
cooler	4.5556	2.9105	1.565	1.23E-01
RNAlater	4.5556	2.9105	1.565	1.23E-01
room temperature	2.8889	2.9105	0.993	3.25E-01
Loc*cooler	0.6667	4.1161	0.162	8.72E-01
Loc*RNAlater	-11.4444	4.1161	-2.78	**7.18E-03**
Loc*room temperature	-8.7619	4.2606	-2.056	**4.40E-02**
Fungi (Simpson Diversity)				
Intercept	0.72849	0.04339	16.789	**<2e-16**
Location (SK)	0.15258	0.06136	2.487	**1.56E-02**
cooler	-0.0174	0.06136	-0.284	7.78E-01
RNAlater	-0.08716	0.06136	-1.42	1.61E-01
room temperature	-0.07553	0.06136	-1.231	2.23E-01
Loc*cooler	0.03373	0.08678	0.389	6.99E-01
Loc*RNAlater	0.02669	0.08678	0.308	7.59E-01
Loc*room temperature	0.02696	0.08983	0.3	7.65E-01
Fungi (Evenness)				
Intercept	0.68217	0.0399	17.097	**< 2e-16**

Location (SK)	0.10028	0.05643	1.777	8.05E-02
cooler	-0.0645	0.05643	-1.143	2.57E-01
RNAlater	-0.16345	0.05643	-2.897	**5.21E-03**
room temperature	-0.08671	0.05643	-1.537	1.29E-01
Loc*cooler	0.07447	0.0798	0.933	3.54E-01
Loc*RNAlater	0.14065	0.0798	1.763	8.29E-02
Loc*room temperature	0.0307	0.0826	0.372	7.11E-01
Fungi (Zygomycota)				
Fungi (OTU Number)				
Intercept	6.5	1.1314	5.745	**1.67E-06**
Location (SK)	1.5	1.2508	1.199	2.39E-01
cooler	0.5	1.9596	0.255	8.00E-01
RNAlater	-1.5	1.9596	-0.765	4.49E-01
room temperature	3	1.3856	2.165	**3.73E-02**
Loc*cooler	0.8333	2.0997	0.397	6.94E-01
Loc*RNAlater	2.125	2.1082	1.008	3.20E-01
Loc*room temperature	-2.4444	1.5776	-1.549	1.30E-01
Fungi (Simpson Diversity)				
Intercept	0.49699	0.042	11.833	**1.38E-11**
Location (SK)	0.19001	0.0486	3.909	**1.10E-03**
cooler	0.0457	0.07274	0.628	5.36E-01
RNAlater	0.05014	0.07274	0.689	4.97E-01
room temperature	0.0422	0.05243	0.805	4.30E-01
Loc*cooler	-0.04167	0.08055	-0.517	6.11E-01
Loc*RNAlater	-0.07956	0.08081	-0.985	3.37E-01
Loc*room temperature	-0.04537	0.06281	-0.722	4.81E-01
Fungi (Evenness)				

Intercept	0.480768	0.047658	10.088	**6.74E-12**
Location (SK)	0.162865	0.052688	3.091	**3.90E-03**
cooler	-0.006711	0.082547	-0.081	9.36E-01
RNAlater	0.013168	0.082547	0.16	8.74E-01
room temperature	-0.007067	0.058369	-0.121	9.04E-01
Loc*cooler	-0.006695	0.08845	-0.076	9.40E-01
Loc*RNAlater	-0.042348	0.088806	-0.477	6.36E-01
Loc*room temperature	0.004228	0.066456	0.064	9.50E-01
b. Over Time (all cooler)				
Bacteria (Actinobacteria)				
Bacteria (OTU Number)				
Intercept	326.91821	3.04892	107.224	**3.00E-13**
Location (SK)	-7.91121	4.31182	-1.835	1.10E-01
time	-0.04045	0.07373	-0.549	5.90E-01
Loc*time	0.07099	0.10427	0.681	5.00E-01
Bacteria (Simpson Diversity)				
Intercept	9.87E-01	2.39E-04	4127.785	**< 2e-16**
Location (SK)	-1.26E-02	3.38E-04	-37.107	**6.00E-10**
time	2.46E-06	5.88E-06	0.418	6.80E-01
Loc*time	-1.37E-05	8.32E-06	-1.646	1.00E-01
Bacteria (Evenness)				
Intercept	8.48E-01	1.16E-03	731.63	**< 2e-16**
Location (SK)	-5.97E-02	1.64E-03	-36.444	**5.00E-13**
time	1.17E-06	3.44E-05	0.034	9.70E-01
Loc*time	-5.78E-05	4.87E-05	-1.188	2.40E-01
Bacteria (Planctomycetes)				
Bacteria (OTU Number)				
Intercept	357.46294	3.70663	96.439	**<2e-16**
Location (SK)		5.26272		
	0.96834		0.184	8.60E-01
time	-0.09654	0.10809	-0.893	3.70E-01

Loc*time	0.05685	0.15294	0.372	7.10E-01
Bacteria (Simpson Diversity)				
Intercept	9.81E-01	4.88E-04	2011.717	**< 2e-16**
Location (SK)		6.93E-04	9.759	**2.00E-06**
	6.76E-03			
time	-9.68E-06	1.38E-05	-0.702	4.90E-01
Loc*time	3.31E-06	1.95E-05	0.17	8.70E-01
Bacteria (Evenness)				
Intercept	8.65E-01	1.78E-03	486.944	**< 2e-16**
Location (SK)	1.61E-02	2.52E-03	6.393	**8.00E-09**
time	-8.35E-06	5.77E-05	-0.145	8.90E-01
Loc*time	2.73E-05	8.17E-05	0.334	7.40E-01
Bacteria (Proteobacteria)				
Bacteria (OTU Number)				
Intercept	254.12077	2.64762	95.981	**< 2e-16**
Location (SK)			4.081	**1.00E-06**
	15.28186	3.74431		
time	-0.01691	0.08605	-0.197	8.50E-01
Loc*time	0.02399	0.12169	0.197	8.40E-01
Bacteria (Simpson Diversity)				
Intercept	8.70E-01	2.34E-03	372.26	**< 2e-16**
Location (SK)	9.37E-02	3.30E-03	28.351	**3.00E-13**
time	1.12E-04	7.34E-05	1.521	1.30E-01
Loc*time	-1.53E-04	1.04E-04	-1.47	1.50E-01
Bacteria (Evenness)				
Intercept	6.72E-01	2.71E-03	248	**<2e-16**
Location (SK)	1.17E-01	3.83E-03	30.551	**<2e-16**
time	1.50E-04	8.81E-05	1.7	9.00E-02
Loc*time	-2.05E-04	1.25E-04	-1.645	1.00E-01
Fungi (Ascomycota)				
Fungi (OTU Number)				
Intercept	106.49359	9.55558	11.145	**<2e-16**
Location (SK)	23.28686	13.51363	1.723	9.00E-02
time	-0.2996	0.31055	-0.965	3.40E-01

Loc*time	0.06697	0.43918	0.152	8.80E-01
Fungi (Simpson Diversity)				
Intercept	0.9483872	0.0100942	93.953	**7.00E-13**
Location (SK)	-0.0387191	0.0142754	-2.712	**3.00E-02**
time	-0.0004032	0.000245	-1.646	1.00E-01
Loc*time	0.0001199	0.0003465	0.346	7.30E-01
Fungi (Evenness)				
Intercept	0.8026188	0.011429	70.227	**1.00E-16**
Location (SK)	-0.0937333	0.016163	-5.799	**2.00E-04**
time	-0.0003642	0.0003224	-1.13	2.60E-01
Loc*time	0.0002313	0.000456	0.507	6.10E-01
Fungi (Basidiomycota)				
Fungi (OTU Number)				
Intercept	20.277528	1.987682	10.202	**<2e-16**
Location (SK)	4.303015	2.811006	1.531	1.30E-01
time	0.001555	0.064598	0.024	9.80E-01
Loc*time	-0.008884	0.091355	-0.097	9.20E-01
Fungi (Simpson Diversity)				
Intercept	0.6746623	0.0239429	28.178	**< 2e-16**
Location (SK)	0.2015002	0.0338603	5.951	**6.00E-08**
time	0.0002316	0.0007781	0.298	7.70E-01
Loc*time	-0.0004707	0.0011004	-0.428	6.70E-01
Fungi (Evenness)				
Intercept	0.5782517	0.0227841	25.38	**7.00E-11**
Location (SK)	0.2097352	0.0322215	6.509	**5.00E-05**
time	0.0006263	0.0006613	0.947	3.50E-01
Loc*time	-0.0011156	0.0009352	-1.193	2.40E-01
Fungi (Zygomycota)				
Fungi (OTU Number)				
Intercept	7.57143	1.39524	5.427	**2.00E-06**
Location (SK)	1.33528	1.44377	0.925	3.60E-01
time	0.07143	0.15798	0.452	6.50E-01
Loc*time	-0.0774	0.15844	-0.489	6.30E-01

Fungi (Simpson Diversity)				
Intercept	0.565336	0.040193	14.066	**2.00E-15**
Location (SK)	0.098609	0.041915	2.353	**3.00E-02**
time	0.004326	0.004403	0.983	3.30E-01
Loc*time	-0.004009	0.004416	-0.908	3.70E-01
Fungi (Evenness)				
Intercept	0.5217674	0.0576722	9.047	**5.00E-07**
Location (SK)	0.0809379	0.0617587	1.311	2.20E-01
time	0.0015105	0.005505	0.274	7.90E-01
Loc*time	-0.0004732	0.0055211	-0.086	9.30E-01

Table SI5. Analyses of qPCR measurements.

Analyses, including PERMANOVA, GLM of diversity metrics, and GLM (with liquid nitrogen as the storage reference), of qPCR measurements, examining impact of batch order in one extraction within Kansas samples.

a. PERMANOVA			
	Parameter	R^2	*p* value
Bacteria			
	storage	0.13554	**1.70E-01**
	order	0.04379	**2.10E-01**
Fungi			
	storage	0.08572	**3.20E-01**
	order	0.07968	**1.20E-03**

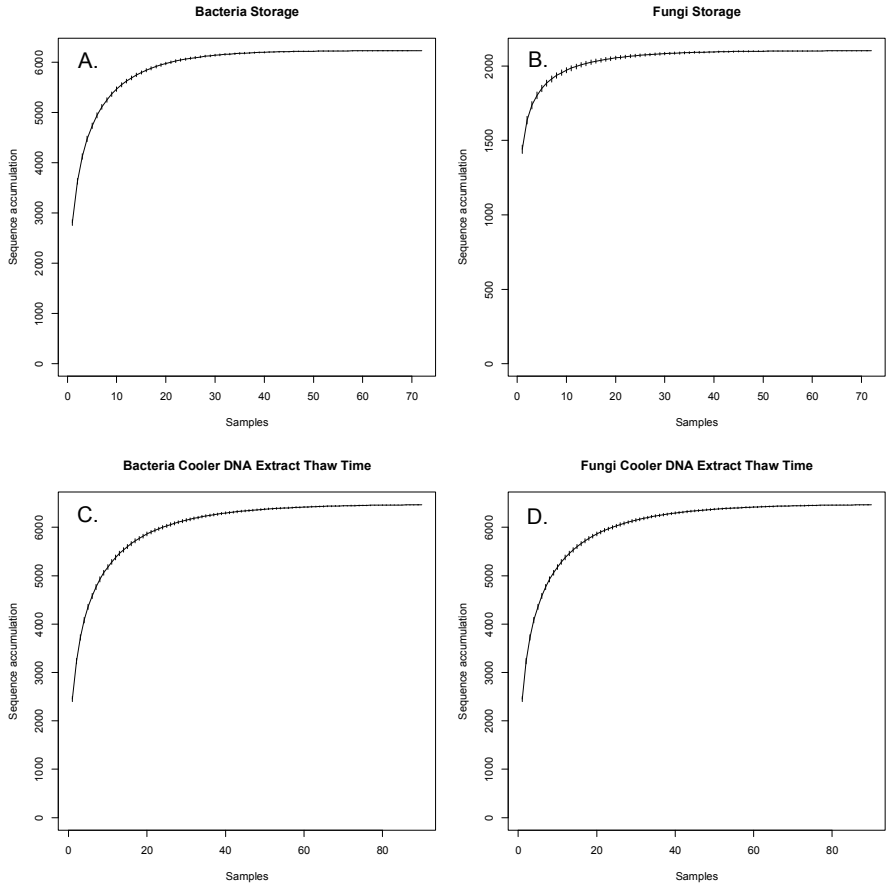
b. GLM Diversity Metrics				
Bacteria (OTU Number)				
Parameter	Estimate (coefficient)	Std. Error	t-value	*p* value
Intercept	1.97E+03	2.24E+02	8.794	**6.29E-10**
cooler	-86.62	1.74E+02	-0.497	6.23E-01
RNAlater	-297.01	2.92E+02	-1.017	3.17E-01
room temperature	148.62	1.74E+02	0.853	4.00E-01

order	11.9	1.50E+01	0.791	**4.35E-01**
Bacteria (Simpson Diversity)				
Intercept	0.897743	3.34E-02	26.911	**1.27E-09**
cooler	-3.07E-02	2.59E-02	-1.184	2.70E-01
RNAlater	7.49E-03	4.34E-02	0.172	8.67E-01
room temperature	-1.76E-02	2.59E-02	-0.678	**5.16E-01**
order	0.001052	0.002235	0.47	6.49E-01
Bacteria (Evenness)				
Intercept	0.634387	0.046886	13.53	**1.50E-14**
cooler	-0.047086	0.036417	-1.293	2.06E-01
RNAlater	0.010665	0.061035	0.175	8.62E-01
room temperature	-0.016455	0.036417	-0.452	6.55E-01
order	0.002025	0.003142	0.645	5.24E-01
Fungi (OTU Number)				
Intercept	212.381	100.99	2.103	**4.37E-02**
cooler	7.332	78.441	0.093	9.26E-01
RNAlater	-24.558	131.466	-0.187	8.53E-01
room temperature	167.779	78.441	2.139	**4.04E-02**
order	6.012	6.768	0.888	3.81E-01
Fungi (Simpson Diversity)				
Intercept	0.91694	0.060024	15.276	**5.63E-16**
cooler	-0.057637	0.046622	-1.236	2.26E-01
RNAlater	-0.073633	0.078138	-0.942	3.53E-01
room temperature	0.008482	0.046622	0.182	8.57E-01
order	0.002897	0.004023	0.72	4.77E-01
Fungi (Evenness)				
Intercept	0.571266	0.146288	3.905	**4.75E-04**
cooler	-0.076438	0.071927	-1.063	2.96E-01
RNAlater	0.103168	0.120549	0.856	3.99E-01
room temperature	0.060355	0.071927	0.839	4.08E-01
order	0.00466	0.006206	0.751	4.58E-01
c. GLM of qPCR measurements				

Parameter	Estimate (coefficient)	Std. Error	t-value	*p* value
Bacteria (16S)				
Intercept	1.06E+08	4.49E+08	0.235	8.14E-01
cooler	-5.40E+08	5.20E+08	-1.038	2.99E-01
RNAlater	-8.36E+08	5.29E+08	-1.581	1.14E-01
room temperature	1.09E+07	5.44E+08	0.02	9.84E-01
order	1.01E+08	1.86E+07	5.423	**5.87E-08**
Fungi (ITS)				
Intercept	-2.15E+06	2.99E+06	-0.72	4.82E-01
cooler	-1.07E+06	3.60E+06	-0.297	7.74E-01
RNAlater	1.77E+06	3.65E+06	0.485	6.40E-01
room temperature	5.02E+06	3.72E+06	1.348	2.10E-01
order	4.70E+05	1.13E+05	4.175	**2.99E-05**

Figure SI1. OTU rarefaction curves.

Rarefaction curves for bacterial and fungal OTUs for (A) bacteria storage, (B) fungi storage, (C) bacteria cooler DNA extract thaw time and (D) fungi cooler DNA extract thaw time. Curves show saturation of sequences, indicating sufficient sequencing.



Supplementary Methods

Amplicon library preparation and Illumina Miseq Sequencing

1. Bacteria (16S)

Amplicon library preparation was completed at the Genome Quebec Innovation Center using a two-step PCR procedure for the 16S rRNA gene. The first step involved amplifying bacterial

DNA using the tagged primers 515-F and 806-R, and second step added barcodes and Illumina adapters. The initial 25 µl PCR included 0.5 U FastStart High Fidelity (Roche), 10X PCR buffer with 18 mM $MgCl_2$ (Roche), 5% DMSO (Roche), 0.2 mM dNTP mix (FroggaBio), 0.6 µM of each primer (515F-CS1 and 806R-CS2), and 1 µl of DNA diluted 1/10. The PCR conditions consisted of 94°C for 2 min, 33 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by 72°C for 7 min. Verification of amplification was assessed on 2% agarose gel. The PCR product was diluted 1/100 for the second PCR step to add 10-bp barcodes and Illumina adapter sequences in 18 µl reactions. The PCR conditions for the second step consisted of 95°C for 10 min, 15 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 60 s, followed by 72°C for 3 min. Verification of successful barcode incorporation was assessed on 2% agarose gel. Amplicons were quantified with Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and libraries generated by pooling the same quantity (ng) of each sample. Libraries were purified with a ratio of 0.85 sparQ Pure Mag Beads (Quantabio). Libraries were then quantified using Kapa SYBR Fast Universal Kit (Kapa Biosystems) and average fragment size was determined using a LabChip GX instrument (PerkinElmer). Prior to sequencing, 10% of Phix control library was spiked into the amplicon library at a final concentration of 8 pM to improve the unbalanced base composition. Paired-end sequencing was performed with a MiSeq reagent kit v2 (500-cycle) (Illumina).

2. Fungi (ITS1)

Amplicon library preparation was completed at the Genome Quebec Innovation Center using a two-step PCR procedure for the fungal ITS1 region. The first step involved amplifying fungal DNA using the tagged primers ITS1F and 58A2R, and second step added barcodes and Illumina

adapters. The initial 8 µl PCR included 0.16 U HotStarTaq (Qiagen), 10X PCR buffer with 15 mM $MgCl_2$ (Qiagen), 5% DMSO (Roche), 0.2 mM dNTP mix (FroggaBio), 0.6 µM of each primer (ITS1F-CS1 and 58A2R-CS2), and 1 µl of DNA diluted 1/100. The PCR conditions consisted of 96°C for 15 min, 35 cycles at 96°C for 30 s, 52°C for 30 s, and 72°C for 60 s, followed by 72°C for 10 min. Verification of amplification was assessed on 2% agarose gel. The PCR product was diluted 1/100 for the second PCR step to add 10-bp barcodes and Illumina adapter sequences in 18 µl reactions. The PCR conditions for the second step consisted of 95°C for 10 min, 15 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 60 s, followed by 72°C for 3 min. Verification of successful barcode incorporation was assessed on 2% agarose gel. Amplicons were quantified with Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) and libraries generated by pooling the same quantity (ng) of each sample. Libraries were purified with a ratio of 0.85 sparQ Pure Mag Beads (Quantabio). Libraries were then quantified using Kapa SYBR Fast Universal Kit (Kapa Biosystems) and average fragment size was determined using a LabChip GX instrument (PerkinElmer). Prior to sequencing, 10% of Phix control library was spiked into the amplicon library at a final concentration of 10 pM to improve the unbalanced base composition. Paired-end sequencing was performed with a MiSeq reagent kit v3 (600-cycle) (Illumina).

Appendix B: Utility of LSU for environmental sequencing of arbuscular mycorrhizal fungi: a new reference database and pipeline

Material from: Delavaux, C. S., Sturmer, S. L., Wagner, M. R., Schütte, U., Morton, J. B., & Bever, J. D. (2020). Utility of large subunit for environmental sequencing of arbuscular mycorrhizal fungi: a new reference database and pipeline. *New Phytologist*. DOI: https://doi.org/10.1111/nph.17080

Background

Arbuscular mycorrhizal fungi (AMF – phylum Glomeromycota) form symbioses with most plant species worldwide and play critical roles in plant nutrient and water uptake, pathogen resistance and soil aggregation (Smith and Read 2008, Delavaux et al. 2017, Brundrett and Tedersoo 2018b). Because AMF community composition influences ecological function (van der Heijden et al. 1998, Vogelsang et al. 2006, Koziol et al. 2018b), understanding patterns of AMF composition is a research priority. Hyphae of AMF species are not morphologically distinguishable, and therefore quantification of AMF species diversity and community composition has increasingly relied on metabarcoding of rRNA gene sequences from field samples (Öpik et al. 2014). However, to date, no single region of the rRNA gene has been universally accepted as optimal for AMF environmental sequencing.

The internal transcribed spacer (ITS) region of the rRNA gene has been suggested as the universal fungal marker (Schoch et al. 2012, Lindahl et al. 2013) and has been used for AMF biogeographical studies (Tedersoo et al. 2014) and environmental sequencing (Öpik et al. 2014).

However, this region is suboptimal as a marker gene for AMF (Stockinger et al. 2010, Schoch et al. 2012). The sequence matching approach used for ITS sequences with other fungi is of limited utility for AMF because of the poor representation and poor curation of AMF sequences in ITS sequence databases (Bidartondo 2008, Stockinger et al. 2010). This database problem cannot be easily rectified because a high proportion of AMF encountered in environmental samples are undescribed. While phylogenetic approaches can be used to identify new sequences as AMF, this approach cannot be used for ITS amplicons because its rapid sequence evolution (Nilsson et al. 2008) does not generate reliable trees. The most commonly used region of the rRNA gene for environmental sequencing of AMF is the small subunit, or SSU (Öpik et al. 2014). The utility of this region is enhanced by a well-developed and curated database for AMF (Öpik et al. 2010, Davison et al. 2015). However, the SSU region has the disadvantage of being slow-evolving and therefore not sufficiently variable to adequately resolve AMF species (Krüger et al. 2009, Bruns and Taylor 2016, Schlaeppi et al. 2016). In contrast, the large subunit (LSU) region consistently shows greater utility for taxonomic resolution for AMF (Krüger et al. 2012, Hart et al. 2015, House et al. 2016), making it potentially more useful in environmental AMF sequencing. Thus far, the LSU region has rarely been used in environmental sequencing of AMF (Gollotte et al. 2004, Lekberg et al. 2013, House and Bever 2018a, Vieira et al. 2018, Schütte et al. 2019), perhaps because of bioinformatical challenges in implementation.

Here, we aim to expand the utility and ease the adoption of the LSU for amplicon sequencing of AMF by providing a well-curated LSU reference database, a reference backbone tree for phylogenetic placement and a computational pipeline easily implemented using current bioinformatical tools.

Reference Database and Pipeline

We present a current curated database and reference tree using AMF sequences from several sources: a subset of sequences published by Kruger et al. (2012) available on the National Center for Biotechnology Information (NCBI), a database of unpublished spore-derived sequences from the International Culture Collection of (Vesicular) and Arbuscular Mycorrhizal Fungi (INVAM, Morgantown, WV, USA) and additional recently described sequences from NCBI. Sequences amplified by Kruger et al. (2012) generally used primers SSUmAf and LSUmAr, with a second amplification round with SSUmCf and LSUmBr or SSU-Glom1-NDL22, while sequences amplified by INVAM used primers 1TS1 and NDL22, followed by a second amplification round using primers LR1 and NDL22 (Morton and Msiska 2010); additional primers used in recently described sequences from NCBI can be found in each respective publication associated with the accession numbers detailed in Figure 1. To build the reference backbone tree, representative sequences were chosen to maintain the tree structure, conserving clear clades within the tree (using the Interactive Tree of Life to view the tree (Letunic and Bork 2019)), but were kept at a minimum, to make the use of the tree as a reference computationally feasible. These sequences were aligned using MAFFT (Katoh and Standley 2013) and a tree constructed using RAxML v 8 (Stamatakis 2014) with 1000 bootstrap replicates and the evolutionary model GTRGAMMA in QIIME2 (https://qiime2.org). Outgroups were *Mortierella elongata* (MH047197, Mucoromycota), *Exophiala spinifera* (MH876260; Basidiomycota) and *Rhodotorula hordea* (AY631901; Ascomycota). In addition, we included LSU sequences of a plant, *Citrus limon* (X05910, Rutaceae), and an animal, *Rutilus rutilus* (EF417167, Cyprinidae). We use the reference database as the reference for open (closed and then denovo) operational taxonomic unit

(OTU) clustering and use a phylogenetic tree generated from these same sequences (Figure 1; Supporting Information Figure S1) as a backbone constraint in constructing phylogenies to place study sequences. All previously unreported reference sequences have been uploaded to Genbank (MT832155 - MT832238).

We present a pipeline starting from raw Illumina LSU sequences (MiSeq V3, 2x300 bp) and ending with an OTU table of phylogenetically defined putative AMF OTUs for downstream analyses (Figure 2). This pipeline is built for use with a high performance computing (HPC) cluster using a Simple Linux Utility for Resource Management (SLURM) workload manager. Our pipeline covers key bioinformatical steps, including primer removal, quality control, and OTU clustering (vsearch algorithm (Rognes et al. 2016); for a discussion of why OTUs may be preferable over amplicon sequence variants (ASVs) for this particular application, see Supporting Information Methods S1). Importantly, our pipeline maintains non-overlapping forward and reverse reads for each sequence, retaining all possible data in long, non-paired reads (700-900 bp; see Table S1 for a forward and reverse read concatenation test). In addition, the pipeline places representative OTU sequences within a tree using our backbone phylogeny and subsequently extracts those that fall within the AMF clade. Operationally, our software handles many batches of OTUs in parallel, thereby greatly improving processing speed. Finally, the output of all batches is joined into a single OTU table containing counts of putative AMF OTUs in each sample, along with a FASTA file containing the representative sequences of all phylogenetically determined putative AMF OTUs. The analogous data files for the OTUs that fall outside of the AMF clade are also provided by this final step. The full bioinformatical pipeline and description can be found in Supporting Information (Supporting Information

Methods S1); all required files are also supplied (Supporting Information Methods S2; https://github.com/c383d893/AMF-LSU-Database-and-Pipeline).

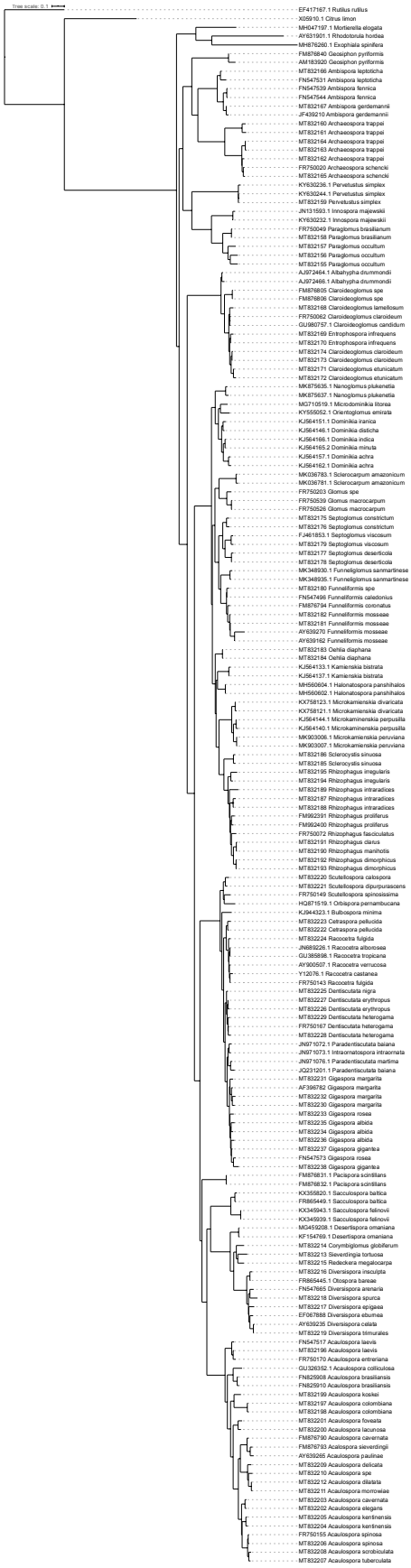
Concluding Remarks

In the age of metagenomics, it is attractive to declare one marker suitable for all fungi (Lekberg et al. 2018). Universal fungal primers would facilitate efforts to compare relative abundances between taxonomic and functional groups and to identify global scale biogeographic patterns. Nonetheless, here we confirm that the general primers targeting the ITS region are not adequate for detecting a majority of undescribed AMF species. We estimate that nearly 90% of phylogenetically defined putative AMF OTUs in our test dataset derived from a Midwestern US grassland are undescribed (i.e. have no Glomeromycota BLAST match in NCBI) and about 30 % do not group with described families in our phylogenetic tree (Supporting Information Table S2). Given this severe limitation in building a database and current database constraints (Stockinger et al. 2010, Schoch et al. 2012, Hart et al. 2015), the sequence matching algorithms are not adequate for environmental sequencing of AMF regardless of rRNA gene region. The phylogenetic approach we use here can accommodate undescribed AMF taxa in environmental samples. While the ITS region evolves too quickly to allow reliable tree construction, the LSU region can be used to build a phylogenetic tree and place all study sequences inside or outside of the conserved AMF clade. This allows identification of undescribed putative AMF through clade placement of any environmental sequence. We illustrate this benefit in two studies in which analyses of LSU amplicons reveals significant environmental patterns in AMF composition in

U.S. grasslands and boreal forests that were not evident in analyses of ITS amplicons (Supporting Information Methods S3, Table S3).

The small subunit (SSU) region, like the LSU region, can be used to build phylogenetic trees and place environmental sequences in the AMF clade (Öpik et al. 2014, Stefani et al. 2020). In addition, the SSU amplicons from commonly used primers have been easier to handle bioinformatically because the small length of about 500 bp allows for merging of short, paired-end Illumina reads (Lee et al. 2008, Dumbrell et al. 2011). Second, environmental sequences can be directly assigned to virtual taxa in a large publicly available database (Öpik et al. 2010, Öpik et al. 2014). However, the SSU region has the disadvantage of being slowly evolving, thereby limiting inferences to taxonomically coarse designations (Krüger et al. 2009, Stockinger et al. 2010, Schoch et al. 2012). Individual virtual taxa assigned using the MaarjAM database include many distinct species and have been suggested to be analogous to genera (Bruns and Taylor 2016). Analogous problems are present in the LSU in that sequence variation within isolates can be attributed to different OTUs, and individual OTUs can include sequences of different species, particularly for the Claroideoglomeraceae (Stockinger et al. 2010, House et al. 2016). Nonetheless, the LSU does a much better job of capturing other AMF families (Krüger et al. 2012, House et al. 2016) and has been suggested as the most suitable region within short read length restrictions (Stockinger et al. 2010). How these differences translate into inferences on issues such as environmental dependence of AMF distributions or frequency of endemism remains to be evaluated. Here we provide a well-curated LSU reference database, a backbone phylogeny and a computational pipeline that uses these resources to process environmentally derived amplicon sequences. We are optimistic that this set of tools will facilitate molecular

work with AMF within the LSU region, leading to finer scale assessments of ecological inferences from AMF community structure.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FR750155 Acaulospora spinosa
MT832206 Acaulospora spinosa
MT832208 Acaulospora scrobiculata
MT832207 Acaulospora tuberculata

Figure 1. AMF LSU backbone tree.

The phylogenetic tree of curated AMF across all known species using 1000 bootstrap replicates and evolutionary model GTRGAMMA. This reference backbone tree can be used to place study sequences into the conserved AMF clade.

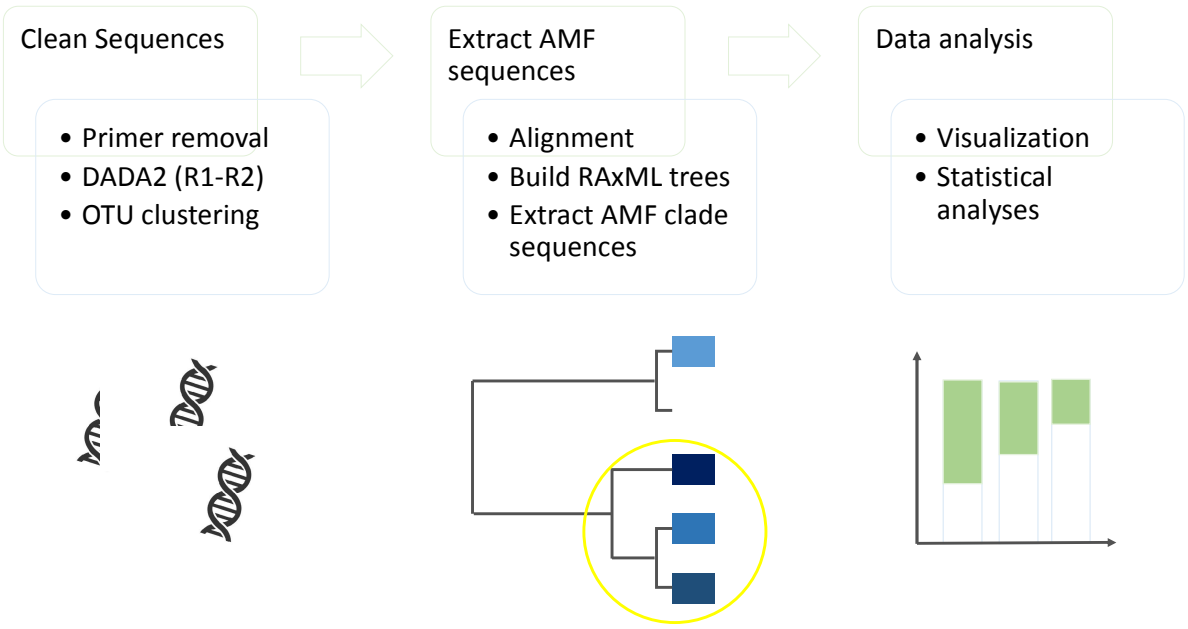


Figure 2. Visual representation of AMF LSU pipeline.

An overview of the steps included in AMF LSU data analysis. The presented pipeline covers steps 1 and 2. The initial step involves cleaning raw sequences, joining forward and reverse reads and OTU clustering. The pipeline then extracts clade-determined putative AMF sequences into an abundance table and a .fasta file.

Supporting Information

Methods S1. Bioinformatical Pipeline.

The bioinformatical pipeline presented was adapted from QIIME2 tutorials (Bolyen et al. 2018). This pipeline is based on using the forward primer LROR (ACCCGCTGAACTTAAGC, 5' to 3') and the reverse primer FLR2 (TCGTTTAAAGCCATTACGTC, 5' to 3') in the LSU region (Bunyard et al. 1994, Trouvelot et al. 1999). Because Illumina sequencing yields relatively low read length (maximum 300 bp), but the LSU region obtained using these primers is between 700-900 bp, the forward and reverse reads cannot be merged by overlapping sections as is traditionally done with 'paired-end' reads. Nonetheless, the taxonomic resolution of AMF likely benefits from including more sequence data in addition to the forward read. To confirm that performance improves when both the forward and reverse reads are included, we repeated our pipeline using known AMF sequences cut at 150 bp and 250 bp for either only the forward read, or the concatenated forward and reverse reads to quantify quality of placement on our backbone tree. We did this by using all known AMF from the reference database and five known outgroups (AF291338, MH697855, MT176543, MT453294, NG_059905). Sequences were trimmed to LROR/FLR2 primers and then cut to 150 or 250 bp and joined using the Biostrings (Pages et al. 2016) and stringr (Wickham 2010) packages in R. Results confirm a slight benefit at the 150 bp length of concatenating the forward and reverse reads, with two additional references being correctly placed in the AMF clade (Table S1). We conclude that including the most possible sequence information (forward and reverse reads) is important for the best phylogenetic placement. Therefore, in our pipeline, we concatenate the forward and reverse reads, placing 10 'N' bp between the reads.

To do this, we use a set of scripts which can be implemented on a high performance computing (HPC) cluster using a Simple Linux Utility for Resource Management (SLURM) workload manager. We use an R script using DADA2, to concatenate paired reads separated by NNNNNNNNNN. The pipeline first uses cutadapt v 2.3 to remove the forward and reverse primers, along with any remaining adaptors preceding the primers (Martin 2011). Then, quality plots are made using FastQC v. 0.11.8 (Andrews 2010) to determine truncation length for the particular study sequences. Next, the DADA2 R script (Callahan et al. 2016) adapted from the 'DADA2 Pipeline Tutorial' by Benjamin Callahan under license CC-BY 4.0 (https://benjjneb.github.io/dada2/tutorial.html) is used to clean and quality filter reads and concatenate matching forward and reverse reads. This step generates an ASV (amplicon sequence variant) table, a FASTA file with corresponding ASV sequences, and a diagnostic tracking table to show the number of reads that made it through each step. Next, an open OTU clustering step is included to group the ASVs into OTUs using vsearch (Rognes et al. 2016). Although there is still considerable debate about whether to use ASVs and OTUs in microbiome data (Callahan et al. 2017, Glassman and Martiny 2018), we recommend using OTUs due to both AMF biology and computational feasibility. In brief, single AMF species have been shown to have high sequence variation among copies of the rRNA gene, distributing into multiple OTUs (Stockinger et al. 2010, House et al. 2016), therefore potentially resulting in dozens of ASVs that map onto intracellular genetic variation rather than taxonomic variation (Schlaeppi et al. 2016).

A final step allows users to place their OTU representative sequences onto our reference backbone tree using RAxML v. 8.2.12 to determine whether they fall within the conserved AMF clade, regardless of whether the sequences have been previously identified as AMF. Implementation in QIIME2 v. 2019.10 phylogeny plugin to use a backbone reference tree is

pending, though currently unavailable. Therefore, we have created an automated pipeline to build several small trees using our phylogeny as a backbone constraint, and then extract all OTUs falling within the AMF clade (with 'FM876840 Geosiphon pyriformis' and 'MT832207 Acaulospora tuberculata' as clade edges) into a combined table. Users can then use this OTU table in any downstream metabarcoding analyses in R or other software. The pipeline output also provides analogous files (OTU table and representative sequences FASTA file) for the sequences that fall outside of the conserved AMF clade that were present in the input data. The full pipeline was tested using a large dataset of 180 soil samples (NCBI Project # PRJNA648993), resulting in 4027 OTUs classified as putative AMF and 2477 OTUs classified as putative non-AMF.

This pipeline is intended as a template; different cutoffs and settings can and should be fine-tuned to balance removing errors and maintaining adequate data for analysis. In the DADA2 implementation, it is essential to set truncation and quality-filtering parameters that are appropriate for each dataset; optimizing these parameters typically requires visual inspection of raw .fastq files using a tool such as FASTQC (Andrews 2010).

Methods S2 Pipeline scripts and instructions.

All associated pipeline files and an instructional text document (README_LSU_AMF_pipeline.txt) can be found on the github page, https://github.com/c383d893/AMF-LSU-Database-and-Pipeline.

Methods S3 Comparing ecological inference between ITS and LSU primers.

Because several studies use general ITS primers proposed by Ihrmark (Ihrmark et al. 2012) to analyze AMF as part of the fungal community (Maestre et al. 2015, Gomes et al. 2017, Carson et

al. 2019), we compare general fungal ITS primers to LSU primers here. To determine if the use of these LSU versus ITS primers leads to consistent ecological inferences, we directly compared inferences for the same samples for two recent studies (House and Bever 2018a, Schütte et al. 2019). Specifically, we compared previous inferences using LSU amplicon data using primers LROR and FLR2 (Bunyard et al. 1994, Trouvelot et al. 1999), with phylogenetically determined AMF, to the ITS amplicon data using general fungal primers fITS7 and ITS4 (Ihrmark et al. 2012), with OTUs assigned to AMF (Glomeromycota) using the UNITE ITS database (Kõljalg et al. 2013). Across both studies, we found that the ecological inferences made initially using LSU data could not be replicated using ITS-generated data.

The study by House and Bever (2018a) used LSU amplicons to demonstrate that AMF community composition shifts with disturbance and across a rainfall gradient for remnant grasslands. When ITS-based OTUs (generated with general primers and determined through database matching) were used instead of LSU-based OTUs (generated with LSU primers and determined through phylogenetic placement), confidence in the effect of disturbance was greatly reduced (Table S3, House and Bever 2018 original $p < 0.001$, ITS-obtained $p = 0.04$) and the effect of the precipitation gradient was no longer detectable (House and Bever 2018, remnant $p < 0.001$, disturbed $p = 0.04$; reanalysis using ITS, remnant $p = 0.22$, disturbed $p = 0.08$, Table S3).

The second study by Schütte et al. (2019) used LSU amplicon sequencing to demonstrate differences in AMF community composition in unthawed and thawed permafrost in Alaska. However, analyses of the ITS sequences using general fungal primers from the same samples were not able to detect any ITS sequences that matched AMF in the UNITE database. These results are consistent with Öpik et al.'s (2008) observations of diverse AMF communities in northern soils when targeting SSU with AMF-specific primers, and other studies' ITS-based

amplicon sequencing that concluded AMF are in low relative abundances in artic soils (Zhang et al. 2016, Voříšková et al. 2019). This suggests that reliance on ITS sequencing using general fungal primers in studies of arctic and boreal systems may have contributed to the perception that AMF are less abundant and potentially functionally less important in northern systems. This low AMF detection when using general fungal ITS primers may result from amplicon competition, biasing against finding AMF; alternatively, the UNITE database may be less complete for AMF than for other taxa, leading to a bioinformatic and taxonomic limitation in AMF identification via database-matching. Nonetheless, we compare these two approaches as a practical comparison between two relatively commonly used approaches in fungal ecology.

Table S1. Forward and reverse read concatenation test.

In silico tests based on 174 known AMF sequences confirmed a slight benefit of concatenating forward and reverse short reads (150 bp), relative to using only the forward reads. There was no difference in sensitivity for 250 bp reads.

	Outgroup OTUs (true negatives)	Incorrect Outgroup OTUs (false negatives)	AMF OTUs (true positives)
True	5	-	174
R1 (150)	5	104	70
R1-NNN-R2 (150)	5	102	72
R1 (250)	5	102	72
R1-NNN-R2 (250)	5	102	72

Table S2. *Family classification of putative AMF OTUs generated from our pipeline with test dataset*

Of the 4072 OTUs resulting from our pipeline using a large dataset of 180 soil samples (NCBI Project # PRJNA648993), 1192 OTUs (29.3 %) fall within the Glomeromycota clade but cannot be classified into any known AMF families.

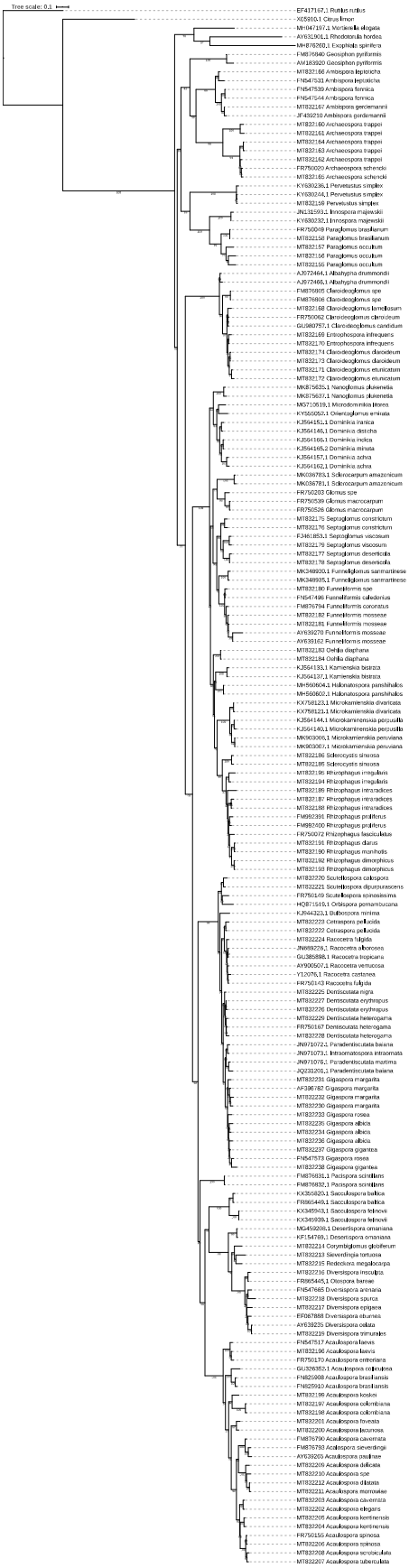
TOTAL OTUs		4072
OTUs placed in known family within Glomeromycota		2880
	Acaulosporaceae	298
	Ambisporaceae	95
	Archaeosporaceae	65
	Claroideoglomeraceae	400
	Diversisporaceae	141
	Gigasporaceae	480
	Glomeraceae	934
	Pacisporaceae	1
	Paraglomeraceae	420
	Sacculosporaceae	46
OTUs not placed in known family within Glomeromycota		1192

Table S3. Comparing ecological inference between ITS database and LSU phylogenetic results.

Results are from sequencing data previously published in House and Bever (2018a) and show that when ITS data generated from database matching were used instead of LSU data generated from AMF clade placement, confidence in the effect of disturbance was reduced, while the effect of the precipitation gradient (side of gradient) was no longer detectable.

Subset of samples	Predictor variables	LSU		ITS	
		R^2	p value	R^2	p value

All samples	Disturbance	0.03226	**0.0002**	0.03695	**0.0408**
	Side of gradient	0.02580	**0.0002**	0.03376	0.0712
	Disturbance × Side of gradient	0.02883	**0.0004**	0.02409	0.2100
Remnant samples only	Side of gradient	0.03761	**0.0002**	0.05308	0.2208
Disturbed samples only	Side of gradient	0.08316	**0.04**	0.06699	0.0774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MT832203 Acaulospora cavernata
MT832202 Acaulospora elegans
MT832205 Acaulospora kentinensis
MT832204 Acaulospora kentinensis
FR750155 Acaulospora spinosa
MT832206 Acaulospora spinosa
MT832208 Acaulospora scrobiculata
MT832207 Acaulospora tuberculata

Figure S1. Backbone tree with bootstrap support values.

The phylogenetic tree of curated AMF across all known species using 1000 bootstrap replicates and evolutionary model GTRGAMMA with bootstrap support values specified at each node.

Appendix C: Supplementary Information for Mycorrhizal fungi influence global plant geography

Material from: Delavaux, C.S., Weigelt, P., Dawson, W. *et al.* (2019). Mycorrhizal fungi influence global plant biogeography. *Nature Ecology and Evolution*. DOI: https://doi.org/10.1038/s41559-019-0823-4

SI Table 1. Model results reported in the manuscript.

GLM explaining proportion mycorrhizal to non-mycorrhizal (M:NM) plant species with AMNM plants assigned to mycorrhizal. These results are those reported in the text.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

M1: Native Model (GLMM), N = 1960				
	Estimate (coefficient)	Std. Error	Z-value	p
(intercept)	4.52188	0.05074	89.120	***
mycorrhizal status (non-mycorrhizal)	-0.62743	0.01549	-40.494	***
land type (mainland)	2.47176	0.06986	35.382	***
mycorrhizal status*land type	-0.15368	0.02056	-7.474	***
M2: Naturalized Model (GLMM), N = 1170				
	Estimate (coefficient)	Std. Error	Z-value	p
(intercept)	3.72292	0.09748	38.191	***
mycorrhizal status (non-mycorrhizal)	-0.72307	0.02677	-27.007	***
land type (mainland)	0.68059	0.11683	5.826	***
mycorrhizal status*land type	0.33721	0.03103	10.867	***
M3: Mainland Native Model (GLM), N = 515				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	2.681e-01	1.820e-02	14.733	
area	-2.847e-02	2.917e-03	-9.762	***
precipitation	6.184e-05	3.249e-06	19.035	***
temperature	2.331e-02	3.529e-04	66.071	***
elevation range	6.830e-02	5.138e-03	13.292	***
spatial autocovariate	5.166e-02	9.376e-04	55.101	***
M4: Island Native Model (GLM), N = 422				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	-1.305e-02	4.292e-02	-0.304	
area	5.310e-02	6.066e-03	8.753	***
distance	-3.553e-02	1.317e-02	-2.698	**
geology (non-oceanic)	-3.331e-02	4.172e-02	-0.798	

precipitation	7.659e-05	6.899e-06	11.101	***
temperature	2.840e-02	7.880e-04	36.045	***
elevation range	8.611e-03	1.103e-02	0.781	
distance*geology (non-oceanic)	-1.752e-02	1.555e-02	-1.126	
spatial autocovariate	8.807e-02	5.360e-03	16.430	***
M5: Island Age Native Model (GLM), N = 246				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	-1.603e-01	6.824e-02	-2.349	*
area	8.437e-02	1.154e-02	7.311	***
distance	-5.733e-02	1.768e-02	-3.242	**
precipitation	7.264e-05	1.041e-05	6.980	***
temperature	3.074e-02	1.577e-03	19.494	***
age	2.312e-02	1.359e-02	1.702	
elevation range	4.199e-02	1.445e-02	2.906	**
spatial autocovariate	1.023e-01	8.324e-03	12.289	***
M6: Mainland Naturalized Model (GLM), N = 294				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	-1.659e-01	8.485e-02	-1.956	
area	-2.954e-02	1.551e-02	-1.905	
precipitation	6.642e-05	1.857e-05	3.577	***
temperature	2.489e-02	1.516e-03	16.415	***
elevation range	7.359e-02	2.020e-02	3.642	***
population density	3.037e-02	9.592e-03	3.166	**
spatial autocovariate	1.449e-01	1.035e-02	13.998	***
M7: Island Naturalized Model (GLM), N = 141				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	1.576e-01	1.424e-01	1.107	
area	1.422e-02	2.097e-02	0.678	
distance	-5.839e-02	4.816e-02	-1.212	
geology (non-oceanic)	-4.612e-01	1.801e-01	-2.561	*
precipitation	6.653e-05	2.417e-05	2.753	**
temperature	3.394e-02	3.844e-03	8.827	***
population density	-7.422e-03	1.910e-02	-0.389	
elevation range	-2.987e-02	2.880e-02	-1.037	
distance*geology (non-oceanic)	1.634e-01	5.892e-02	2.774	**

spatial autocovariate	1.528e-01	2.455e-02	6.221	***
M8: Island Age Naturalized Model (GLM), N = 97				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	1.664e-01	2.409e-01	0.691	
area	1.092e-01	3.114e-02	3.508	***
distance	-2.302e-01	7.771e-02	-2.963	**
precipitation	7.901e-05	2.718e-05	2.906	**
temperature	5.134e-02	5.517e-03	9.306	***
age	-2.112e-02	3.592e-02	-0.588	
elevation range	-3.649e-02	3.296e-02	-1.107	
population density	-3.242e-02	2.196e-02	-1.476	
spatial autocovariate	1.059e-01	3.525e-02	3.004	**

SI Table 2. Families and corresponding consensus proportions of mycorrhizal status.

Families and proportions for mycorrhizal (M), non-mycorrhizal (NM) and both arbuscular mycorrhizal and non-mycorrhizal (AMNM) for each the three references used, along with a final average consensus proportion used in the manuscript.

Family	1_MP	2_MP	3_MP	1_AMNMP	2_AMNMP	3_AMNMP	1_NMP	2_NMP	3_NMP	avgp.M	avgp.NM	avgp.AMNM
Acanthaceae	0.60	NA	NA	0.00	NA	NA	0.40	NA	NA	0.60	0.40	0.00
Acoraceae	0.00	0.00	NA	1.00	1.00	NA	0.00	0.00	NA	0.00	0.00	1.00
Actinidiaceae	0.33	NA	NA	0.00	NA	NA	0.67	NA	NA	0.33	0.67	0.00
Adiantaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Adoxaceae	0.45	0.00	NA	0.45	1.00	NA	0.09	0.00	NA	0.23	0.05	0.73
Agavaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Aizoaceae	0.50	0.00	0	0.25	0.00	1	0.25	1.00	0	0.17	0.42	0.42
Alismataceae	0.50	0.00	NA	0.38	1.00	NA	0.13	0.00	NA	0.25	0.06	0.69
Alliaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alstroemeriaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Altingiaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Amaranthaceae	0.20	NA	0	0.16	NA	1	0.64	NA	0	0.10	0.32	0.58
Amaryllidaceae	0.93	NA	NA	0.04	NA	NA	0.02	NA	NA	0.93	0.02	0.04
Anacardiaceae	0.91	NA	NA	0.05	NA	NA	0.05	NA	NA	0.91	0.05	0.05
Aneuraceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Anisophylleaceae	0.50	NA	NA	0.00	NA	NA	0.50	NA	NA	0.50	0.50	0.00
Annonaceae	0.91	NA	NA	0.00	NA	NA	0.09	NA	NA	0.91	0.09	0.00

Anarthriaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Anisophylleaceae	0.50	NA	NA	0.00	NA	NA	0.50	NA	NA	0.50	0.50	0.00
Anthocerotaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Apiaceae	0.73	NA	NA	0.18	NA	NA	0.09	NA	NA	0.73	0.09	0.18
Apocynaceae	0.84	NA	NA	0.09	NA	NA	0.07	NA	NA	0.84	0.07	0.09
Apodanthaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Aponogetonaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Aquifoliaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Araceae	0.73	0.00	NA	0.03	1.00	NA	0.23	0.00	NA	0.37	0.12	0.52
Araliaceae	0.61	NA	NA	0.22	NA	NA	0.17	NA	NA	0.61	0.17	0.22
Araucariaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Arecaceae	0.94	NA	NA	0.06	NA	NA	0.00	NA	NA	0.94	0.00	0.06
Aristolochiaceae	0.00	NA	NA	1.00	NA	NA	0.00	NA	NA	0.00	0.00	1.00
Arnelliaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Asparagaceae	0.83	NA	NA	0.12	NA	NA	0.06	NA	NA	0.83	0.06	0.12
Asphodelaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Aspleniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Asteliaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Asteraceae	0.83	NA	NA	0.13	NA	NA	0.04	NA	NA	0.83	0.04	0.13
Asteropeiceae	NA	1.00	NA	NA	0.00	NA	NA	0.00	NA	1.00	0.00	0.00
Avicenniaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Aytoniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Azollaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Balanophoraceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00

Balsaminaceae	0.43	NA	NA	0.57	NA	NA	0.00	NA	NA	0.43	0.00	0.57
Bataceae	0.00	NA	NA	0.00	NA	NA	1.00	NA	NA	0.00	1.00	0.00
Begoniaceae	0.90	NA	NA	0.00	NA	NA	0.10	NA	NA	0.90	0.10	0.00
Berberidaceae	0.71	NA	NA	0.21	NA	NA	0.07	NA	NA	0.71	0.07	0.21
Betulaceae	1.00	1.00	1	0.00	0.00	0	0.00	0.00	0	1.00	0.00	0.00
Bieberstein iaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Bignoniaceae	0.91	NA	NA	0.09	NA	NA	0.00	NA	NA	0.91	0.00	0.09
Bixaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Blasiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Blechnaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Boraginaceae	0.56	NA	NA	0.23	NA	NA	0.21	NA	NA	0.56	0.21	0.23
Brassicaceae	0.06	0.00	NA	0.19	0.00	NA	0.74	1.00	NA	0.03	0.87	0.10
Bromeliaceae	0.75	0.00	NA	0.00	1.00	NA	0.25	0.00	NA	0.38	0.13	0.50
Bruniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Burmanniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Burseraceae	0.86	NA	NA	0.14	NA	NA	0.00	NA	NA	0.86	0.00	0.14
Butomaceae	0.00	0.00	NA	0.00	1.00	NA	1.00	0.00	NA	0.00	0.50	0.50
Buxaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Byblidaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Cactaceae	0.69	NA	NA	0.00	NA	NA	0.31	NA	NA	0.69	0.31	0.00
Calceolariaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Callitrichaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Calophyllaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Calypogeiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Campanulaceae	0.73	NA	NA	0.22	NA	NA	0.04	NA	NA	0.73	0.04	0.22

Cannabaceae	0.71	NA	NA	0.14	NA	NA	0.14	NA	NA	0.71	0.14	0.14
Cannaceae	0.00	NA	NA	0.00	NA	NA	1.00	NA	NA	0.00	1.00	0.00
Capparaceae	0.50	0.00	NA	0.00	0.00	NA	0.50	1.00	NA	0.25	0.75	0.00
Capparidaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Caprifoliaceae	0.70	NA	NA	0.21	NA	NA	0.09	NA	NA	0.70	0.09	0.21
Caricaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Carlemanniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Caryocaraceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Caryophyllaceae	0.06	NA	0	0.17	NA	1	0.76	NA	0	0.03	0.38	0.59
Casuarinaceae	1.00	1.00	NA	0.00	0.00	NA	0.00	0.00	NA	1.00	0.00	0.00
Ceasalpiniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Celastraceae	0.62	NA	NA	0.31	NA	NA	0.08	NA	NA	0.62	0.08	0.31
Centrolepidaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Cephaloziaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cephaloziellaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ceratophyllaceae	0.00	0.00	NA	0.00	0.00	NA	1.00	1.00	NA	0.00	1.00	0.00
Chenopodiaceae	NA	NA	0	NA	NA	1	NA	NA	0	0.00	0.00	1.00
Chrysobalanaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Cistaceae	1.00	1.00	NA	0.00	0.00	NA	0.00	0.00	NA	1.00	0.00	0.00
Cleomaceae	NA	1.00	NA	NA	0.00	NA	NA	0.00	NA	1.00	0.00	0.00
Clethraceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Clusiaceae	0.90	NA	NA	0.00	NA	NA	0.10	NA	NA	0.90	0.10	0.00
Codoniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Colchicaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Combretaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Commelinaceae	0.17	0.00	0	0.17	0.00	1	0.67	1.00	0	0.06	0.56	0.39
Conocephalaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Convolvulaceae	0.81	0.00	NA	0.06	0.00	NA	0.13	1.00	NA	0.41	0.56	0.03
Coriariaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Cornaceae	0.75	NA	NA	0.25	NA	NA	0.00	NA	NA	0.75	0.00	0.25
Corsiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Costaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Crassulaceae	0.21	0.00	NA	0.13	0.00	NA	0.67	1.00	NA	0.10	0.83	0.06
Cruciferae	NA	NA	0	NA	NA	1	NA	NA	0	0.00	0.00	1.00
Cucurbitaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Cunoniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Cupressaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Cyatheaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Cycadaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Cyclanthaceae	0.00	1.00	NA	0.00	0.00	NA	1.00	0.00	NA	0.50	0.50	0.00
Cymodoceaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Cynomoriaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Cyperaceae	0.32	1.00	0	0.12	0.00	1	0.56	0.00	0	0.44	0.19	0.37
Cytinaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Dasypogonaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Davalliaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dennstaedtiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Diapensiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dichapetalaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Dicksoniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dilleniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Dioscoreaceae	0.50	NA	NA	0.00	NA	NA	0.50	NA	NA	0.50	0.50	0.00
Dipsacaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Dipterocarpaceae	0.95	1.00	NA	0.00	0.00	NA	0.05	0.00	NA	0.98	0.03	0.00
Droseraceae	0.00	0.00	NA	0.50	0.00	NA	0.50	1.00	NA	0.00	0.75	0.25
Drosophyllaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Dryopteridaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ebenaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Elaeagnaceae	0.60	NA	NA	0.40	NA	NA	0.00	NA	NA	0.60	0.00	0.40
Elaeocarpaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Elatinaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Ephedraceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Equisetaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Eremolepidaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Ericaceae	0.72	1.00	NA	0.00	0.00	NA	0.28	0.00	NA	0.86	0.14	0.00
Erythroxylaceae	0.00	1.00	NA	0.00	0.00	NA	1.00	0.00	NA	0.50	0.50	0.00
Escalloniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Euphorbiaceae	0.91	1.00	NA	0.06	0.00	NA	0.03	0.00	NA	0.96	0.01	0.03
Fabaceae	0.90	1.00	NA	0.06	0.00	NA	0.04	0.00	NA	0.95	0.02	0.03
Fagaceae	0.92	1.00	1	0.04	0.00	0	0.04	0.00	0	0.97	0.01	0.01

Fouquieriaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Frankeniaceae	0.33	0.00	NA	0.00	0.00	NA	0.67	1.00	NA	0.17	0.83	0.00
Fumariaceae	NA	NA	0	NA	NA	1	NA	NA	0	0.00	0.00	1.00
Garryaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Gentianaceae	0.75	NA	NA	0.19	NA	NA	0.06	NA	NA	0.75	0.06	0.19
Geocalycaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Geraniaceae	0.78	NA	NA	0.22	NA	NA	0.00	NA	NA	0.78	0.00	0.22
Gesneriaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Ginkgoaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Gleicheniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Gnetaceae	1.00	1.00	NA	0.00	0.00	NA	0.00	0.00	NA	1.00	0.00	0.00
Goodeniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Grammitidaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Griseliniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Grossulariaceae	0.83	NA	NA	0.17	NA	NA	0.00	NA	NA	0.83	0.00	0.17
Gunneraceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Gymnomitriaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Haemodoraceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Haloragaceae	0.33	0.00	NA	0.33	1.00	NA	0.33	0.00	NA	0.17	0.17	0.67
Hamamelidaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Haplomitriaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Heliconiaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Hemerocallidaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Herbertaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hippuridaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Humiriaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Hyacinthaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hydatellaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Hydnoraceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Hydrangeaceae	0.88	NA	NA	0.06	NA	NA	0.06	NA	NA	0.88	0.06	0.06
Hydrocharitaceae	0.33	0.00	NA	0.00	1.00	NA	0.67	0.00	NA	0.17	0.33	0.50
Hydrophyllaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Hymenophyllaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Hypericaceae	0.42	NA	NA	0.25	NA	NA	0.33	NA	NA	0.42	0.33	0.25
Hypoxidaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Icacinaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Iridaceae	0.95	NA	NA	0.05	NA	NA	0.00	NA	NA	0.95	0.00	0.05
Irvingiaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Isoetaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ixioliriaceae	0.00	NA	NA	1.00	NA	NA	0.00	NA	NA	0.00	0.00	1.00
Jubulaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Juglandaceae	0.75	NA	1	0.25	NA	0	0.00	NA	0	0.88	0.00	0.13
Junglandaceae	NA	1.00	NA	NA	0.00	NA	NA	0.00	NA	1.00	0.00	0.00
Juncaceae	0.13	0.00	0	0.45	0.00	1	0.43	1.00	0	0.04	0.48	0.48
Juncaginaceae	0.00	0.00	NA	0.25	1.00	NA	0.75	0.00	NA	0.00	0.38	0.63
Jungermanniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Krameriaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00

Lamiaceae	0.84	NA	NA	0.12	NA	NA	0.04	NA	NA	0.84	0.04	0.12
Lauraceae	1.00	0.00	NA	0.00	0.00	NA	0.00	1.00	NA	0.50	0.50	0.00
Lecythidaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Lejeuneaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lennoaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Lentibulariaceae	0.00	0.00	NA	0.00	0.00	NA	1.00	1.00	NA	0.00	1.00	0.00
Lepidoziaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Liliaceae	0.89	NA	NA	0.07	NA	NA	0.04	NA	NA	0.89	0.04	0.07
Limnocharitaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Linaceae	0.77	NA	NA	0.23	NA	NA	0.00	NA	NA	0.77	0.00	0.23
Linderniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Loasaceae	0.00	0.00	NA	0.00	0.00	NA	1.00	1.00	NA	0.00	1.00	0.00
Loganiaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Loranthaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Lunulariaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lycopodiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lythraceae	0.71	NA	NA	0.14	NA	NA	0.14	NA	NA	0.71	0.14	0.14
Magnoliaceae	0.89	NA	NA	0.11	NA	NA	0.00	NA	NA	0.89	0.00	0.11
Malpighiaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Malvaceae	0.98	NA	NA	0.00	NA	NA	0.02	NA	NA	0.98	0.02	0.00
Bombacoideae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sterculioideae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tilioideae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Marantaceae	0.80	NA	NA	0.00	NA	NA	0.20	NA	NA	0.80	0.20	0.00

Marattiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Marchantiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Marsileaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Melanthiaceae	0.75	NA	NA	0.25	NA	NA	0.00	NA	NA	0.75	0.00	0.25
Melastomataceae	0.88	NA	NA	0.13	NA	NA	0.00	NA	NA	0.88	0.00	0.13
Meliaceae	0.95	1.00	NA	0.05	0.00	NA	0.00	0.00	NA	0.98	0.00	0.03
Menispermaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Menyanthaceae	0.00	0.00	NA	0.50	1.00	NA	0.50	0.00	NA	0.00	0.25	0.75
Mesembranthaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Metzgeriaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mimosaceae	NA	1.00	NA	NA	0.00	NA	NA	0.00	NA	1.00	0.00	0.00
Misodendraceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Mitrastemonaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Molluginaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Montiaceae	0.25	NA	NA	0.25	NA	NA	0.50	NA	NA	0.25	0.50	0.25
Moraceae	0.78	NA	NA	0.11	NA	NA	0.11	NA	NA	0.78	0.11	0.11
Moringaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Musaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Myricaceae	0.60	0.00	NA	0.40	0.00	NA	0.00	1.00	NA	0.30	0.50	0.20
Myristicaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Myrsinaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Myrtaceae	0.97	1.00	1	0.01	0.00	0	0.01	0.00	0	0.99	0.00	0.00
Najadaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Nartheciaceae	0.00	NA	NA	1.00	NA	NA	0.00	NA	NA	0.00	0.00	1.00

Nelumbonaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Nepenthaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Nephrolepidaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Nitrariaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Nothofagaceae	1.00	1.00	NA	0.00	0.00	NA	0.00	0.00	NA	1.00	0.00	0.00
Nyctaginaceae	0.33	1.00	0	0.17	0.00	1	0.50	0.00	0	0.44	0.17	0.39
Nymphaeaceae	0.00	0.00	NA	0.00	1.00	NA	1.00	0.00	NA	0.00	0.50	0.50
Ochnaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Olacaceae	1.00	0.00	NA	0.00	0.00	NA	0.00	1.00	NA	0.50	0.50	0.00
Oleaceae	0.66	NA	NA	0.24	NA	NA	0.10	NA	NA	0.66	0.10	0.24
Onagraceae	0.72	NA	NA	0.21	NA	NA	0.07	NA	NA	0.72	0.07	0.21
Ophioglossaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Opiliaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Orobanchaceae	0.08	0.00	NA	0.13	0.00	NA	0.79	1.00	NA	0.04	0.90	0.06
Osmundaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Oxalidaceae	0.86	NA	NA	0.14	NA	NA	0.00	NA	NA	0.86	0.00	0.14
Paeoniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Pandaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Pandanaceae	0.33	NA	NA	0.00	NA	NA	0.67	NA	NA	0.33	0.67	0.00
Papaveraceae	0.21	0.00	NA	0.16	1.00	NA	0.63	0.00	NA	0.11	0.32	0.58
Papilionaceae	NA	1.00	NA	NA	0.00	NA	NA	0.00	NA	1.00	0.00	0.00
Parnassiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Passifloraceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Paulowniaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00

Pedaliaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Pelliaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Petrosaviaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Phrymaceae	0.50	NA	NA	0.50	NA	NA	0.00	NA	NA	0.50	0.00	0.50
Phyllanthaceae	0.79	1.00	NA	0.05	0.00	NA	0.16	0.00	NA	0.89	0.08	0.03
Phytolaccaceae	NA	0.00	0	NA	0.00	1	NA	1.00	0	0.00	0.50	0.50
Picrodendraceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Pinaceae	1.00	1.00	1	0.00	0.00	0	0.00	0.00	0	1.00	0.00	0.00
Piperaceae	0.71	0.00	NA	0.14	1.00	NA	0.14	0.00	NA	0.36	0.07	0.57
Pittosporaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Plagiochilaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Plagiogyriaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Plantaginaceae	0.70	NA	NA	0.16	NA	NA	0.14	NA	NA	0.70	0.14	0.16
Plantanaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Plumbaginaceae	0.40	0.00	NA	0.20	1.00	NA	0.40	0.00	NA	0.20	0.20	0.60
Poaceae	0.73	NA	NA	0.19	NA	NA	0.07	NA	NA	0.73	0.07	0.19
Podocarpaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Podostemaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Polemoniaceae	0.67	NA	NA	0.17	NA	NA	0.17	NA	NA	0.67	0.17	0.17
Polygalaceae	0.75	NA	NA	0.25	NA	NA	0.00	NA	NA	0.75	0.00	0.25
Polygonaceae	0.22	1.00	0	0.35	0.00	1	0.43	0.00	0	0.41	0.14	0.45
Polypodiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pontederiaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Porellaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Portulacaceae	0.00	0.00	0	0.20	1.00	1	0.80	0.00	0	0.00	0.27	0.73
Posidoniaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Potamogetonaceae	0.11	0.00	NA	0.11	1.00	NA	0.78	0.00	NA	0.06	0.39	0.56
Primulaceae	0.56	NA	NA	0.28	NA	NA	0.16	NA	NA	0.56	0.16	0.28
Proteaceae	0.67	0.00	NA	0.00	0.00	NA	0.33	1.00	NA	0.33	0.67	0.00
Pseudolepicoleaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Psilotaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pteridaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Putranjivaceae	0.50	NA	NA	0.00	NA	NA	0.50	NA	NA	0.50	0.50	0.00
Quiinaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Radulaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Rafflesiaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Ranunculaceae	0.77	NA	NA	0.16	NA	NA	0.07	NA	NA	0.77	0.07	0.16
Resedaceae	0.00	0.00	NA	1.00	1.00	NA	0.00	0.00	NA	0.00	0.00	1.00
Restoniaceae	1.00	0.00	NA	0.00	0.00	NA	0.00	1.00	NA	0.50	0.50	0.00
Rhamnaceae	0.78	1.00	NA	0.17	0.00	NA	0.04	0.00	NA	0.89	0.02	0.09
Rhizophoraceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Ricciaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Roridulaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Rosaceae	0.70	1.00	NA	0.26	0.00	NA	0.04	0.00	NA	0.85	0.02	0.13
Rubiaceae	0.76	NA	NA	0.13	NA	NA	0.11	NA	NA	0.76	0.11	0.13
Ruppiaceae	NA	0.00	NA	NA	1.00	NA	NA	0.00	NA	0.00	0.00	1.00
Ruscaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Rutaceae	0.96	NA	NA	0.04	NA	NA	0.00	NA	NA	0.96	0.00	0.04

Salicaceae	0.97	1.00	1	0.01	0.00	0	0.01	0.00	0	0.99	0.00	0.00
Santalaceae	0.20	0.00	NA	0.00	1.00	NA	0.80	0.00	NA	0.10	0.40	0.50
Sapindaceae	0.71	NA	NA	0.29	NA	NA	0.00	NA	NA	0.71	0.00	0.29
Sapotaceae	1.00	1.00	NA	0.00	0.00	NA	0.00	0.00	NA	1.00	0.00	0.00
Sarcolaenaceae	NA	1.00	NA	NA	0.00	NA	NA	0.00	NA	1.00	0.00	0.00
Sarraceniaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Saxifragaceae	0.10	0.00	NA	0.23	1.00	NA	0.67	0.00	NA	0.05	0.33	0.62
Scapaniaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Scheuchzeriaceae	0.00	NA	NA	0.00	NA	NA	1.00	NA	NA	0.00	1.00	0.00
Schisandraceae	0.75	NA	NA	0.25	NA	NA	0.00	NA	NA	0.75	0.00	0.25
Schizaeaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Scrophulariaceae	0.65	0.00	NA	0.15	0.00	NA	0.20	1.00	NA	0.33	0.60	0.08
Selaginellaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Simaroubaceae	0.67	NA	NA	0.33	NA	NA	0.00	NA	NA	0.67	0.00	0.33
Smilacaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Solanaceae	0.90	NA	NA	0.06	NA	NA	0.03	NA	NA	0.90	0.03	0.06
Sparganiaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Staphyleaceae	0.00	NA	NA	0.50	NA	NA	0.50	NA	NA	0.00	0.50	0.50
Stegnospermataceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Styracaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tamaricaceae	0.80	0.00	NA	0.20	1.00	NA	0.00	0.00	NA	0.40	0.00	0.60
Taxaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Taxodiaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00

Tetramelaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Tetrameristaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Theaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Thelypteridaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Themidaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Theophrastaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Thymelaeaceae	0.88	NA	NA	0.13	NA	NA	0.00	NA	NA	0.88	0.00	0.13
Tiliaceae	NA	0.00	1	NA	1.00	0	NA	0.00	0	0.50	0.00	0.50
Tofieldiaceae	0.33	NA	NA	0.33	NA	NA	0.33	NA	NA	0.33	0.33	0.33
Triuridaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Tropaeolaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Turneraceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Typhaceae	0.25	0.00	NA	0.50	0.00	NA	0.25	1.00	NA	0.13	0.63	0.25
Ulmaceae	0.33	NA	NA	0.67	NA	NA	0.00	NA	NA	0.33	0.00	0.67
Urticaceae	0.71	0.00	0	0.14	1.00	1	0.14	0.00	0	0.24	0.05	0.71
Valerianaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Verbenaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Violaceae	0.69	NA	NA	0.24	NA	NA	0.07	NA	NA	0.69	0.07	0.24
Viscaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Vitaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Vittariaceae	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Welwitschiaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Xanthorrhoeaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Xyridaceae	NA	0.00	NA	NA	0.00	NA	NA	1.00	NA	0.00	1.00	0.00
Zamiaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00

Zingiberaceae	1.00	NA	NA	0.00	NA	NA	0.00	NA	NA	1.00	0.00	0.00
Zosteraceae	0.00	0.00	NA	0.00	0.00	NA	1.00	1.00	NA	0.00	1.00	0.00
Zygophyllaceae	0.64	0.00	NA	0.09	1.00	NA	0.27	0.00	NA	0.32	0.14	0.55

SI Table 3. Model results with AMNM plants assigned to NM.

GLM explaining proportion mycorrhizal to non-mycorrhizal (M:NM) plant species with AMNM plants assigned to non-mycorrhizal.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

M1.2: Native Model (GLMM), N = 1961				
	Estimate (coefficient)	Std. Error	Z-value	p
(intercept)	4.76610	0.05101	93.444	***
mycorrhizal status (non-mycorrhizal)	-1.69763	0.01464	-115.977	***
land type (mainland)	2.45925	0.07030	34.984	***
mycorrhizal status*land type	-0.12324	0.01883	-6.545	***
M2.2: Naturalized Model (GLMM), N = 1176				
	Estimate (coefficient)	Std. Error	Z-value	p
(intercept)	3.92857	0.09792	40.120	***
mycorrhizal status (non-mycorrhizal)	-1.65671	0.02656	-62.376	***
land type (mainland)	0.77747	0.11747	6.619	***
mycorrhizal status*land type	0.21854	0.03019	7.238	***
M3.2: Mainland Native Model (GLM), N = 515				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	1.391e+00	2.369e-02	58.735	***
area	1.352e-02	3.902e-03	-3.466	***
precipitation	1.205e-04	4.558e-06	26.438	
temperature	1.440e-02	4.617e-04	31.197	***
elevation range	4.255e-02	6.849e-03	6.212	***
spatial autocovariate	6.485e-02	1.368e-03	47.404	***

M4.2: Island Native Model (GLM), N = 422				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	1.077e+00	5.622e-02	19.156	***
area	3.349e-02	8.036e-03	4.168	***
distance	-6.050e-03	1.740e-02	-0.348	
geology (non-oceanic)	-3.184e-02	5.501e-02	-0.579	
precipitation	1.011e-04	9.309e-06	10.856	***
temperature	2.055e-02	9.996e-04	20.559	***
elevation range	2.549e-02	1.458e-02	1.749	
distance*geology (non-oceanic)	1.795e-03	2.061e-02	0.087	
spatial autocovariate	1.328e-01	8.978e-03	14.796	***
M5.2: Island Age Native Model (GLM), N = 246				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	8.980e-01	9.039e-02	9.934	***
area	5.690e-02	1.535e-02	3.708	***
distance	6.308e-03	2.361e-02	0.267	
precipitation	8.414e-05	1.410e-05	5.968	***
temperature	2.222e-02	2.032e-03	10.936	***
age	1.609e-02	1.805e-02	0.891	
elevation range	5.579e-02	1.883e-02	2.962	**
spatial autocovariate	1.512e-01	1.427e-02	10.599	***
M6.2: Mainland Naturalized Model (GLM), N = 294				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	8.728e-01	1.058e-01	8.249	***
area	-2.059e-02	1.940e-02	-1.061	
precipitation	9.305e-05	2.418e-05	3.847	***
temperature	2.375e-02	1.890e-03	12.568	***
elevation range	6.520e-02	2.540e-02	2.567	*
population density	2.510e-02	1.201e-02	2.089	*
spatial autocovariate	1.730e-01	1.374e-02	12.584	***
M7.2: Island Naturalized Model (GLM), N = 142				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	1.281e+00	1.837e-01	6.974	***

area	2.387e-02	2.544e-02	0.938	
distance	-5.840e-02	6.087e-02	-0.959	
geology (non-oceanic)	-4.320e-01	2.270e-01	-1.903	
precipitation	5.241e-05	3.140e-05	1.669	
temperature	2.777e-02	4.775e-03	5.815	***
population density	-4.960e-03	2.485e-02	-0.200	
elevation range	-4.030e-02	3.674e-02	-1.097	
distance*geology (non-oceanic)	1.577e-01	7.409e-02	2.129	
spatial autocovariate	9.311e-02	5.131e-02	1.815	***
M8.2: Island Age Naturalized Model (GLM), N = 97				
	Estimate (coefficient)	Std. Error	Z-value	*p*
(intercept)	1.496e+00	3.234e-01	4.626	***
area	7.508e-02	3.974e-02	1.889	
distance	-2.459e-01	1.059e-01	-2.323	*
precipitation	6.413e-05	3.574e-05	1.794	
temperature	4.068e-02	6.767e-03	6.012	***
age	-2.902e-03	4.614e-02	-0.063	
elevation range	-3.325e-02	4.246e-02	-0.783	
population density	-1.443e-02	2.856e-02	-0.505	
spatial autocovariate	8.379e-02	7.010e-02	1.195	***

SI Table 4. Regions used in study with associated coordinates, mycorrhizal data and native status.

Each region along with associated latitude, longitude, mycorrhizal species counts (either 1 where AMNM is categorized as M or 2 where AMNM is categorized as NM), non-mycorrhizal species counts (either 1 or 2 as described above) and native status (either native or naturalized).

region	longitude	latitude	myc1	nonmyc1	myc2	nonmyc2	native
1	123.08	-12.27	12	10	18	4	native
1	123.08	-12.27	1	2	3	0	naturalized
3	105.64	-10.49	211	77	252	36	native
3	105.64	-10.49	88	43	111	20	naturalized
4	152.12	-19.01	12	9	17	4	native
4	152.12	-19.01	2	2	3	1	naturalized
21	NA	NA	1	3	3	1	native
21	NA	NA	0	0	0	0	naturalized
27	NA	NA	4	4	6	2	native
27	NA	NA	0	0	0	0	naturalized
29	113.60	-28.30	27	25	43	9	native
29	113.60	-28.30	0	0	0	0	naturalized
32	NA	NA	1	2	2	1	native
32	NA	NA	0	0	0	0	naturalized
40	113.73	-28.44	56	36	77	15	native
40	113.73	-28.44	11	13	18	6	naturalized
42	NA	NA	7	11	11	7	native
42	NA	NA	0	0	0	0	naturalized
93	NA	NA	1	1	2	0	native
93	NA	NA	0	0	0	0	naturalized
100	113.97	-28.95	11	18	21	8	native
100	113.97	-28.95	8	9	12	5	naturalized
125	96.87	-12.16	41	22	51	12	native
125	96.87	-12.16	30	12	36	6	naturalized
126	96.82	-11.83	37	19	48	8	native
126	96.82	-11.83	8	4	10	2	naturalized
127	159.08	-31.55	259	126	331	54	native
127	159.08	-31.55	137	69	175	31	naturalized

128	167.95	-29.03	75	41	99	17	native
128	167.95	-29.03	134	79	176	37	naturalized
129	158.86	-54.63	87	78	131	34	native
129	158.86	-54.63	1	2	2	1	naturalized
131	-31.11	39.70	57	42	79	20	native
131	-31.11	39.70	148	141	224	65	naturalized
132	-31.20	39.44	71	51	98	24	native
132	-31.20	39.44	276	202	386	92	naturalized
133	-28.70	38.58	75	50	102	23	native
133	-28.70	38.58	421	267	568	120	naturalized
134	-28.33	38.47	79	55	109	25	native
134	-28.33	38.47	302	224	423	103	naturalized
135	-28.01	39.05	44	30	60	14	native
135	-28.01	39.05	250	187	355	82	naturalized
136	-28.03	38.64	73	50	101	22	native
136	-28.03	38.64	258	200	368	90	naturalized
137	-27.21	38.72	77	53	108	22	native
137	-27.21	38.72	351	277	502	126	naturalized
138	-25.48	37.80	81	55	112	24	native
138	-25.48	37.80	393	293	553	133	naturalized
139	-25.10	36.97	58	42	80	20	native
139	-25.10	36.97	384	254	523	115	naturalized
140	-120.37	34.04	121	83	161	43	native
140	-120.37	34.04	0	0	0	0	naturalized
141	-120.11	33.97	226	144	303	67	native
141	-120.11	33.97	0	0	0	0	naturalized
142	-119.75	34.01	284	174	375	83	native
142	-119.75	34.01	0	0	0	0	naturalized
143	-119.42	34.01	113	70	149	34	native
143	-119.42	34.01	0	0	0	0	naturalized
144	-119.04	33.48	46	37	66	17	native
144	-119.04	33.48	0	0	0	0	naturalized
145	-18.01	27.75	221	136	288	69	native
145	-18.01	27.75	0	0	0	0	naturalized
146	-17.86	28.69	259	167	337	89	native
146	-17.86	28.69	0	0	0	0	naturalized
147	-17.23	28.12	282	179	368	93	native
147	-17.23	28.12	0	0	0	0	naturalized
148	-16.56	28.29	407	270	538	139	native

148	-16.56	28.29	0	0	0	0	naturalized
149	-15.59	27.95	349	223	461	111	native
149	-15.59	27.95	0	0	0	0	naturalized
150	-14.04	28.41	223	154	308	69	native
150	-14.04	28.41	0	0	0	0	naturalized
151	-13.64	29.02	207	144	284	67	native
151	-13.64	29.02	0	0	0	0	naturalized
152	-60.11	-51.75	145	91	197	39	native
152	-60.11	-51.75	0	0	0	0	naturalized
153	-58.75	-51.74	143	91	196	38	native
153	-58.75	-51.74	0	0	0	0	naturalized
154	-92.00	1.65	9	7	13	3	native
154	-92.00	1.65	0	0	0	0	naturalized
155	-89.68	-1.38	69	39	91	17	native
155	-89.68	-1.38	6	3	8	1	naturalized
156	-91.51	-0.39	101	49	129	21	native
156	-91.51	-0.39	2	0	2	0	naturalized
157	-90.44	-1.29	142	76	185	33	native
157	-90.44	-1.29	74	27	88	13	naturalized
158	-89.96	0.33	28	22	39	11	native
158	-89.96	0.33	1	0	1	0	naturalized
159	-91.18	-0.56	191	102	247	46	native
159	-91.18	-0.56	93	41	115	19	naturalized
160	-90.48	0.33	34	17	43	8	native
160	-90.48	0.33	0	0	0	0	naturalized
161	-90.76	0.59	95	46	119	22	native
161	-90.76	0.59	2	3	4	1	naturalized
162	-90.67	-0.61	64	42	87	19	native
162	-90.67	-0.61	0	1	1	0	naturalized
163	-89.43	-0.83	162	85	210	37	native
163	-89.43	-0.83	108	46	132	22	naturalized
164	-90.36	-0.63	223	119	288	54	native
164	-90.36	-0.63	123	54	151	26	naturalized
165	-90.06	-0.82	45	27	60	12	native
165	-90.06	-0.82	0	1	1	0	naturalized
166	-90.71	-0.27	154	90	203	41	native
166	-90.71	-0.27	23	10	28	5	naturalized
167	-91.82	1.38	13	14	20	7	native
167	-91.82	1.38	0	0	0	0	naturalized

168	NA	NA	2	1	3	0	native
168	NA	NA	0	0	0	0	naturalized
171	-155.52	19.60	557	248	717	88	native
171	-155.52	19.60	466	240	591	115	naturalized
172	-159.52	22.06	725	306	920	111	native
172	-159.52	22.06	364	182	459	87	naturalized
173	-156.61	20.55	79	46	106	19	native
173	-156.61	20.55	62	28	76	14	naturalized
176	-156.93	20.83	352	171	460	63	native
176	-156.93	20.83	195	90	240	45	naturalized
177	-171.73	25.77	48	40	69	19	native
177	-171.73	25.77	6	4	8	2	naturalized
178	-160.10	22.02	14	11	21	4	native
178	-160.10	22.02	15	8	21	2	naturalized
179	-173.97	26.06	15	15	24	6	native
179	-173.97	26.06	2	1	3	0	naturalized
180	-156.34	20.79	718	314	926	106	native
180	-156.34	20.79	459	226	580	105	naturalized
181	-177.37	28.21	34	24	46	12	native
181	-177.37	28.21	62	34	80	16	naturalized
183	-157.01	21.13	521	237	673	85	native
183	-157.01	21.13	253	126	320	59	naturalized
186	-160.15	21.90	121	68	160	29	native
186	-160.15	21.90	46	20	57	9	naturalized
187	-157.97	21.46	677	291	862	106	native
187	-157.97	21.46	493	229	616	106	naturalized
189	-18.59	64.99	321	338	512	147	native
189	-18.59	64.99	0	0	0	0	naturalized
191	134.49	-33.72	65	47	93	19	native
191	134.49	-33.72	0	0	0	0	naturalized
192	134.80	-33.60	10	15	19	6	native
192	134.80	-33.60	0	0	0	0	naturalized
197	134.27	-33.95	37	32	56	13	native
197	134.27	-33.95	0	0	0	0	naturalized
200	-7.90	53.43	724	593	1052	265	native
200	-7.90	53.43	0	0	0	0	naturalized
201	137.24	-35.83	500	271	643	128	native
201	137.24	-35.83	146	95	197	44	naturalized
202	55.53	-21.13	950	388	1191	147	native

202	55.53	-21.13	316	145	389	72	naturalized
203	-140.69	-8.00	47	25	61	11	native
203	-140.69	-8.00	13	2	14	1	naturalized
204	-138.64	-10.47	146	66	186	26	native
204	-138.64	-10.47	130	38	150	18	naturalized
206	-140.57	-7.92	32	16	42	6	native
206	-140.57	-7.92	12	3	14	1	naturalized
207	-139.01	-9.77	173	82	223	32	native
207	-139.01	-9.77	161	54	186	29	naturalized
208	-138.83	-9.99	47	20	59	8	native
208	-138.83	-9.99	22	6	25	3	naturalized
211	-140.14	-8.87	200	92	253	39	native
211	-140.14	-8.87	169	60	198	31	naturalized
212	-139.09	-9.94	125	60	163	22	native
212	-139.09	-9.94	60	19	70	9	naturalized
213	-139.55	-8.91	112	57	148	21	native
213	-139.55	-8.91	118	34	134	18	naturalized
214	-140.07	-9.40	112	51	144	19	native
214	-140.07	-9.40	101	33	119	15	naturalized
215	172.60	-41.79	1337	663	1717	283	native
215	172.60	-41.79	878	537	1161	254	naturalized
216	-130.11	-25.07	61	29	77	13	native
216	-130.11	-25.07	0	0	0	0	naturalized
217	-128.32	-24.38	67	36	85	18	native
217	-128.32	-24.38	0	0	0	0	naturalized
218	-130.74	-23.93	17	10	21	6	native
218	-130.74	-23.93	0	0	0	0	naturalized
219	-124.78	-24.68	3	1	3	1	native
219	-124.78	-24.68	0	0	0	0	naturalized
220	166.88	11.29	17	8	22	3	native
220	166.88	11.29	23	10	28	5	naturalized
221	166.43	11.13	12	7	16	3	native
221	166.43	11.13	5	3	6	2	naturalized
231	130.42	30.81	100	48	130	18	native
231	130.42	30.81	0	0	0	0	naturalized
232	130.29	30.79	97	46	125	18	native
232	130.29	30.79	0	0	0	0	naturalized
233	129.93	30.83	249	129	325	53	native
233	129.93	30.83	0	0	0	0	naturalized

234	130.98	30.58	468	267	627	108	native
234	130.98	30.58	0	0	0	0	naturalized
235	130.52	30.34	569	300	754	115	native
235	130.52	30.34	0	0	0	0	naturalized
236	130.21	30.46	194	94	254	34	native
236	130.21	30.46	0	0	0	0	naturalized
237	129.92	29.97	210	101	272	39	native
237	129.92	29.97	0	0	0	0	naturalized
238	129.87	29.85	283	157	374	66	native
238	129.87	29.85	0	0	0	0	naturalized
239	129.54	29.90	143	71	185	29	native
239	129.54	29.90	0	0	0	0	naturalized
240	129.53	29.69	151	78	197	32	native
240	129.53	29.69	0	0	0	0	naturalized
241	129.71	29.64	160	76	205	31	native
241	129.71	29.64	0	0	0	0	naturalized
242	129.60	29.46	196	94	252	38	native
242	129.60	29.46	0	0	0	0	naturalized
243	129.33	29.22	100	46	126	20	native
243	129.33	29.22	0	0	0	0	naturalized
244	129.21	29.15	259	129	336	52	native
244	129.21	29.15	0	0	0	0	naturalized
245	128.99	28.80	41	17	51	7	native
245	128.99	28.80	0	0	0	0	naturalized
246	129.43	28.31	487	262	641	108	native
246	129.43	28.31	0	0	0	0	naturalized
247	129.97	28.32	280	147	365	62	native
247	129.97	28.32	0	0	0	0	naturalized
248	128.95	27.77	398	205	520	83	native
248	128.95	27.77	0	0	0	0	naturalized
249	128.60	27.38	361	174	463	72	native
249	128.60	27.38	0	0	0	0	naturalized
250	128.43	27.04	231	116	298	49	native
250	128.43	27.04	0	0	0	0	naturalized
251	127.96	26.49	570	300	743	127	native
251	127.96	26.49	0	0	0	0	naturalized
252	126.77	26.35	369	174	468	75	native
252	126.77	26.35	0	0	0	0	naturalized
253	125.33	24.77	353	178	453	78	native

253	125.33	24.77	0	0	0	0	naturalized
254	124.21	24.43	527	252	670	109	native
254	124.21	24.43	0	0	0	0	naturalized
255	123.81	24.34	524	263	674	113	native
255	123.81	24.34	0	0	0	0	naturalized
256	122.99	24.46	364	187	470	81	native
256	122.99	24.46	0	0	0	0	naturalized
257	19.05	74.45	22	36	40	18	native
257	19.05	74.45	0	0	0	0	naturalized
258	16.58	76.49	3	9	7	5	native
258	16.58	76.49	0	0	0	0	naturalized
259	25.23	76.58	5	13	12	6	native
259	25.23	76.58	0	0	0	0	naturalized
260	22.50	77.80	23	44	45	22	native
260	22.50	77.80	0	0	0	0	naturalized
261	21.34	78.42	19	39	38	20	native
261	21.34	78.42	0	0	0	0	naturalized
262	26.65	78.72	9	21	20	10	native
262	26.65	78.72	0	0	0	0	naturalized
263	11.22	78.57	17	48	40	25	native
263	11.22	78.57	0	0	0	0	naturalized
264	28.69	78.90	11	24	23	12	native
264	28.69	78.90	0	0	0	0	naturalized
265	10.91	79.68	8	22	19	11	native
265	10.91	79.68	0	0	0	0	naturalized
266	10.83	79.76	7	13	15	5	native
266	10.83	79.76	0	0	0	0	naturalized
267	14.49	80.03	2	4	4	2	native
267	14.49	80.03	0	0	0	0	naturalized
268	15.88	78.61	67	87	111	43	native
268	15.88	78.61	0	0	0	0	naturalized
269	28.02	80.13	1	1	2	0	native
269	28.02	80.13	0	0	0	0	naturalized
271	32.57	80.15	1	5	3	3	native
271	32.57	80.15	0	0	0	0	naturalized
272	18.36	80.31	0	4	2	2	native
272	18.36	80.31	0	0	0	0	naturalized
273	22.76	79.85	28	51	52	27	native
273	22.76	79.85	0	0	0	0	naturalized

274	20.66	80.65	1	3	2	2	native
274	20.66	80.65	0	0	0	0	naturalized
275	25.01	80.66	1	2	2	1	native
275	25.01	80.66	0	0	0	0	naturalized
276	20.79	80.72	3	8	7	4	native
276	20.79	80.72	0	0	0	0	naturalized
277	46.37	-9.43	329	178	418	89	native
277	46.37	-9.43	33	9	38	4	naturalized
278	46.51	-9.73	175	81	217	39	native
278	46.51	-9.73	25	7	29	3	naturalized
279	47.57	-9.73	173	89	219	43	native
279	47.57	-9.73	18	5	21	2	naturalized
280	47.74	-10.09	184	79	226	37	native
280	47.74	-10.09	23	4	26	1	naturalized
281	50.73	-9.33	26	15	35	6	native
281	50.73	-9.33	6	2	6	2	naturalized
282	50.99	-9.53	16	9	20	5	native
282	50.99	-9.53	4	2	4	2	naturalized
283	51.17	-10.16	65	33	84	14	native
283	51.17	-10.16	24	8	28	4	naturalized
284	56.64	-10.42	7	4	10	1	native
284	56.64	-10.42	0	0	0	0	naturalized
285	59.59	-16.60	15	8	18	5	native
285	59.59	-16.60	0	0	0	0	naturalized
288	53.67	-5.69	56	23	68	11	native
288	53.67	-5.69	17	3	18	2	naturalized
289	56.28	-7.13	58	30	74	14	native
289	56.28	-7.13	30	13	37	6	naturalized
290	53.30	-5.42	62	30	76	16	native
290	53.30	-5.42	29	11	34	6	naturalized
293	-64.34	18.73	203	82	250	35	native
293	-64.34	18.73	0	0	0	0	naturalized
294	-63.05	18.21	165	64	197	32	native
294	-63.05	18.21	0	0	0	0	naturalized
295	-61.80	17.08	412	170	503	79	native
295	-61.80	17.08	0	0	0	0	naturalized
296	-76.64	24.26	1043	420	1282	181	native
296	-76.64	24.26	27	7	32	2	naturalized
297	-59.56	13.17	338	130	410	58	native

297	-59.56	13.17	0	0	0	0	naturalized
298	-61.79	17.63	131	52	157	26	native
298	-61.79	17.63	0	0	0	0	naturalized
299	-71.52	17.58	123	45	149	19	native
299	-71.52	17.58	0	0	0	0	naturalized
300	-66.52	17.89	83	28	98	13	native
300	-66.52	17.89	0	0	0	0	naturalized
303	-79.03	21.63	5871	1986	7040	817	native
303	-79.03	21.63	300	126	362	64	naturalized
304	-65.29	18.32	146	53	173	26	native
304	-65.29	18.32	0	0	0	0	naturalized
306	-67.48	18.38	105	38	125	18	native
306	-67.48	18.38	0	0	0	0	naturalized
307	-61.35	15.43	544	212	660	96	native
307	-61.35	15.43	0	0	0	0	naturalized
308	-73.05	18.84	508	175	607	76	native
308	-73.05	18.84	0	0	0	0	naturalized
310	-61.68	12.11	422	167	514	75	native
310	-61.68	12.11	0	0	0	0	naturalized
311	-61.58	16.23	720	296	883	133	native
311	-61.58	16.23	0	0	0	0	naturalized
312	-64.57	18.48	190	63	224	29	native
312	-64.57	18.48	0	0	0	0	naturalized
315	-77.31	18.16	2331	972	2938	365	native
315	-77.31	18.16	13	4	16	1	naturalized
316	-64.75	18.45	52	17	61	8	native
316	-64.75	18.45	0	0	0	0	naturalized
318	-61.27	15.93	170	76	208	38	native
318	-61.27	15.93	0	0	0	0	naturalized
319	-61.02	14.65	692	275	841	126	native
319	-61.02	14.65	0	0	0	0	naturalized
320	-67.89	18.09	238	87	287	38	native
320	-67.89	18.09	0	0	0	0	naturalized
322	-62.19	16.74	333	137	408	62	native
322	-62.19	16.74	14	4	16	2	naturalized
323	-75.01	18.40	71	21	83	9	native
323	-75.01	18.40	15	6	19	2	naturalized
324	-62.59	17.15	84	42	110	16	native
324	-62.59	17.15	0	0	0	0	naturalized

326	-66.47	18.22	0	0	0	0	native
326	-66.47	18.22	457	170	543	84	naturalized
327	-63.24	17.63	462	185	569	78	native
327	-63.24	17.63	20	8	24	4	naturalized
329	-62.83	17.90	204	79	241	42	native
329	-62.83	17.90	0	0	0	0	naturalized
330	-64.77	17.73	429	168	522	75	native
330	-64.77	17.73	0	0	0	0	naturalized
331	-62.98	17.49	449	166	538	77	native
331	-62.98	17.49	21	8	26	3	naturalized
332	-64.74	18.34	384	136	456	64	native
332	-64.74	18.34	0	0	0	0	naturalized
333	-62.77	17.34	266	112	330	48	native
333	-62.77	17.34	0	0	0	0	naturalized
334	-60.97	13.90	589	223	714	98	native
334	-60.97	13.90	0	0	0	0	naturalized
335	-63.06	18.06	542	213	659	96	native
335	-63.06	18.06	21	7	25	3	naturalized
336	-64.93	18.34	442	175	538	79	native
336	-64.93	18.34	0	0	0	0	naturalized
337	-61.19	13.25	515	177	613	79	native
337	-61.19	13.25	0	0	0	0	naturalized
338	-61.28	12.84	204	70	237	37	native
338	-61.28	12.84	0	0	0	0	naturalized
339	-64.62	18.42	356	129	426	59	native
339	-64.62	18.42	0	0	0	0	naturalized
340	-72.79	20.04	423	138	507	54	native
340	-72.79	20.04	0	0	0	0	naturalized
342	-65.44	18.12	402	146	484	64	native
342	-65.44	18.12	0	0	0	0	naturalized
343	-64.40	18.48	255	89	303	41	native
343	-64.40	18.48	0	0	0	0	naturalized
344	109.75	19.20	3428	1274	4128	574	native
344	109.75	19.20	93	55	120	28	naturalized
345	114.13	22.38	1056	374	1254	176	native
345	114.13	22.38	54	41	75	20	naturalized
346	113.56	22.16	647	242	778	111	native
346	113.56	22.16	30	27	42	15	naturalized
347	120.97	23.75	2273	1055	2893	435	native

347	120.97	23.75	490	288	638	140	naturalized
348	-110.98	18.79	142	48	170	20	native
348	-110.98	18.79	26	10	32	4	naturalized
349	-110.80	19.31	7	4	10	1	native
349	-110.80	19.31	0	0	0	0	naturalized
350	-114.72	18.36	29	14	36	7	native
350	-114.72	18.36	0	0	0	0	naturalized
351	-118.29	29.04	84	57	111	30	native
351	-118.29	29.04	0	0	0	0	naturalized
352	178.77	-49.69	50	40	73	17	native
352	178.77	-49.69	0	0	0	0	naturalized
354	-159.30	3.85	12	11	16	7	native
354	-159.30	3.85	0	0	0	0	naturalized
355	150.93	8.62	49	23	64	8	native
355	150.93	8.62	0	0	0	0	naturalized
356	-9.94	-40.32	41	25	56	10	native
356	-9.94	-40.32	6	4	9	1	naturalized
357	53.80	12.49	714	349	895	168	native
357	53.80	12.49	0	0	0	0	naturalized
358	-150.21	-9.96	10	9	14	5	native
358	-150.21	-9.96	0	0	0	0	naturalized
359	-169.86	-19.05	161	52	187	26	native
359	-169.86	-19.05	0	0	0	0	naturalized
360	154.80	1.07	26	9	31	4	native
360	154.80	1.07	0	0	0	0	naturalized
361	-87.06	5.53	78	29	96	11	native
361	-87.06	5.53	0	0	0	0	naturalized
362	-177.99	-29.38	68	43	93	18	native
362	-177.99	-29.38	72	44	94	22	naturalized
363	7.40	1.62	436	150	519	67	native
363	7.40	1.62	0	0	0	0	naturalized
364	6.60	0.24	917	308	1089	136	native
364	6.60	0.24	0	0	0	0	naturalized
365	5.63	-1.44	300	120	368	52	native
365	5.63	-1.44	0	0	0	0	naturalized
366	37.94	-46.64	11	12	16	7	native
366	37.94	-46.64	1	2	2	1	naturalized
367	37.74	-46.91	12	15	19	8	native
367	37.74	-46.91	6	5	9	2	naturalized

368	-159.78	-21.23	125	59	160	24	native
368	-159.78	-21.23	0	0	0	0	naturalized
369	-5.71	-15.97	83	52	114	21	native
369	-5.71	-15.97	136	73	176	33	naturalized
370	-14.37	-7.94	27	18	38	7	native
370	-14.37	-7.94	0	0	0	0	naturalized
371	134.52	7.43	371	165	461	75	native
371	134.52	7.43	0	0	0	0	naturalized
372	-134.96	-23.12	42	26	53	15	native
372	-134.96	-23.12	0	0	0	0	naturalized
373	-174.82	-19.86	397	138	472	63	native
373	-174.82	-19.86	152	66	188	30	naturalized
374	-169.53	16.74	3	3	5	1	native
374	-169.53	16.74	5	3	7	1	naturalized
375	166.63	19.29	12	11	17	6	native
375	166.63	19.29	0	0	0	0	naturalized
376	-69.97	12.51	336	149	420	65	native
376	-69.97	12.51	20	6	24	2	naturalized
377	-68.29	12.18	276	119	342	53	native
377	-68.29	12.18	23	8	28	3	naturalized
378	-68.97	12.19	390	149	473	66	native
378	-68.97	12.19	30	9	36	3	naturalized
379	-159.78	-18.87	43	18	51	10	native
379	-159.78	-18.87	0	0	0	0	naturalized
380	-158.11	-20.00	76	34	93	17	native
380	-158.11	-20.00	0	0	0	0	naturalized
381	-157.92	-21.93	83	38	105	16	native
381	-157.92	-21.93	0	0	0	0	naturalized
382	-161.00	-10.42	20	10	25	5	native
382	-161.00	-10.42	0	0	0	0	naturalized
383	-158.94	-19.27	19	12	25	6	native
383	-158.94	-19.27	0	0	0	0	naturalized
384	-157.34	-20.16	66	32	82	16	native
384	-157.34	-20.16	0	0	0	0	naturalized
385	-157.70	-19.87	72	37	90	19	native
385	-157.70	-19.87	0	0	0	0	naturalized
386	-165.41	-11.56	16	10	21	5	native
386	-165.41	-11.56	0	0	0	0	naturalized
387	-163.15	-18.06	25	11	30	6	native

387	-163.15	-18.06	0	0	0	0	naturalized
388	-161.09	-10.02	22	11	27	6	native
388	-161.09	-10.02	0	0	0	0	naturalized
389	-165.85	-10.88	22	13	29	6	native
389	-165.85	-10.88	0	0	0	0	naturalized
390	-163.13	-13.25	20	10	25	5	native
390	-163.13	-13.25	0	0	0	0	naturalized
391	-158.29	-19.81	19	9	23	5	native
391	-158.29	-19.81	0	0	0	0	naturalized
392	-157.98	-8.99	19	12	25	6	native
392	-157.98	-8.99	0	0	0	0	naturalized
394	-12.29	-37.11	37	24	52	9	native
394	-12.29	-37.11	39	26	53	12	naturalized
395	-12.68	-37.30	35	22	49	8	native
395	-12.68	-37.30	11	6	14	3	naturalized
396	-12.48	-37.42	18	12	26	4	native
396	-12.48	-37.42	2	2	3	1	naturalized
397	-36.69	-54.38	23	22	36	9	native
397	-36.69	-54.38	16	9	22	3	naturalized
398	51.62	-46.36	0	0	0	0	native
398	51.62	-46.36	22	21	33	10	naturalized
399	69.48	-49.30	34	28	47	15	native
399	69.48	-49.30	28	27	42	13	naturalized
400	72.60	-53.04	4	4	5	3	native
400	72.60	-53.04	0	0	0	0	naturalized
401	73.52	-53.09	10	7	13	4	native
401	73.52	-53.09	1	0	1	0	naturalized
402	-8.39	71.01	26	38	45	19	native
402	-8.39	71.01	0	0	0	0	naturalized
404	-29.30	-20.51	19	11	26	4	native
404	-29.30	-20.51	15	9	19	5	naturalized
405	149.95	45.91	166	106	226	46	native
405	149.95	45.91	0	0	0	0	naturalized
406	152.00	46.95	133	86	180	39	native
406	152.00	46.95	2	2	3	1	naturalized
407	152.48	47.34	115	79	158	36	native
407	152.48	47.34	1	0	1	0	naturalized
408	152.82	47.52	56	42	79	19	native
408	152.82	47.52	0	1	1	0	naturalized

409	153.01	47.75	114	78	156	36	native
409	153.01	47.75	0	0	0	0	naturalized
410	153.22	48.08	75	49	102	22	native
410	153.22	48.08	0	2	1	1	naturalized
411	171.22	7.12	32	16	41	7	native
411	171.22	7.12	9	1	9	1	naturalized
412	175.42	-36.20	240	137	318	59	native
412	175.42	-36.20	0	0	0	0	naturalized
413	150.87	46.52	66	30	83	13	native
413	150.87	46.52	0	0	0	0	naturalized
414	-17.00	32.75	401	245	534	112	native
414	-17.00	32.75	348	141	422	67	naturalized
415	-16.51	32.51	114	70	152	32	native
415	-16.51	32.51	8	5	10	3	naturalized
416	-16.34	33.07	206	139	278	67	native
416	-16.34	33.07	46	28	61	13	naturalized
417	-15.92	30.11	62	48	88	22	native
417	-15.92	30.11	8	4	10	2	naturalized
418	-25.17	17.06	106	62	138	30	native
418	-25.17	17.06	0	0	0	0	naturalized
419	-24.97	16.85	80	45	104	21	native
419	-24.97	16.85	0	0	0	0	naturalized
421	-24.26	16.60	80	46	103	23	native
421	-24.26	16.60	0	0	0	0	naturalized
422	-22.93	16.74	48	30	67	11	native
422	-22.93	16.74	0	0	0	0	naturalized
423	-22.81	16.10	61	35	80	16	native
423	-22.81	16.10	0	0	0	0	naturalized
424	-23.16	15.22	57	33	75	15	native
424	-23.16	15.22	0	0	0	0	naturalized
425	-23.62	15.08	99	49	126	22	native
425	-23.62	15.08	0	0	0	0	naturalized
426	-24.38	14.93	84	40	104	20	native
426	-24.38	14.93	0	0	0	0	naturalized
427	-24.71	14.85	59	27	72	14	native
427	-24.71	14.85	0	0	0	0	naturalized
428	-64.77	32.31	128	74	170	32	native
428	-64.77	32.31	140	65	175	30	naturalized
429	-24.75	16.77	31	21	43	9	native

429	-24.75	16.77	0	0	0	0	naturalized
430	-24.67	16.66	26	18	36	8	native
430	-24.67	16.66	0	0	0	0	naturalized
431	-24.59	16.62	30	22	42	10	native
431	-24.59	16.62	0	0	0	0	naturalized
432	-59.91	43.95	68	65	103	30	native
432	-59.91	43.95	0	0	0	0	naturalized
433	-56.04	48.73	0	0	0	0	native
433	-56.04	48.73	165	150	238	77	naturalized
451	-151.74	-16.49	120	55	153	22	native
451	-151.74	-16.49	0	0	0	0	naturalized
460	-151.00	-16.75	129	64	169	24	native
460	-151.00	-16.75	0	0	0	0	naturalized
465	-150.63	-17.66	14	13	21	6	native
465	-150.63	-17.66	0	0	0	0	naturalized
467	-148.26	-15.84	0	0	0	0	native
467	-148.26	-15.84	38	14	44	8	naturalized
469	-134.97	-23.11	0	0	0	0	native
469	-134.97	-23.11	22	7	24	5	naturalized
479	-152.27	-16.44	153	57	183	27	native
479	-152.27	-16.44	0	0	0	0	naturalized
480	-148.07	-17.88	36	20	47	9	native
480	-148.07	-17.88	0	0	0	0	naturalized
481	-149.83	-17.53	182	73	224	31	native
481	-149.83	-17.53	60	22	72	10	naturalized
482	-153.94	-16.80	27	18	36	9	native
482	-153.94	-16.80	0	0	0	0	naturalized
490	-143.05	-20.70	4	3	6	1	native
490	-143.05	-20.70	0	0	0	0	naturalized
494	-151.44	-16.82	245	105	307	43	native
494	-151.44	-16.82	52	18	62	8	naturalized
495	-147.65	-23.87	0	0	0	0	native
495	-147.65	-23.87	50	15	56	9	naturalized
496	-147.57	-15.14	18	13	25	6	native
496	-147.57	-15.14	0	0	0	0	naturalized
497	-144.35	-27.61	0	0	0	0	native
497	-144.35	-27.61	19	9	22	6	naturalized
499	-142.42	-16.07	7	6	10	3	native
499	-142.42	-16.07	0	0	0	0	naturalized

501	-152.81	-22.65	0	0	0	0	native
501	-152.81	-22.65	50	20	59	11	naturalized
502	-151.35	-22.47	0	0	0	0	native
502	-151.35	-22.47	53	19	60	12	naturalized
503	NA	NA	18	11	24	5	native
503	NA	NA	0	0	0	0	naturalized
505	-151.49	-16.62	133	58	167	24	native
505	-151.49	-16.62	22	8	27	3	naturalized
507	-149.40	-17.68	385	156	478	63	native
507	-149.40	-17.68	116	47	140	23	naturalized
509	-145.21	-14.63	16	11	21	6	native
509	-145.21	-14.63	0	0	0	0	naturalized
521	-149.57	-17.00	42	26	55	13	native
521	-149.57	-17.00	0	0	0	0	naturalized
527	-149.48	-23.37	0	0	0	0	native
527	-149.48	-23.37	38	15	42	11	naturalized
528	-151.82	-16.26	34	22	44	12	native
528	-151.82	-16.26	0	0	0	0	naturalized
537	-26.68	-57.08	1	0	1	0	native
537	-26.68	-57.08	0	0	0	0	naturalized
545	NA	NA	3	6	5	4	native
545	NA	NA	0	0	0	0	naturalized
546	103.82	1.36	2941	859	3372	428	native
546	103.82	1.36	107	46	135	18	naturalized
547	2.94	39.15	216	117	278	55	native
547	2.94	39.15	0	0	0	0	naturalized
548	2.32	39.58	66	30	83	13	native
548	2.32	39.58	0	0	0	0	naturalized
549	1.46	38.69	310	191	415	86	native
549	1.46	38.69	0	0	0	0	naturalized
550	1.41	38.98	411	244	542	113	native
550	1.41	38.98	0	0	0	0	naturalized
551	2.96	39.61	589	332	776	145	native
551	2.96	39.61	0	0	0	0	naturalized
552	4.07	39.96	475	269	626	118	native
552	4.07	39.96	0	0	0	0	naturalized
553	166.10	-50.74	206	140	286	60	native
553	166.10	-50.74	9	10	15	4	naturalized
554	-66.76	44.58	50	47	78	19	native

554	-66.76	44.58	0	0	0	0	naturalized
557	146.83	-19.14	424	164	509	79	native
557	146.83	-19.14	0	0	0	0	naturalized
558	-78.85	-33.65	67	41	94	14	native
558	-78.85	-33.65	90	57	122	25	naturalized
559	-80.79	-33.77	40	30	60	10	native
559	-80.79	-33.77	51	36	72	15	naturalized
560	-78.94	-33.71	4	3	6	1	native
560	-78.94	-33.71	6	8	11	3	naturalized
561	-171.42	-14.06	93	27	109	11	native
561	-171.42	-14.06	0	0	0	0	naturalized
566	-170.72	-14.30	163	62	202	23	native
566	-170.72	-14.30	109	44	134	19	naturalized
567	-170.55	-14.28	62	21	75	8	native
567	-170.55	-14.28	49	23	62	10	naturalized
568	-169.66	-14.17	110	37	131	16	native
568	-169.66	-14.17	60	21	72	9	naturalized
569	-169.62	-14.18	101	33	121	13	native
569	-169.62	-14.18	29	10	34	5	naturalized
570	-169.47	-14.24	125	47	152	20	native
570	-169.47	-14.24	85	36	105	16	naturalized
571	-171.08	-11.06	16	10	21	5	native
571	-171.08	-11.06	22	12	29	5	naturalized
574	-109.22	10.30	6	8	11	3	native
574	-109.22	10.30	10	3	11	2	naturalized
575	72.43	-7.34	20	9	24	5	native
575	72.43	-7.34	72	32	90	14	naturalized
576	153.49	48.98	18	16	26	8	native
576	153.49	48.98	0	0	0	0	naturalized
579	146.00	43.37	49	29	65	13	native
579	146.00	43.37	4	7	8	3	naturalized
580	146.06	43.42	101	51	130	22	native
580	146.06	43.42	8	6	12	2	naturalized
581	146.32	43.63	74	39	98	15	native
581	146.32	43.63	4	4	6	2	naturalized
582	145.92	43.44	94	45	121	18	native
582	145.92	43.44	7	8	12	3	naturalized
583	146.14	43.51	101	48	130	19	native
583	146.14	43.51	9	6	13	2	naturalized

587	146.59	-41.94	2041	1061	2626	476	native
587	146.59	-41.94	387	296	540	143	naturalized
593	154.53	49.12	95	71	132	34	native
593	154.53	49.12	1	0	1	0	naturalized
594	-157.35	1.85	9	9	14	4	native
594	-157.35	1.85	0	0	0	0	naturalized
595	145.95	44.17	341	212	466	87	native
595	145.95	44.17	7	12	14	5	naturalized
596	146.74	43.80	269	167	368	68	native
596	146.74	43.80	4	8	9	3	naturalized
597	147.88	45.08	287	181	390	78	native
597	147.88	45.08	5	9	10	4	naturalized
598	155.72	50.37	181	126	250	57	native
598	155.72	50.37	0	2	1	1	naturalized
599	156.36	50.74	143	98	196	45	native
599	156.36	50.74	0	2	1	1	naturalized
600	155.57	50.86	84	51	111	24	native
600	155.57	50.86	0	0	0	0	naturalized
601	-179.05	-17.98	133	31	150	14	native
601	-179.05	-17.98	12	2	13	1	naturalized
602	-178.79	-18.21	189	56	220	25	native
602	-178.79	-18.21	18	6	20	4	naturalized
603	-178.69	-18.33	58	17	67	8	native
603	-178.69	-18.33	1	0	1	0	naturalized
604	166.93	-0.53	45	18	54	9	native
604	166.93	-0.53	10	3	10	3	naturalized
605	153.25	48.29	35	26	48	13	native
605	153.25	48.29	0	0	0	0	naturalized
606	177.08	-12.50	175	63	208	30	native
606	177.08	-12.50	0	0	0	0	naturalized
607	55.21	-3.72	64	28	79	13	native
607	55.21	-3.72	65	17	73	9	naturalized
610	55.73	-4.28	122	50	149	23	native
610	55.73	-4.28	64	19	71	12	naturalized
611	55.67	-3.81	73	32	91	14	native
611	55.67	-3.81	62	18	70	10	naturalized
612	55.87	-4.33	106	38	127	17	native
612	55.87	-4.33	41	10	45	6	naturalized
614	55.92	-4.34	57	21	68	10	native

614	55.92	-4.34	49	8	53	4	naturalized
615	55.24	-4.39	87	40	110	17	native
615	55.24	-4.39	105	25	118	12	naturalized
617	133.29	-32.51	44	31	61	14	native
617	133.29	-32.51	0	0	0	0	naturalized
623	133.29	-32.56	25	22	37	10	native
623	133.29	-32.56	0	0	0	0	naturalized
624	133.28	-32.58	28	26	41	13	native
624	133.28	-32.58	0	0	0	0	naturalized
626	-45.63	-60.71	1	1	2	0	native
626	-45.63	-60.71	0	0	0	0	naturalized
627	-176.53	-43.94	216	170	318	68	native
627	-176.53	-43.94	259	185	352	92	naturalized
632	153.42	-27.84	98	43	124	17	native
632	153.42	-27.84	0	0	0	0	naturalized
637	NA	NA	0	1	1	0	native
637	NA	NA	0	0	0	0	naturalized
639	NA	NA	7	5	9	3	native
639	NA	NA	0	0	0	0	naturalized
643	NA	NA	1	3	3	1	native
643	NA	NA	0	0	0	0	naturalized
646	NA	NA	31	19	42	8	native
646	NA	NA	0	0	0	0	naturalized
648	174.74	-35.48	93	55	124	24	native
648	174.74	-35.48	0	0	0	0	naturalized
649	-77.12	34.67	119	57	154	22	native
649	-77.12	34.67	0	0	0	0	naturalized
650	-75.26	38.03	248	129	325	52	native
650	-75.26	38.03	0	0	0	0	naturalized
660	NA	NA	167	89	218	38	native
660	NA	NA	0	0	0	0	naturalized
661	-73.03	40.69	162	100	218	44	native
661	-73.03	40.69	0	0	0	0	naturalized
663	NA	NA	35	21	48	8	native
663	NA	NA	0	0	0	0	naturalized
664	NA	NA	20	10	26	4	native
664	NA	NA	0	0	0	0	naturalized
666	175.15	-35.94	40	14	49	5	native
666	175.15	-35.94	0	0	0	0	naturalized

667	NA	NA	21	10	27	4	native
667	NA	NA	0	0	0	0	naturalized
668	NA	NA	16	8	21	3	native
668	NA	NA	0	0	0	0	naturalized
669	NA	NA	11	7	14	4	native
669	NA	NA	0	0	0	0	naturalized
674	-72.46	40.97	100	46	126	20	native
674	-72.46	40.97	0	0	0	0	naturalized
675	27.12	36.66	160	70	196	34	native
675	27.12	36.66	0	0	0	0	naturalized
676	142.18	26.98	250	127	326	51	native
676	142.18	26.98	0	0	0	0	naturalized
678	175.92	52.36	61	44	86	19	native
678	175.92	52.36	0	0	0	0	naturalized
680	145.77	18.12	84	33	103	14	native
680	145.77	18.12	0	0	0	0	naturalized
691	NA	NA	1	1	2	0	native
691	NA	NA	0	0	0	0	naturalized
694	-87.86	17.37	23	13	30	6	native
694	-87.86	17.37	0	0	0	0	naturalized
695	-87.54	17.35	37	20	46	11	native
695	-87.54	17.35	0	0	0	0	naturalized
697	NA	NA	79	34	97	16	native
697	NA	NA	0	0	0	0	naturalized
698	NA	NA	64	29	79	14	native
698	NA	NA	0	0	0	0	naturalized
700	-80.04	19.68	154	61	186	29	native
700	-80.04	19.68	0	0	0	0	naturalized
704	NA	NA	7	7	11	3	native
704	NA	NA	0	0	0	0	naturalized
705	-162.07	5.88	9	9	13	5	native
705	-162.07	5.88	0	0	0	0	naturalized
706	-160.38	4.68	11	11	17	5	native
706	-160.38	4.68	0	0	0	0	naturalized
708	162.30	11.50	27	19	34	12	native
708	162.30	11.50	12	6	16	2	naturalized
717	-2.58	49.46	353	248	489	112	native
717	-2.58	49.46	0	0	0	0	naturalized
718	-2.20	49.72	280	192	384	88	native

718	-2.20	49.72	0	0	0	0	naturalized
719	-2.36	49.43	222	142	301	63	native
719	-2.36	49.43	0	0	0	0	naturalized
720	-2.45	49.47	131	93	184	40	native
720	-2.45	49.47	0	0	0	0	naturalized
725	-2.13	49.21	378	273	529	122	native
725	-2.13	49.21	0	0	0	0	naturalized
727	-1.30	50.67	495	324	679	140	native
727	-1.30	50.67	0	0	0	0	naturalized
728	-4.38	53.29	445	334	630	149	native
728	-4.38	53.29	0	0	0	0	naturalized
729	-4.54	54.23	345	244	481	108	native
729	-4.54	54.23	0	0	0	0	naturalized
730	-3.04	59.03	240	203	349	94	native
730	-3.04	59.03	0	0	0	0	naturalized
731	-1.26	60.38	200	183	298	85	native
731	-1.26	60.38	0	0	0	0	naturalized
733	-5.20	55.63	343	260	487	116	native
733	-5.20	55.63	0	0	0	0	naturalized
734	-6.86	57.92	320	269	461	128	native
734	-6.86	57.92	0	0	0	0	naturalized
736	9.11	42.15	4361	2569	5775	1155	native
736	9.11	42.15	184	113	242	55	naturalized
737	24.95	35.25	3293	1866	4318	841	native
737	24.95	35.25	0	0	0	0	naturalized
738	14.16	37.59	1793	1084	2372	505	native
738	14.16	37.59	73	43	95	21	naturalized
739	9.04	40.09	1699	1015	2270	444	native
739	9.04	40.09	51	38	73	16	naturalized
742	145.67	18.77	57	26	73	10	native
742	145.67	18.77	0	0	0	0	naturalized
743	145.56	14.85	33	18	42	9	native
743	145.56	14.85	0	0	0	0	naturalized
747	145.83	17.60	63	27	79	11	native
747	145.83	17.60	0	0	0	0	naturalized
748	145.68	16.35	52	24	66	10	native
748	145.68	16.35	0	0	0	0	naturalized
753	145.42	19.68	34	14	42	6	native
753	145.42	19.68	0	0	0	0	naturalized

775	144.89	20.54	6	6	10	2	native
775	144.89	20.54	0	0	0	0	naturalized
780	144.78	13.44	236	112	302	46	native
780	144.78	13.44	0	0	0	0	naturalized
781	145.84	17.31	32	14	40	6	native
781	145.84	17.31	0	0	0	0	naturalized
803	145.22	20.02	31	18	42	7	native
803	145.22	20.02	0	0	0	0	naturalized
844	145.21	14.16	163	61	199	25	native
844	145.21	14.16	0	0	0	0	naturalized
845	145.75	15.19	155	68	193	30	native
845	145.75	15.19	0	0	0	0	naturalized
846	145.78	16.70	51	24	65	10	native
846	145.78	16.70	0	0	0	0	naturalized
854	145.63	15.01	122	52	151	23	native
854	145.63	15.01	0	0	0	0	naturalized
903	-171.67	-2.81	14	10	19	5	native
903	-171.67	-2.81	0	0	0	0	naturalized
904	-174.52	-4.67	12	9	18	3	native
904	-174.52	-4.67	0	0	0	0	naturalized
905	-172.18	-4.51	13	9	18	4	native
905	-172.18	-4.51	0	0	0	0	naturalized
908	-171.09	-3.13	11	9	17	3	native
908	-171.09	-3.13	0	0	0	0	naturalized
910	-171.24	-4.46	11	8	16	3	native
910	-171.24	-4.46	0	0	0	0	naturalized
914	-32.43	-3.86	118	40	138	20	native
914	-32.43	-3.86	0	0	0	0	naturalized
915	NA	NA	41	14	48	7	native
915	NA	NA	0	0	0	0	naturalized
916	NA	NA	5	2	6	1	native
916	NA	NA	0	0	0	0	naturalized
917	NA	NA	9	3	10	2	native
917	NA	NA	0	0	0	0	naturalized
918	NA	NA	6	2	7	1	native
918	NA	NA	0	0	0	0	naturalized
919	NA	NA	12	26	25	13	native
919	NA	NA	0	0	0	0	naturalized
920	26.15	37.60	486	284	635	135	native

920	26.15	37.60	0	0	0	0	naturalized
921	27.17	36.59	299	155	383	71	native
921	27.17	36.59	0	0	0	0	naturalized
922	-179.46	71.20	198	197	297	98	native
922	-179.46	71.20	0	0	0	0	naturalized
923	24.17	39.39	241	124	306	59	native
923	24.17	39.39	0	0	0	0	naturalized
925	-112.83	28.64	55	26	69	12	native
925	-112.83	28.64	0	0	0	0	naturalized
927	-112.58	28.70	87	36	103	20	native
927	-112.58	28.70	0	0	0	0	naturalized
928	-112.31	28.38	18	7	20	5	native
928	-112.31	28.38	0	0	0	0	naturalized
929	-111.38	27.97	40	15	46	9	native
929	-111.38	27.97	0	0	0	0	naturalized
930	-111.88	27.44	57	19	65	11	native
930	-111.88	27.44	0	0	0	0	naturalized
931	-112.07	27.22	105	35	123	17	native
931	-112.07	27.22	0	0	0	0	naturalized
932	-111.43	26.63	23	11	28	6	native
932	-111.43	26.63	0	0	0	0	naturalized
933	-111.27	26.12	86	36	105	17	native
933	-111.27	26.12	0	0	0	0	naturalized
934	-111.15	25.96	135	55	163	27	native
934	-111.15	25.96	0	0	0	0	naturalized
935	-111.25	25.79	89	38	106	21	native
935	-111.25	25.79	0	0	0	0	naturalized
936	-111.04	25.68	89	34	103	20	native
936	-111.04	25.68	0	0	0	0	naturalized
937	-110.78	25.65	86	32	101	17	native
937	-110.78	25.65	0	0	0	0	naturalized
938	-110.72	25.29	71	25	81	15	native
938	-110.72	25.29	0	0	0	0	naturalized
940	-110.62	24.98	151	63	180	34	native
940	-110.62	24.98	0	0	0	0	naturalized
941	-110.57	24.83	77	32	92	17	native
941	-110.57	24.83	0	0	0	0	naturalized
942	-110.35	24.49	165	73	201	37	native
942	-110.35	24.49	0	0	0	0	naturalized

943	-109.87	24.23	167	61	197	31	native
943	-109.87	24.23	0	0	0	0	naturalized
946	-112.29	28.72	69	28	83	14	native
946	-112.29	28.72	0	0	0	0	naturalized
949	NA	NA	17	9	20	6	native
949	NA	NA	0	0	0	0	naturalized
950	-113.57	29.56	21	9	25	5	native
950	-113.57	29.56	0	0	0	0	naturalized
951	-113.04	28.89	11	5	13	3	native
951	-113.04	28.89	0	0	0	0	naturalized
954	-112.96	28.73	8	7	11	4	native
954	-112.96	28.73	0	0	0	0	naturalized
957	-113.51	29.07	43	27	57	13	native
957	-113.51	29.07	0	0	0	0	naturalized
958	-113.51	29.00	38	23	49	12	native
958	-113.51	29.00	0	0	0	0	naturalized
968	NA	NA	11	7	14	4	native
968	NA	NA	0	0	0	0	naturalized
969	NA	NA	7	5	9	3	native
969	NA	NA	0	0	0	0	naturalized
971	50.23	-46.10	8	6	10	4	native
971	50.23	-46.10	0	0	0	0	naturalized
972	51.76	-46.41	8	6	10	4	native
972	51.76	-46.41	0	0	0	0	naturalized
973	77.56	-37.84	9	5	12	2	native
973	77.56	-37.84	0	0	0	0	naturalized
974	77.52	-38.72	4	3	6	1	native
974	77.52	-38.72	0	0	0	0	naturalized
975	169.16	-52.54	92	63	127	28	native
975	169.16	-52.54	0	0	0	0	naturalized
976	166.59	-48.03	13	13	19	7	native
976	166.59	-48.03	0	0	0	0	naturalized
982	154.76	49.42	80	40	102	18	native
982	154.76	49.42	0	0	0	0	naturalized
983	154.11	48.82	62	42	83	21	native
983	154.11	48.82	0	0	0	0	naturalized
984	165.70	-21.31	3441	869	3851	459	native
984	165.70	-21.31	0	0	0	0	naturalized
986	55.47	-4.68	159	73	201	31	native

986	55.47	-4.68	171	52	197	26	naturalized
987	NA	NA	3	2	4	1	native
987	NA	NA	0	0	0	0	naturalized
988	NA	NA	10	3	11	2	native
988	NA	NA	0	0	0	0	naturalized
989	55.50	-4.61	34	10	39	5	native
989	55.50	-4.61	41	9	45	5	naturalized
990	55.50	-4.63	16	5	19	2	native
990	55.50	-4.63	18	4	20	2	naturalized
991	NA	NA	3	1	3	1	native
991	NA	NA	0	0	0	0	naturalized
994	55.53	-4.66	20	7	23	4	native
994	55.53	-4.66	21	4	22	3	naturalized
995	NA	NA	4	3	6	1	native
995	NA	NA	0	0	0	0	naturalized
996	NA	NA	4	3	6	1	native
996	NA	NA	0	0	0	0	naturalized
998	NA	NA	0	1	1	0	native
998	NA	NA	0	0	0	0	naturalized
1000	NA	NA	11	4	13	2	native
1000	NA	NA	0	0	0	0	naturalized
1001	NA	NA	4	1	5	0	native
1001	NA	NA	0	0	0	0	naturalized
1002	NA	NA	4	1	4	1	native
1002	NA	NA	0	0	0	0	naturalized
1003	55.73	-4.32	106	48	132	22	native
1003	55.73	-4.32	66	20	77	9	naturalized
1004	55.84	-4.36	38	15	45	8	native
1004	55.84	-4.36	45	16	53	8	naturalized
1008	NA	NA	3	1	4	0	native
1008	NA	NA	0	0	0	0	naturalized
1009	NA	NA	4	1	4	1	native
1009	NA	NA	0	0	0	0	naturalized
1014	55.94	-4.59	78	27	92	13	native
1014	55.94	-4.59	64	18	73	9	naturalized
1016	NA	NA	7	3	9	1	native
1016	NA	NA	0	0	0	0	naturalized
1021	53.36	-5.44	19	10	24	5	native
1021	53.36	-5.44	7	3	8	2	naturalized

1022	53.31	-5.76	23	11	30	4	native
1022	53.31	-5.76	24	8	28	4	naturalized
1025	52.73	-7.01	26	13	34	5	native
1025	52.73	-7.01	18	5	20	3	naturalized
1029	NA	NA	3	1	3	1	native
1029	NA	NA	0	0	0	0	naturalized
1030	55.23	-4.49	128	55	158	25	native
1030	55.23	-4.49	84	25	97	12	naturalized
1031	NA	NA	10	3	11	2	native
1031	NA	NA	0	0	0	0	naturalized
1032	92.79	12.30	1817	625	2151	291	native
1032	92.79	12.30	0	0	0	0	naturalized
1033	2.91	39.57	0	0	0	0	native
1033	2.91	39.57	84	59	113	30	naturalized
1044	138.01	37.60	0	0	0	0	native
1044	138.01	37.60	1413	1015	1940	488	naturalized
1048	57.57	-20.28	419	161	510	70	native
1048	57.57	-20.28	372	174	462	84	naturalized
1049	46.70	-19.38	0	0	0	0	native
1049	46.70	-19.38	269	112	328	53	naturalized
1054	93.60	7.53	1242	428	1479	191	native
1054	93.60	7.53	0	0	0	0	naturalized
1058	122.87	11.74	0	0	0	0	native
1058	122.87	11.74	367	131	434	64	naturalized
1059	63.42	-19.72	155	67	195	27	native
1059	63.42	-19.72	159	73	196	36	naturalized
1064	80.70	7.62	3033	1255	3721	567	native
1064	80.70	7.62	202	93	253	42	naturalized
1224	175.73	-38.58	0	0	0	0	native
1224	175.73	-38.58	792	466	1041	217	naturalized
1225	57.79	-19.85	26	9	30	5	native
1225	57.79	-19.85	0	0	0	0	naturalized
1236	-178.58	-19.14	80	24	93	11	native
1236	-178.58	-19.14	0	0	0	0	naturalized
1254	179.89	-18.60	87	23	101	9	native
1254	179.89	-18.60	0	0	0	0	naturalized
1272	178.79	-17.68	265	76	311	30	native
1272	178.79	-17.68	0	0	0	0	naturalized
1281	179.25	-16.60	549	156	638	67	native

1281	179.25	-16.60	0	0	0	0	naturalized
1283	-178.97	-17.23	111	31	130	12	native
1283	-178.97	-17.23	0	0	0	0	naturalized
1285	-179.53	-17.43	4	1	4	1	native
1285	-179.53	-17.43	0	0	0	0	naturalized
1287	177.96	-17.84	710	212	828	94	native
1287	177.96	-17.84	0	0	0	0	naturalized
1306	NA	NA	119	47	147	19	native
1306	NA	NA	0	0	0	0	naturalized
1307	NA	NA	344	123	403	64	native
1307	NA	NA	0	0	0	0	naturalized
1311	-16.04	11.29	314	112	369	57	native
1311	-16.04	11.29	0	0	0	0	naturalized
1313	-79.98	-26.32	11	10	17	4	native
1313	-79.98	-26.32	0	0	0	0	naturalized
1314	20.43	38.62	1394	781	1810	365	native
1314	20.43	38.62	0	0	0	0	naturalized
1315	25.23	40.24	1389	795	1819	365	native
1315	25.23	40.24	0	0	0	0	naturalized
1317	25.13	37.16	1183	697	1551	329	native
1317	25.13	37.16	0	0	0	0	naturalized
1497	NA	NA	522	173	618	77	native
1497	NA	NA	0	0	0	0	naturalized
1500	-81.52	12.95	220	65	256	29	native
1500	-81.52	12.95	21	9	27	3	naturalized
1501	NA	NA	217	47	242	22	native
1501	NA	NA	0	0	0	0	naturalized
1503	126.12	37.24	289	167	389	67	native
1503	126.12	37.24	0	0	0	0	naturalized
1504	126.10	37.18	159	80	208	31	native
1504	126.10	37.18	0	0	0	0	naturalized
1507	126.17	37.21	212	118	283	47	native
1507	126.17	37.21	0	0	0	0	naturalized
10004	132.75	-12.88	1078	481	1313	246	native
10004	132.75	-12.88	0	0	0	0	naturalized
10007	-57.90	-26.76	905	341	1115	131	native
10007	-57.90	-26.76	0	0	0	0	naturalized
10010	-59.49	5.17	644	249	773	120	native
10010	-59.49	5.17	0	0	0	0	naturalized

10013	51.19	25.31	143	115	209	49	native
10013	51.19	25.31	55	35	71	19	naturalized
10014	77.43	31.12	251	107	314	44	native
10014	77.43	31.12	0	0	0	0	naturalized
10015	5.34	45.44	2425	1424	3181	668	native
10015	5.34	45.44	0	0	0	0	naturalized
10017	140.87	-19.44	1534	661	1891	304	native
10017	140.87	-19.44	114	51	143	22	naturalized
10018	151.27	-25.72	1324	468	1587	205	native
10018	151.27	-25.72	203	107	260	50	naturalized
10019	143.31	-15.72	3133	1172	3726	579	native
10019	143.31	-15.72	364	170	453	81	naturalized
10020	150.76	-27.62	1630	654	1987	297	native
10020	150.76	-27.62	291	168	381	78	naturalized
10021	140.43	-23.11	787	447	1027	207	native
10021	140.43	-23.11	38	19	48	9	naturalized
10022	141.67	-26.51	532	363	721	174	native
10022	141.67	-26.51	35	23	47	11	naturalized
10023	148.75	-24.01	1606	600	1935	271	native
10023	148.75	-24.01	186	98	238	46	naturalized
10024	147.94	-27.41	1047	485	1318	214	native
10024	147.94	-27.41	119	77	158	38	naturalized
10025	144.54	-23.38	1113	549	1411	251	native
10025	144.54	-23.38	88	55	113	30	naturalized
10026	152.64	-27.43	1799	703	2177	325	native
10026	152.64	-27.43	481	250	613	118	naturalized
10027	146.02	-19.51	2360	913	2843	430	native
10027	146.02	-19.51	288	134	358	64	naturalized
10028	150.52	-23.51	1625	663	1979	309	native
10028	150.52	-23.51	262	130	327	65	naturalized
10029	147.00	-21.91	1719	687	2091	315	native
10029	147.00	-21.91	224	115	280	59	naturalized
10030	145.13	-27.06	939	508	1220	227	native
10030	145.13	-27.06	104	64	137	31	naturalized
10031	152.39	-25.64	1416	546	1709	253	native
10031	152.39	-25.64	294	157	377	74	naturalized
10032	13.35	51.05	2844	1677	3782	739	native
10032	13.35	51.05	204	151	280	75	naturalized
10034	11.24	42.79	1111	613	1443	281	native

10034	11.24	42.79	0	0	0	0	naturalized
10036	11.79	-0.62	0	0	0	0	native
10036	11.79	-0.62	53	18	62	9	naturalized
10039	29.25	45.09	646	472	902	216	native
10039	29.25	45.09	0	0	0	0	naturalized
10040	34.94	0.23	496	161	579	78	native
10040	34.94	0.23	0	0	0	0	naturalized
10050	-70.59	-26.09	126	73	165	34	native
10050	-70.59	-26.09	0	0	0	0	naturalized
10051	-59.41	-20.59	1767	621	2129	259	native
10051	-59.41	-20.59	0	0	0	0	naturalized
10052	-61.06	-21.90	667	263	820	110	native
10052	-61.06	-21.90	0	0	0	0	naturalized
10053	-58.69	-23.61	1511	551	1840	222	native
10053	-58.69	-23.61	0	0	0	0	naturalized
10056	8.23	46.80	2693	1736	3625	804	native
10056	8.23	46.80	72	64	105	31	naturalized
10057	-75.21	3.14	165	50	191	24	native
10057	-75.21	3.14	0	0	0	0	naturalized
10059	18.67	15.36	2240	1069	2828	481	native
10059	18.67	15.36	85	44	105	24	naturalized
10063	-70.07	-37.14	139	64	175	28	native
10063	-70.07	-37.14	0	0	0	0	naturalized
10064	142.41	-37.25	773	334	950	157	native
10064	142.41	-37.25	0	0	0	0	naturalized
10065	141.48	-34.68	429	308	600	137	native
10065	141.48	-34.68	0	0	0	0	naturalized
10066	148.44	-37.26	688	255	832	111	native
10066	148.44	-37.26	0	0	0	0	naturalized
10071	-3.55	40.39	0	0	0	0	native
10071	-3.55	40.39	381	240	506	115	naturalized
10072	-7.96	39.69	1493	935	2007	421	native
10072	-7.96	39.69	211	154	293	72	naturalized
10078	26.19	30.08	581	413	805	189	native
10078	26.19	30.08	0	0	0	0	naturalized
10080	-55.41	-24.14	836	232	975	93	native
10080	-55.41	-24.14	0	0	0	0	naturalized
10081	-1.74	12.29	1232	538	1538	232	native
10081	-1.74	12.29	0	0	0	0	naturalized

10082	23.81	-22.19	3834	1539	4642	731	native
10082	23.81	-22.19	177	135	253	59	naturalized
10083	26.47	-32.14	3656	1685	4470	871	native
10083	26.47	-32.14	526	308	687	147	naturalized
10084	26.87	-28.61	2072	933	2575	430	native
10084	26.87	-28.61	297	209	410	96	naturalized
10085	28.14	-26.12	1379	568	1666	281	native
10085	28.14	-26.12	379	247	513	113	naturalized
10086	30.73	-28.71	4724	1785	5686	823	native
10086	30.73	-28.71	518	287	672	133	naturalized
10087	28.25	-29.58	1517	696	1886	327	native
10087	28.25	-29.58	277	170	373	74	naturalized
10088	29.33	-23.75	2538	954	3002	490	native
10088	29.33	-23.75	309	174	400	83	naturalized
10089	30.21	-25.86	2717	981	3201	497	native
10089	30.21	-25.86	392	212	507	97	naturalized
10090	17.22	-22.14	3185	1538	3912	811	native
10090	17.22	-22.14	250	192	360	82	naturalized
10091	21.35	-29.53	2674	1705	3459	920	native
10091	21.35	-29.53	234	177	329	82	naturalized
10092	25.34	-26.32	1351	561	1628	284	native
10092	25.34	-26.32	238	149	315	72	naturalized
10093	31.50	-26.56	2749	1001	3282	468	native
10093	31.50	-26.56	190	102	246	46	naturalized
10094	20.61	-33.01	6331	3034	7691	1674	native
10094	20.61	-33.01	476	312	635	153	naturalized
10095	34.50	-18.82	454	148	530	72	native
10095	34.50	-18.82	0	0	0	0	naturalized
10101	6.42	50.58	410	234	546	98	native
10101	6.42	50.58	0	0	0	0	naturalized
10103	-64.77	-32.69	433	157	527	63	native
10103	-64.77	-32.69	0	0	0	0	naturalized
10104	1.16	43.37	1331	837	1774	394	native
10104	1.16	43.37	0	0	0	0	naturalized
10106	-60.57	-36.68	1172	545	1497	220	native
10106	-60.57	-36.68	163	153	246	70	naturalized
10107	-66.95	-27.33	1435	608	1777	266	native
10107	-66.95	-27.33	33	37	51	19	naturalized
10108	-60.77	-26.39	1204	502	1498	208	native

10108	-60.77	-26.39	21	19	31	9	naturalized
10109	-68.52	-43.79	799	370	1004	165	native
10109	-68.52	-43.79	57	75	91	41	naturalized
10110	-57.81	-28.78	1999	753	2437	315	native
10110	-57.81	-28.78	45	34	62	17	naturalized
10111	-63.80	-32.14	0	0	0	0	native
10111	-63.80	-32.14	72	72	109	35	naturalized
10112	-58.44	-34.62	183	93	238	38	native
10112	-58.44	-34.62	49	32	67	14	naturalized
10113	-59.21	-32.05	1371	559	1698	232	native
10113	-59.21	-32.05	78	66	112	32	naturalized
10114	-59.93	-24.89	1124	435	1373	186	native
10114	-59.93	-24.89	20	17	28	9	naturalized
10115	-65.76	-23.32	2014	852	2493	373	native
10115	-65.76	-23.32	43	35	60	18	naturalized
10116	-65.45	-37.13	534	221	667	88	native
10116	-65.45	-37.13	72	73	107	38	naturalized
10117	-67.18	-29.68	1045	451	1296	200	native
10117	-67.18	-29.68	22	30	37	15	naturalized
10118	-68.59	-34.63	1096	499	1374	221	native
10118	-68.59	-34.63	73	74	110	37	naturalized
10119	-54.65	-26.87	2112	728	2546	294	native
10119	-54.65	-26.87	30	29	44	15	naturalized
10120	-70.12	-38.64	0	0	0	0	native
10120	-70.12	-38.64	65	75	101	39	naturalized
10121	-67.23	-40.41	897	414	1124	187	native
10121	-67.23	-40.41	76	95	122	49	naturalized
10122	-64.82	-24.30	2288	955	2828	415	native
10122	-64.82	-24.30	53	47	73	27	naturalized
10123	-69.94	-48.81	602	306	766	142	native
10123	-69.94	-48.81	43	60	69	34	naturalized
10124	-63.26	-27.78	653	272	805	120	native
10124	-63.26	-27.78	23	30	39	14	naturalized
10125	-60.95	-30.70	1105	475	1388	192	native
10125	-60.95	-30.70	42	43	64	21	naturalized
10126	-68.87	-30.86	934	442	1183	193	native
10126	-68.87	-30.86	27	47	49	25	naturalized
10127	-66.03	-33.77	738	316	919	135	native
10127	-66.03	-33.77	31	38	50	19	naturalized

10128	-67.40	-54.33	310	174	413	71	native
10128	-67.40	-54.33	52	52	78	26	naturalized
10129	-65.37	-26.94	1687	744	2112	319	native
10129	-65.37	-26.94	61	50	89	22	naturalized
10130	-51.62	-24.64	7233	2397	8672	958	native
10130	-51.62	-24.64	127	83	170	40	naturalized
10131	-53.32	-29.74	5573	1938	6736	775	native
10131	-53.32	-29.74	116	89	165	40	naturalized
10132	-50.49	-27.25	5924	2126	7205	845	native
10132	-50.49	-27.25	124	86	171	39	naturalized
10133	-69.46	-19.71	505	226	628	103	native
10133	-69.46	-19.71	0	0	0	0	naturalized
10134	-69.10	-23.46	630	307	792	145	native
10134	-69.10	-23.46	0	0	0	0	naturalized
10136	-70.96	-32.71	912	406	1124	194	native
10136	-70.96	-32.71	0	0	0	0	naturalized
10137	-71.07	-34.43	703	281	856	128	native
10137	-71.07	-34.43	0	0	0	0	naturalized
10138	-71.42	-35.58	863	354	1051	166	native
10138	-71.42	-35.58	0	0	0	0	naturalized
10139	-72.24	-37.15	897	406	1114	189	native
10139	-72.24	-37.15	0	0	0	0	naturalized
10140	-72.28	-38.70	738	327	916	149	native
10140	-72.28	-38.70	0	0	0	0	naturalized
10141	-72.82	-41.57	617	321	792	146	native
10141	-72.82	-41.57	0	0	0	0	naturalized
10142	-73.26	-46.42	349	171	444	76	native
10142	-73.26	-46.42	0	0	0	0	naturalized
10143	-71.90	-52.48	434	236	566	104	native
10143	-71.90	-52.48	0	0	0	0	naturalized
10144	-70.65	-33.59	945	420	1161	204	native
10144	-70.65	-33.59	0	0	0	0	naturalized
10145	-56.06	-22.88	1474	411	1718	167	native
10145	-56.06	-22.88	0	0	0	0	naturalized
10146	-54.95	-25.39	852	251	1003	100	native
10146	-54.95	-25.39	0	0	0	0	naturalized
10147	-56.06	-26.23	628	182	736	74	native
10147	-56.06	-26.23	0	0	0	0	naturalized
10149	-55.88	-25.19	871	241	1016	96	native

10149	-55.88	-25.19	0	0	0	0	naturalized
10150	-57.49	-25.53	1031	373	1253	151	native
10150	-57.49	-25.53	0	0	0	0	naturalized
10151	-56.95	-25.19	1231	409	1466	174	native
10151	-56.95	-25.19	0	0	0	0	naturalized
10152	-57.12	-22.84	945	257	1094	108	native
10152	-57.12	-22.84	0	0	0	0	naturalized
10153	-56.30	-25.82	933	298	1111	120	native
10153	-56.30	-25.82	0	0	0	0	naturalized
10154	-55.75	-26.83	486	137	570	53	native
10154	-55.75	-26.83	0	0	0	0	naturalized
10155	-57.09	-26.95	429	125	508	46	native
10155	-57.09	-26.95	0	0	0	0	naturalized
10156	-57.13	-26.04	1228	380	1451	157	native
10156	-57.13	-26.04	0	0	0	0	naturalized
10157	-56.65	-24.16	820	238	960	98	native
10157	-56.65	-24.16	0	0	0	0	naturalized
10158	-56.95	-30.55	454	154	542	66	native
10158	-56.95	-30.55	0	0	0	0	naturalized
10159	-55.96	-34.52	413	161	512	62	native
10159	-55.96	-34.52	0	0	0	0	naturalized
10160	-54.37	-32.40	458	149	549	58	native
10160	-54.37	-32.40	0	0	0	0	naturalized
10161	-57.68	-34.11	370	124	442	52	native
10161	-57.68	-34.11	0	0	0	0	naturalized
10162	-56.11	-32.94	152	53	184	21	native
10162	-56.11	-32.94	0	0	0	0	naturalized
10163	-55.90	-33.70	328	121	403	46	native
10163	-55.90	-33.70	0	0	0	0	naturalized
10164	-56.94	-33.51	131	49	163	17	native
10164	-56.94	-33.51	0	0	0	0	naturalized
10165	-55.01	-33.87	302	94	360	36	native
10165	-55.01	-33.87	0	0	0	0	naturalized
10166	-54.86	-34.55	480	161	581	60	native
10166	-54.86	-34.55	0	0	0	0	naturalized
10167	-56.23	-34.83	711	286	886	111	native
10167	-56.23	-34.83	0	0	0	0	naturalized
10168	-57.38	-32.03	477	143	564	56	native
10168	-57.38	-32.03	0	0	0	0	naturalized

10169	-55.03	-31.65	556	167	655	68	native
10169	-55.03	-31.65	0	0	0	0	naturalized
10170	-57.47	-32.72	410	154	506	58	native
10170	-57.47	-32.72	0	0	0	0	naturalized
10171	-54.02	-33.94	405	162	505	62	native
10171	-54.02	-33.94	0	0	0	0	naturalized
10172	-57.05	-31.24	461	146	550	57	native
10172	-57.05	-31.24	0	0	0	0	naturalized
10173	-56.75	-34.27	425	157	526	56	native
10173	-56.75	-34.27	0	0	0	0	naturalized
10174	-57.76	-33.48	391	136	475	52	native
10174	-57.76	-33.48	0	0	0	0	naturalized
10175	-55.81	-32.04	529	169	630	68	native
10175	-55.81	-32.04	0	0	0	0	naturalized
10176	-54.30	-33.00	287	102	349	40	native
10176	-54.30	-33.00	0	0	0	0	naturalized
10178	-65.94	3.41	4479	1495	5300	674	native
10178	-65.94	3.41	22	9	28	3	naturalized
10179	-63.49	6.21	4233	1478	5051	660	native
10179	-63.49	6.21	30	14	39	5	naturalized
10180	-61.33	8.76	3650	1116	4291	475	native
10180	-61.33	8.76	21	9	27	3	naturalized
10181	-53.23	3.93	5029	1599	5964	664	native
10181	-53.23	3.93	116	48	145	19	naturalized
10182	-58.98	4.79	0	0	0	0	native
10182	-58.98	4.79	63	24	79	8	naturalized
10183	-55.92	4.14	4510	1561	5398	673	native
10183	-55.92	4.14	58	24	73	9	naturalized
10184	11.78	43.87	751	401	970	182	native
10184	11.78	43.87	0	0	0	0	naturalized
10202	30.37	-3.12	390	107	445	52	native
10202	30.37	-3.12	0	0	0	0	naturalized
10207	-157.81	56.87	199	182	293	88	native
10207	-157.81	56.87	0	0	0	0	naturalized
10209	-109.59	38.72	376	209	490	95	native
10209	-109.59	38.72	0	0	0	0	naturalized
10215	-112.67	36.18	955	516	1235	236	native
10215	-112.67	36.18	0	0	0	0	naturalized
10216	-109.81	34.95	269	154	350	73	native

10216	-109.81	34.95	0	0	0	0	naturalized
10221	-113.03	37.30	641	345	826	160	native
10221	-113.03	37.30	0	0	0	0	naturalized
10222	-102.48	43.69	305	138	386	57	native
10222	-102.48	43.69	0	0	0	0	naturalized
10223	-103.23	29.29	921	434	1145	210	native
10223	-103.23	29.29	0	0	0	0	naturalized
10224	-107.67	38.55	427	254	556	125	native
10224	-107.67	38.55	0	0	0	0	naturalized
10225	-112.18	37.58	491	273	636	128	native
10225	-112.18	37.58	0	0	0	0	naturalized
10226	-109.88	38.24	461	230	584	107	native
10226	-109.88	38.24	0	0	0	0	naturalized
10227	-111.18	38.17	591	334	771	154	native
10227	-111.18	38.17	0	0	0	0	naturalized
10228	-104.55	32.14	591	247	722	116	native
10228	-104.55	32.14	0	0	0	0	naturalized
10230	-122.13	42.94	561	342	756	147	native
10230	-122.13	42.94	0	0	0	0	naturalized
10231	-81.57	41.26	580	292	769	103	native
10231	-81.57	41.26	0	0	0	0	naturalized
10232	-117.13	36.48	642	401	854	189	native
10232	-117.13	36.48	0	0	0	0	naturalized
10233	-151.06	63.29	386	343	566	163	native
10233	-151.06	63.29	0	0	0	0	naturalized
10234	-80.88	25.47	449	208	563	94	native
10234	-80.88	25.47	0	0	0	0	naturalized
10235	-153.34	67.68	326	299	475	150	native
10235	-153.34	67.68	0	0	0	0	naturalized
10236	-136.87	58.84	311	247	445	113	native
10236	-136.87	58.84	0	0	0	0	naturalized
10237	-113.80	48.68	549	391	765	175	native
10237	-113.80	48.68	0	0	0	0	naturalized
10238	-110.71	43.82	721	459	963	217	native
10238	-110.71	43.82	0	0	0	0	naturalized
10239	-114.26	38.95	423	276	570	129	native
10239	-114.26	38.95	0	0	0	0	naturalized
10240	-105.56	37.77	347	202	462	87	native
10240	-105.56	37.77	0	0	0	0	naturalized

10241	-83.51	35.60	793	367	1022	138	native
10241	-83.51	35.60	0	0	0	0	naturalized
10242	-104.87	31.92	643	279	793	129	native
10242	-104.87	31.92	0	0	0	0	naturalized
10244	-115.84	33.91	515	272	659	128	native
10244	-115.84	33.91	0	0	0	0	naturalized
10245	-155.07	58.58	342	300	499	143	native
10245	-155.07	58.58	0	0	0	0	naturalized
10246	-150.13	59.81	313	255	452	116	native
10246	-150.13	59.81	0	0	0	0	naturalized
10247	-159.20	67.35	232	203	335	100	native
10247	-159.20	67.35	0	0	0	0	naturalized
10248	-153.57	60.58	533	470	781	222	native
10248	-153.57	60.58	0	0	0	0	naturalized
10249	-121.41	40.49	437	301	604	134	native
10249	-121.41	40.49	0	0	0	0	naturalized
10250	-86.13	37.20	859	394	1104	149	native
10250	-86.13	37.20	0	0	0	0	naturalized
10251	-108.46	37.24	462	259	597	124	native
10251	-108.46	37.24	0	0	0	0	naturalized
10252	-121.70	46.86	424	294	584	134	native
10252	-121.70	46.86	0	0	0	0	naturalized
10253	-121.21	48.71	633	468	888	213	native
10253	-121.21	48.71	0	0	0	0	naturalized
10254	-123.66	47.80	541	412	758	195	native
10254	-123.66	47.80	0	0	0	0	naturalized
10255	-121.18	36.49	313	190	415	88	native
10255	-121.18	36.49	0	0	0	0	naturalized
10256	-123.97	41.19	557	307	728	136	native
10256	-123.97	41.19	0	0	0	0	naturalized
10257	-105.70	40.36	532	346	726	152	native
10257	-105.70	40.36	0	0	0	0	naturalized
10258	-110.76	32.21	758	339	944	153	native
10258	-110.76	32.21	0	0	0	0	naturalized
10259	-118.59	36.71	816	525	1100	241	native
10259	-118.59	36.71	0	0	0	0	naturalized
10260	-78.47	38.49	656	298	842	112	native
10260	-78.47	38.49	0	0	0	0	naturalized
10261	-92.84	48.48	463	314	652	125	native

10261	-92.84	48.48	0	0	0	0	naturalized
10262	-103.44	43.59	391	174	494	71	native
10262	-103.44	43.59	0	0	0	0	naturalized
10263	-142.58	61.40	602	542	880	264	native
10263	-142.58	61.40	0	0	0	0	naturalized
10264	-110.55	44.60	655	462	902	215	native
10264	-110.55	44.60	0	0	0	0	naturalized
10265	-119.56	37.85	817	538	1113	242	native
10265	-119.56	37.85	0	0	0	0	naturalized
10268	39.39	-4.26	964	296	1108	152	native
10268	39.39	-4.26	0	0	0	0	naturalized
10270	-116.12	51.54	456	320	627	149	native
10270	-116.12	51.54	0	0	0	0	naturalized
10271	-81.48	45.19	476	311	651	136	native
10271	-81.48	45.19	0	0	0	0	naturalized
10272	-117.52	51.27	316	231	444	103	native
10272	-117.52	51.27	0	0	0	0	naturalized
10273	-106.69	49.08	250	131	329	52	native
10273	-106.69	49.08	0	0	0	0	naturalized
10274	-139.86	69.10	139	107	193	53	native
10274	-139.86	69.10	0	0	0	0	naturalized
10275	-117.98	52.85	424	294	580	138	native
10275	-117.98	52.85	0	0	0	0	naturalized
10276	-65.30	44.37	318	194	426	86	native
10276	-65.30	44.37	0	0	0	0	naturalized
10277	-139.20	60.64	441	382	633	190	native
10277	-139.20	60.64	0	0	0	0	naturalized
10278	-116.04	50.96	355	233	480	108	native
10278	-116.04	50.96	0	0	0	0	naturalized
10279	-64.94	46.82	419	260	570	109	native
10279	-64.94	46.82	0	0	0	0	naturalized
10280	-72.97	46.79	236	151	324	63	native
10280	-72.97	46.79	0	0	0	0	naturalized
10281	-63.61	50.22	217	171	312	76	native
10281	-63.61	50.22	0	0	0	0	naturalized
10283	-125.65	61.53	304	227	424	107	native
10283	-125.65	61.53	0	0	0	0	naturalized
10284	-106.37	53.96	334	221	464	91	native
10284	-106.37	53.96	0	0	0	0	naturalized

10285	-100.20	50.83	365	228	504	89	native
10285	-100.20	50.83	0	0	0	0	naturalized
10286	-121.06	68.70	116	105	168	53	native
10286	-121.06	68.70	0	0	0	0	naturalized
10287	-89.90	65.90	53	53	82	24	native
10287	-89.90	65.90	0	0	0	0	naturalized
10288	-139.89	68.39	171	145	245	71	native
10288	-139.89	68.39	0	0	0	0	naturalized
10289	-93.27	57.79	348	289	499	138	native
10289	-93.27	57.79	0	0	0	0	naturalized
10290	-116.53	51.38	304	213	418	99	native
10290	-116.53	51.38	0	0	0	0	naturalized
10309	16.19	2.98	766	194	860	100	native
10309	16.19	2.98	0	0	0	0	naturalized
10310	-14.34	12.16	675	253	800	128	native
10310	-14.34	12.16	0	0	0	0	naturalized
10311	-15.64	12.22	639	231	752	118	native
10311	-15.64	12.22	0	0	0	0	naturalized
10312	-15.08	11.48	622	211	727	106	native
10312	-15.08	11.48	0	0	0	0	naturalized
10319	81.19	28.24	410	132	484	58	native
10319	81.19	28.24	0	0	0	0	naturalized
10320	-115.98	30.53	207	133	274	66	native
10320	-115.98	30.53	0	0	0	0	naturalized
10321	-79.80	0.42	779	283	949	113	native
10321	-79.80	0.42	0	0	0	0	naturalized
10324	33.97	52.51	421	269	576	114	native
10324	33.97	52.51	0	0	0	0	naturalized
10325	26.11	56.19	250	160	343	67	native
10325	26.11	56.19	0	0	0	0	naturalized
10327	17.68	45.51	697	375	906	166	native
10327	17.68	45.51	0	0	0	0	naturalized
10329	74.89	42.70	1082	639	1419	302	native
10329	74.89	42.70	0	0	0	0	naturalized
10330	77.54	42.38	791	493	1055	229	native
10330	77.54	42.38	0	0	0	0	naturalized
10331	79.11	41.98	202	139	273	68	native
10331	79.11	41.98	0	0	0	0	naturalized
10332	72.15	42.09	1188	626	1517	297	native

10332	72.15	42.09	0	0	0	0	naturalized
10333	72.23	40.45	1600	894	2076	418	native
10333	72.23	40.45	0	0	0	0	naturalized
10334	75.62	41.42	892	526	1175	243	native
10334	75.62	41.42	0	0	0	0	naturalized
10335	72.75	39.54	370	241	494	117	native
10335	72.75	39.54	0	0	0	0	naturalized
10336	-85.04	12.84	4738	1821	5831	728	native
10336	-85.04	12.84	0	0	0	0	naturalized
10337	-65.15	-13.86	2328	725	2758	295	native
10337	-65.15	-13.86	26	9	30	5	naturalized
10338	-64.66	-16.71	0	0	0	0	native
10338	-64.66	-16.71	109	77	141	45	naturalized
10339	-64.29	-20.04	1592	610	1934	268	native
10339	-64.29	-20.04	28	23	38	13	naturalized
10340	-65.63	-17.23	2757	1092	3411	438	native
10340	-65.63	-17.23	50	40	68	22	naturalized
10341	-68.13	-15.22	4814	1834	5905	743	native
10341	-68.13	-15.22	78	59	104	33	naturalized
10342	-67.69	-18.63	257	140	336	61	native
10342	-67.69	-18.63	4	7	6	5	naturalized
10343	-67.32	-11.09	1405	380	1626	159	native
10343	-67.32	-11.09	12	5	15	2	naturalized
10344	-66.73	-20.54	642	277	792	127	native
10344	-66.73	-20.54	7	11	12	6	naturalized
10345	-61.46	-17.29	4747	1562	5632	677	native
10345	-61.46	-17.29	61	38	76	23	naturalized
10346	-63.88	-21.59	1594	642	1960	276	native
10346	-63.88	-21.59	23	18	31	10	naturalized
10347	-75.59	6.91	4506	1644	5519	631	native
10347	-75.59	6.91	78	50	105	23	naturalized
10349	-79.15	-3.01	1222	530	1536	216	native
10349	-79.15	-3.01	0	0	0	0	naturalized
10350	-79.10	-1.59	643	258	794	107	native
10350	-79.10	-1.59	0	0	0	0	naturalized
10351	-79.02	-2.53	602	229	742	89	native
10351	-79.02	-2.53	0	0	0	0	naturalized
10352	-78.05	0.74	1352	547	1693	206	native
10352	-78.05	0.74	0	0	0	0	naturalized

10353	-78.72	-1.97	1101	468	1378	191	native
10353	-78.72	-1.97	0	0	0	0	naturalized
10354	-78.86	-0.86	914	414	1164	164	native
10354	-78.86	-0.86	0	0	0	0	naturalized
10355	-79.83	-3.51	673	282	841	114	native
10355	-79.83	-3.51	0	0	0	0	naturalized
10358	-80.01	-2.12	450	173	547	76	native
10358	-80.01	-2.12	0	0	0	0	naturalized
10359	-78.36	0.41	1045	447	1312	180	native
10359	-78.36	0.41	0	0	0	0	naturalized
10360	-79.65	-4.09	1524	599	1876	247	native
10360	-79.65	-4.09	0	0	0	0	naturalized
10361	-79.49	-1.34	941	368	1177	132	native
10361	-79.49	-1.34	0	0	0	0	naturalized
10363	-78.01	-2.56	1509	554	1856	207	native
10363	-78.01	-2.56	0	0	0	0	naturalized
10364	-76.90	-0.73	3131	1112	3804	439	native
10364	-76.90	-0.73	0	0	0	0	naturalized
10365	-76.87	-1.71	1724	574	2067	231	native
10365	-76.87	-1.71	0	0	0	0	naturalized
10366	-78.78	-0.15	2150	956	2756	350	native
10366	-78.78	-0.15	0	0	0	0	naturalized
10367	-76.56	-0.02	1023	314	1212	125	native
10367	-76.56	-0.02	0	0	0	0	naturalized
10368	-78.50	-1.29	896	396	1137	155	native
10368	-78.50	-1.29	0	0	0	0	naturalized
10369	-78.90	-4.17	1045	377	1275	147	native
10369	-78.90	-4.17	0	0	0	0	naturalized
10406	-74.36	-9.17	14528	5535	17909	2154	native
10406	-74.36	-9.17	0	0	0	0	naturalized
10407	20.98	40.05	1982	1137	2587	532	native
10407	20.98	40.05	0	0	0	0	naturalized
10408	21.21	39.38	2021	1153	2647	527	native
10408	21.21	39.38	0	0	0	0	naturalized
10410	22.44	38.52	2340	1381	3075	646	native
10410	22.44	38.52	0	0	0	0	naturalized
10411	22.60	39.38	1529	894	1994	429	native
10411	22.60	39.38	0	0	0	0	naturalized
10412	22.00	40.43	2385	1421	3144	662	native

10412	22.00	40.43	0	0	0	0	naturalized
10413	24.29	40.99	2504	1437	3280	661	native
10413	24.29	40.99	0	0	0	0	naturalized
10417	36.27	50.00	458	278	611	125	native
10417	36.27	50.00	0	0	0	0	naturalized
10418	36.13	56.38	509	361	703	167	native
10418	36.13	56.38	0	0	0	0	naturalized
10419	45.34	54.44	364	231	494	101	native
10419	45.34	54.44	0	0	0	0	naturalized
10420	47.96	53.90	884	572	1193	263	native
10420	47.96	53.90	0	0	0	0	naturalized
10422	13.84	41.79	1191	680	1551	320	native
10422	13.84	41.79	0	0	0	0	naturalized
10429	35.02	31.47	1984	1182	2610	556	native
10429	35.02	31.47	0	0	0	0	naturalized
10430	-1.79	35.05	363	151	446	68	native
10430	-1.79	35.05	0	0	0	0	naturalized
10448	116.41	40.18	1399	688	1814	273	native
10448	116.41	40.18	46	38	65	19	naturalized
10449	117.33	39.31	693	344	903	134	native
10449	117.33	39.31	31	29	44	16	naturalized
10450	116.13	39.55	1717	864	2237	344	native
10450	116.13	39.55	76	69	111	34	naturalized
10451	112.29	37.58	1659	789	2137	311	native
10451	112.29	37.58	50	53	74	29	naturalized
10452	113.91	44.09	2009	1110	2663	456	native
10452	113.91	44.09	56	55	84	27	naturalized
10453	122.61	41.30	1379	710	1804	285	native
10453	122.61	41.30	59	57	85	31	naturalized
10454	126.19	43.66	1429	796	1900	325	native
10454	126.19	43.66	49	48	72	25	naturalized
10455	127.78	47.84	1200	678	1607	271	native
10455	127.78	47.84	58	57	86	29	naturalized
10456	121.45	31.20	1041	503	1342	202	native
10456	121.45	31.20	36	30	50	16	naturalized
10457	119.46	32.97	1522	678	1927	273	native
10457	119.46	32.97	101	72	136	37	naturalized
10458	120.09	29.18	2407	1047	3033	421	native
10458	120.09	29.18	99	72	137	34	naturalized

10459	117.23	31.83	1882	859	2403	338	native
10459	117.23	31.83	81	62	112	31	naturalized
10460	117.99	26.08	2511	1005	3094	422	native
10460	117.99	26.08	153	83	195	41	naturalized
10461	115.72	27.61	2728	1117	3387	458	native
10461	115.72	27.61	102	64	135	31	naturalized
10462	118.15	36.35	944	474	1227	191	native
10462	118.15	36.35	69	66	100	35	naturalized
10463	113.61	33.88	2233	1017	2850	400	native
10463	113.61	33.88	74	57	103	28	naturalized
10464	112.27	30.98	2964	1283	3736	511	native
10464	112.27	30.98	86	61	117	30	naturalized
10465	111.71	27.61	3073	1199	3784	488	native
10465	111.71	27.61	87	60	117	30	naturalized
10466	113.42	23.34	4000	1451	4809	642	native
10466	113.42	23.34	180	89	222	47	naturalized
10467	108.79	23.83	5059	1769	6052	776	native
10467	108.79	23.83	148	73	181	40	naturalized
10469	107.87	30.06	2221	911	2777	355	native
10469	107.87	30.06	22	15	31	6	naturalized
10470	102.71	30.62	7030	3371	8942	1459	native
10470	102.71	30.62	107	73	145	35	naturalized
10471	106.87	26.82	4059	1527	4956	630	native
10471	106.87	26.82	101	62	133	30	naturalized
10472	101.49	24.98	9065	3810	11210	1665	native
10472	101.49	24.98	188	95	236	47	naturalized
10473	88.13	31.69	5342	2799	6949	1192	native
10473	88.13	31.69	63	48	86	25	naturalized
10474	108.87	35.20	2902	1342	3713	531	native
10474	108.87	35.20	62	58	91	29	naturalized
10475	100.93	37.82	3099	1563	4025	637	native
10475	100.93	37.82	56	51	80	27	naturalized
10476	96.00	35.75	2358	1362	3136	584	native
10476	96.00	35.75	49	44	70	23	naturalized
10477	106.16	37.27	1037	537	1365	209	native
10477	106.16	37.27	42	42	63	21	naturalized
10478	85.20	41.11	3041	1700	4016	725	native
10478	85.20	41.11	63	63	92	34	naturalized
10482	10.33	1.70	0	0	0	0	native

10482	10.33	1.70	98	36	115	19	naturalized
10483	-71.49	-1.55	2655	689	3053	291	native
10483	-71.49	-1.55	14	9	20	3	naturalized
10484	-74.90	10.72	552	187	653	86	native
10484	-74.90	10.72	26	13	34	5	naturalized
10485	-70.95	6.55	372	116	442	46	native
10485	-70.95	6.55	12	5	16	1	naturalized
10486	-74.51	8.71	919	269	1073	115	native
10486	-74.51	8.71	21	14	29	6	naturalized
10487	-73.12	5.76	2041	718	2469	290	native
10487	-73.12	5.76	59	38	78	19	naturalized
10488	-75.34	5.33	1225	384	1459	150	native
10488	-75.34	5.33	37	17	47	7	naturalized
10489	-73.99	0.78	2160	638	2549	249	native
10489	-73.99	0.78	14	8	19	3	naturalized
10490	-71.62	5.37	652	199	772	79	native
10490	-71.62	5.37	15	8	20	3	naturalized
10491	-76.86	2.39	2473	903	3041	335	native
10491	-76.86	2.39	36	24	49	11	naturalized
10492	-73.54	9.52	836	307	1014	129	native
10492	-73.54	9.52	21	12	29	4	naturalized
10493	-76.97	5.99	3047	1086	3738	395	native
10493	-76.97	5.99	24	17	34	7	naturalized
10494	-75.80	8.35	598	183	705	76	native
10494	-75.80	8.35	23	12	31	4	naturalized
10495	-74.13	4.76	2925	1049	3561	413	native
10495	-74.13	4.76	93	63	126	30	naturalized
10496	-68.82	2.71	1033	305	1188	150	native
10496	-68.82	2.71	7	5	11	1	naturalized
10497	-72.13	1.90	814	228	957	85	native
10497	-72.13	1.90	9	4	12	1	naturalized
10498	-75.64	2.54	0	0	0	0	native
10498	-75.64	2.54	46	22	60	8	naturalized
10499	-72.46	11.46	858	302	1025	135	native
10499	-72.46	11.46	23	8	28	3	naturalized
10500	-74.26	10.19	2111	731	2538	304	native
10500	-74.26	10.19	43	26	59	10	naturalized
10501	-72.97	3.32	2315	753	2773	295	native
10501	-72.97	3.32	30	19	41	8	naturalized

10502	-77.87	1.56	2448	945	3057	336	native
10502	-77.87	1.56	50	32	68	14	naturalized
10503	-72.89	8.08	1460	503	1770	193	native
10503	-72.89	8.08	30	19	41	8	naturalized
10504	-75.88	0.45	1870	656	2284	242	native
10504	-75.88	0.45	25	14	33	6	naturalized
10505	-75.69	4.48	1093	397	1327	163	native
10505	-75.69	4.48	32	21	43	10	naturalized
10506	-75.92	5.07	1242	450	1530	162	native
10506	-75.92	5.07	24	15	32	7	naturalized
10507	-73.52	6.68	2913	1009	3531	391	native
10507	-73.52	6.68	56	34	74	16	naturalized
10508	-75.12	9.06	292	81	338	35	native
10508	-75.12	9.06	10	4	13	1	naturalized
10509	-75.27	4.04	1560	481	1854	187	native
10509	-75.27	4.04	52	23	68	7	naturalized
10510	-76.53	3.87	2211	863	2731	343	native
10510	-76.53	3.87	26	13	33	6	naturalized
10511	-70.57	0.60	1455	405	1671	189	native
10511	-70.57	0.60	7	3	8	2	naturalized
10512	-69.42	4.68	802	246	933	115	native
10512	-69.42	4.68	17	6	21	2	naturalized
10513	-73.09	3.90	0	0	0	0	native
10513	-73.09	3.90	300	182	400	82	naturalized
10566	-114.51	55.17	0	0	0	0	native
10566	-114.51	55.17	133	149	201	81	naturalized
10567	66.03	33.84	3879	2005	5019	865	native
10567	66.03	33.84	0	0	0	0	naturalized
10571	-86.83	32.79	2566	1404	3394	576	native
10571	-86.83	32.79	559	331	744	146	naturalized
10572	20.07	41.14	0	0	0	0	native
10572	20.07	41.14	50	34	68	16	naturalized
10574	2.63	28.16	0	0	0	0	native
10574	2.63	28.16	36	25	48	13	naturalized
10579	17.57	-12.34	0	0	0	0	native
10579	17.57	-12.34	97	59	128	28	naturalized
10580	20.67	-80.41	0	0	0	0	native
10580	20.67	-80.41	1	1	2	0	naturalized
10581	-111.66	34.30	0	0	0	0	native

10581	-111.66	34.30	314	213	428	99	naturalized
10582	-92.44	34.90	1953	1049	2591	411	native
10582	-92.44	34.90	340	225	464	101	naturalized
10585	-152.47	64.31	0	0	0	0	native
10585	-152.47	64.31	109	119	165	63	naturalized
10594	2.34	9.65	2624	984	3142	466	native
10594	2.34	9.65	0	0	0	0	naturalized
10599	28.05	53.54	1100	713	1510	303	native
10599	28.05	53.54	61	76	96	41	naturalized
10601	-88.68	17.22	0	0	0	0	native
10601	-88.68	17.22	40	15	49	6	naturalized
10606	-124.76	54.75	0	0	0	0	native
10606	-124.76	54.75	520	451	760	211	naturalized
10607	109.50	53.55	0	0	0	0	native
10607	109.50	53.55	53	57	81	29	naturalized
10608	25.23	42.76	0	0	0	0	native
10608	25.23	42.76	250	226	366	110	naturalized
10609	29.89	-3.36	0	0	0	0	native
10609	29.89	-3.36	22	9	26	5	naturalized
10617	-119.59	37.24	0	0	0	0	native
10617	-119.59	37.24	842	560	1147	255	naturalized
10636	12.74	5.69	7058	2338	8301	1095	native
10636	12.74	5.69	0	0	0	0	naturalized
10637	-72.73	41.63	1476	953	2063	366	native
10637	-72.73	41.63	521	356	717	160	naturalized
10639	-105.53	39.00	0	0	0	0	native
10639	-105.53	39.00	245	225	361	109	naturalized
10644	-84.20	9.97	0	0	0	0	native
10644	-84.20	9.97	165	83	214	34	naturalized
10650	116.20	52.84	0	0	0	0	native
10650	116.20	52.84	10	7	13	4	naturalized
10654	33.22	35.04	0	0	0	0	native
10654	33.22	35.04	67	39	89	17	naturalized
10656	-75.51	39.00	1343	849	1864	328	native
10656	-75.51	39.00	367	257	507	117	naturalized
10657	10.05	55.96	962	747	1382	327	native
10657	10.05	55.96	195	152	268	79	naturalized
10659	-70.49	18.89	0	0	0	0	native
10659	-70.49	18.89	32	6	37	1	naturalized

10669	39.62	8.63	0	0	0	0	native
10669	39.62	8.63	42	21	50	13	naturalized
10672	26.26	64.49	0	0	0	0	native
10672	26.26	64.49	58	39	79	18	naturalized
10673	-82.48	28.65	0	0	0	0	native
10673	-82.48	28.65	758	393	977	174	naturalized
10674	-6.86	62.05	0	0	0	0	native
10674	-6.86	62.05	10	6	14	2	naturalized
10675	2.45	46.63	0	0	0	0	native
10675	2.45	46.63	354	203	462	95	naturalized
10680	-83.45	32.65	2707	1450	3570	587	native
10680	-83.45	32.65	457	283	615	125	naturalized
10681	10.39	51.11	0	0	0	0	native
10681	10.39	51.11	206	145	282	69	naturalized
10683	-1.21	7.96	0	0	0	0	native
10683	-1.21	7.96	139	61	173	27	naturalized
10686	-41.39	74.72	307	323	480	150	native
10686	-41.39	74.72	49	59	77	31	naturalized
10698	19.41	47.17	0	0	0	0	native
10698	19.41	47.17	59	50	87	22	naturalized
10700	-114.65	44.40	1744	1172	2400	516	native
10700	-114.65	44.40	263	221	377	107	naturalized
10701	-89.15	40.12	1948	1129	2650	427	native
10701	-89.15	40.12	511	355	703	163	naturalized
10703	-86.29	39.91	1761	1044	2403	402	native
10703	-86.29	39.91	375	269	516	128	naturalized
10704	-93.50	42.08	1373	788	1864	297	native
10704	-93.50	42.08	289	214	398	105	naturalized
10706	106.36	57.10	0	0	0	0	native
10706	106.36	57.10	72	79	110	41	naturalized
10709	12.16	43.53	0	0	0	0	native
10709	12.16	43.53	251	150	340	61	naturalized
10716	-98.37	38.48	1578	841	2093	326	native
10716	-98.37	38.48	259	196	363	92	naturalized
10719	37.86	0.53	0	0	0	0	native
10719	37.86	0.53	78	37	92	23	naturalized
10721	74.56	41.46	0	0	0	0	native
10721	74.56	41.46	22	30	37	15	naturalized
10727	-85.29	37.53	0	0	0	0	native

10727	-85.29	37.53	421	276	574	123	naturalized
10731	-61.96	54.29	498	454	755	197	native
10731	-61.96	54.29	50	55	75	30	naturalized
10733	-9.31	6.45	2476	838	2923	391	native
10733	-9.31	6.45	0	0	0	0	naturalized
10740	-92.02	31.08	2111	1179	2805	485	native
10740	-92.02	31.08	464	299	629	134	naturalized
10743	-69.23	45.40	1330	907	1883	354	native
10743	-69.23	45.40	407	307	571	143	naturalized
10744	-97.43	54.93	0	0	0	0	native
10744	-97.43	54.93	150	144	218	76	naturalized
10746	-71.81	42.26	1584	1028	2215	397	native
10746	-71.81	42.26	704	485	967	222	naturalized
10753	-85.74	44.88	1642	1053	2289	406	native
10753	-85.74	44.88	507	359	702	164	naturalized
10754	-94.20	46.35	0	0	0	0	native
10754	-94.20	46.35	271	213	380	104	naturalized
10758	-109.62	47.03	0	0	0	0	native
10758	-109.62	47.03	240	223	352	111	naturalized
10767	-76.80	39.05	0	0	0	0	native
10767	-76.80	39.05	567	387	782	172	naturalized
10768	-89.67	32.76	2103	1179	2802	480	native
10768	-89.67	32.76	404	242	539	107	naturalized
10769	-92.49	38.37	1867	1037	2510	394	native
10769	-92.49	38.37	420	327	593	154	naturalized
10781	-66.37	46.63	0	0	0	0	native
10781	-66.37	46.63	246	201	351	96	naturalized
10782	-79.38	35.55	0	0	0	0	native
10782	-79.38	35.55	554	342	744	152	naturalized
10785	-100.46	47.45	965	586	1327	224	native
10785	-100.46	47.45	141	128	202	67	naturalized
10786	-99.79	41.53	1263	726	1711	278	native
10786	-99.79	41.53	222	180	315	87	naturalized
10787	83.94	28.25	0	0	0	0	native
10787	83.94	28.25	99	65	135	29	naturalized
10788	5.60	52.25	1139	831	1607	363	native
10788	5.60	52.25	101	84	143	42	naturalized
10789	-116.65	39.36	0	0	0	0	native
10789	-116.65	39.36	180	170	262	88	naturalized

10797	14.08	64.45	0	0	0	0	native
10797	14.08	64.45	279	199	372	106	naturalized
10799	-63.30	45.15	0	0	0	0	native
10799	-63.30	45.15	300	230	421	109	naturalized
10801	133.37	-19.41	0	0	0	0	native
10801	133.37	-19.41	206	111	266	51	naturalized
10803	-88.91	71.05	0	0	0	0	native
10803	-88.91	71.05	2	8	4	6	naturalized
10806	-71.58	43.69	1264	846	1782	328	native
10806	-71.58	43.69	308	241	434	115	naturalized
10807	-74.66	40.19	1714	1102	2391	425	native
10807	-74.66	40.19	552	404	775	181	naturalized
10808	-106.11	34.42	0	0	0	0	native
10808	-106.11	34.42	252	202	353	101	naturalized
10809	-118.98	66.33	0	0	0	0	native
10809	-118.98	66.33	43	50	65	28	naturalized
10810	-75.69	42.99	0	0	0	0	native
10810	-75.69	42.99	741	517	1026	232	naturalized
10815	-82.71	40.42	0	0	0	0	native
10815	-82.71	40.42	545	373	751	167	naturalized
10816	-97.51	35.58	2078	1067	2723	422	native
10816	-97.51	35.58	258	193	359	92	naturalized
10817	56.11	20.60	0	0	0	0	native
10817	56.11	20.60	19	10	23	6	naturalized
10818	-85.83	50.07	0	0	0	0	native
10818	-85.83	50.07	513	366	707	172	naturalized
10819	-120.54	43.94	0	0	0	0	native
10819	-120.54	43.94	499	395	710	184	naturalized
10823	-58.39	-23.24	0	0	0	0	native
10823	-58.39	-23.24	89	54	120	23	naturalized
10824	-63.24	46.40	497	377	728	146	native
10824	-63.24	46.40	171	138	241	68	naturalized
10825	-77.84	40.90	1864	1131	2563	432	native
10825	-77.84	40.90	678	457	929	206	naturalized
10830	19.40	52.12	0	0	0	0	native
10830	19.40	52.12	185	116	250	51	naturalized
10835	-71.75	53.38	0	0	0	0	native
10835	-71.75	53.38	385	307	540	152	naturalized
10837	-71.56	41.68	1145	737	1594	288	native

10837	-71.56	41.68	328	234	453	109	naturalized
10839	24.97	45.84	0	0	0	0	native
10839	24.97	45.84	43	34	64	13	naturalized
10845	29.92	-2.00	0	0	0	0	native
10845	29.92	-2.00	34	15	39	10	naturalized
10849	-105.89	54.42	0	0	0	0	native
10849	-105.89	54.42	147	152	219	80	naturalized
10850	44.59	24.02	1875	962	2399	438	native
10850	44.59	24.02	42	32	58	16	naturalized
10851	-80.90	33.92	2321	1308	3106	523	native
10851	-80.90	33.92	447	310	618	139	naturalized
10855	-100.22	44.44	0	0	0	0	native
10855	-100.22	44.44	157	143	227	73	naturalized
10877	16.74	62.79	2959	1411	3826	544	native
10877	16.74	62.79	681	476	936	221	naturalized
10881	34.82	-6.27	0	0	0	0	native
10881	34.82	-6.27	75	33	88	20	naturalized
10886	-86.35	35.85	0	0	0	0	native
10886	-86.35	35.85	355	247	490	112	naturalized
10887	-99.36	31.50	0	0	0	0	native
10887	-99.36	31.50	527	320	702	145	naturalized
10896	27.30	41.26	0	0	0	0	native
10896	27.30	41.26	47	30	65	12	naturalized
10900	94.79	51.58	0	0	0	0	native
10900	94.79	51.58	7	6	11	2	naturalized
10903	32.39	1.28	0	0	0	0	native
10903	32.39	1.28	49	28	61	16	naturalized
10905	-56.01	-32.80	0	0	0	0	native
10905	-56.01	-32.80	86	54	117	23	naturalized
10906	-111.67	39.33	0	0	0	0	native
10906	-111.67	39.33	298	217	404	111	naturalized
10909	-66.17	7.12	0	0	0	0	native
10909	-66.17	7.12	70	14	79	5	naturalized
10910	-72.66	44.08	1261	829	1770	320	native
10910	-72.66	44.08	346	268	486	128	naturalized
10911	144.31	-36.86	0	0	0	0	native
10911	144.31	-36.86	740	462	974	228	naturalized
10914	-78.83	37.52	0	0	0	0	native
10914	-78.83	37.52	508	346	696	158	naturalized

10917	-120.43	47.39	0	0	0	0	native
10917	-120.43	47.39	503	373	703	173	naturalized
10922	-89.74	44.64	1503	966	2093	376	native
10922	-89.74	44.64	389	299	549	139	naturalized
10925	-80.61	38.64	1554	858	2086	326	native
10925	-80.61	38.64	366	260	505	121	naturalized
10926	-107.54	43.00	0	0	0	0	native
10926	-107.54	43.00	168	146	239	75	naturalized
10929	47.57	15.84	0	0	0	0	native
10929	47.57	15.84	33	5	36	2	naturalized
10931	-135.50	63.64	0	0	0	0	native
10931	-135.50	63.64	69	60	102	27	naturalized
10932	23.65	-2.88	8833	3010	10371	1472	native
10932	23.65	-2.88	0	0	0	0	naturalized
10935	-70.45	-9.31	2837	848	3332	353	native
10935	-70.45	-9.31	35	23	47	11	naturalized
10936	-36.62	-9.52	1167	468	1439	196	native
10936	-36.62	-9.52	41	21	53	9	naturalized
10937	-51.96	1.44	1792	560	2119	233	native
10937	-51.96	1.44	29	12	37	4	naturalized
10938	-64.70	-4.18	5589	1642	6491	740	native
10938	-64.70	-4.18	54	25	68	11	naturalized
10939	-41.73	-12.47	0	0	0	0	native
10939	-41.73	-12.47	110	63	143	30	naturalized
10940	-39.62	-5.09	1613	580	1928	265	native
10940	-39.62	-5.09	66	37	86	17	naturalized
10941	-47.80	-15.78	2094	678	2466	306	native
10941	-47.80	-15.78	69	39	90	18	naturalized
10942	-40.66	-19.64	0	0	0	0	native
10942	-40.66	-19.64	61	36	80	17	naturalized
10943	-49.62	-16.04	3790	1223	4448	565	native
10943	-49.62	-16.04	73	36	94	15	naturalized
10944	-45.29	-5.07	1960	589	2285	264	native
10944	-45.29	-5.07	47	26	61	12	naturalized
10945	-55.92	-12.95	3788	1262	4480	570	native
10945	-55.92	-12.95	52	31	70	13	naturalized
10946	-54.85	-20.33	2585	821	3052	354	native
10946	-54.85	-20.33	68	35	86	17	naturalized
10947	-44.66	-18.46	7007	2267	8268	1006	native

10947	-44.66	-18.46	126	74	165	35	naturalized
10948	-53.06	-3.97	4197	1273	4912	558	native
10948	-53.06	-3.97	59	30	77	12	naturalized
10949	-36.87	-7.14	1195	487	1462	220	native
10949	-36.87	-7.14	54	35	73	16	naturalized
10950	-38.00	-8.33	2026	735	2435	326	native
10950	-38.00	-8.33	82	47	107	22	naturalized
10951	-42.97	-7.38	1396	450	1644	202	native
10951	-42.97	-7.38	43	24	57	10	naturalized
10952	-42.66	-22.19	4517	1594	5441	670	native
10952	-42.66	-22.19	101	69	137	33	naturalized
10953	-36.68	-5.85	0	0	0	0	native
10953	-36.68	-5.85	48	25	62	11	naturalized
10954	-62.86	-10.90	2264	656	2642	278	native
10954	-62.86	-10.90	23	10	28	5	naturalized
10955	-61.40	2.08	1885	656	2248	293	native
10955	-61.40	2.08	27	15	35	7	naturalized
10956	-48.73	-22.27	4698	1577	5614	661	native
10956	-48.73	-22.27	155	100	207	48	naturalized
10957	-37.45	-10.59	997	415	1213	199	native
10957	-37.45	-10.59	32	18	42	8	naturalized
10958	-48.33	-10.15	1493	554	1786	261	native
10958	-48.33	-10.15	21	13	28	6	naturalized
10960	15.33	49.74	1661	992	2195	458	native
10960	15.33	49.74	101	83	143	41	naturalized
10961	16.39	45.03	0	0	0	0	native
10961	16.39	45.03	41	30	59	12	naturalized
10962	154.04	62.70	0	0	0	0	native
10962	154.04	62.70	94	72	132	34	naturalized
10963	-77.02	38.91	0	0	0	0	native
10963	-77.02	38.91	262	184	363	83	naturalized
10964	87.21	54.78	0	0	0	0	native
10964	87.21	54.78	62	68	96	34	naturalized
10965	82.13	58.48	0	0	0	0	native
10965	82.13	58.48	72	66	106	32	naturalized
10966	82.60	52.61	0	0	0	0	native
10966	82.60	52.61	81	76	121	36	naturalized
10967	87.01	50.74	0	0	0	0	native
10967	87.01	50.74	101	88	148	41	naturalized

10968	96.06	67.48	0	0	0	0	native
10968	96.06	67.48	22	25	36	11	naturalized
10971	89.85	53.36	0	0	0	0	native
10971	89.85	53.36	39	46	60	25	naturalized
10972	79.77	55.27	0	0	0	0	native
10972	79.77	55.27	50	45	73	22	naturalized
10973	73.36	56.09	0	0	0	0	native
10973	73.36	56.09	23	26	37	12	naturalized
10978	-3.14	37.07	1301	782	1707	376	native
10978	-3.14	37.07	0	0	0	0	naturalized
10979	69.89	40.66	0	0	0	0	native
10979	69.89	40.66	2	0	2	0	naturalized
10980	69.48	40.30	0	0	0	0	native
10980	69.48	40.30	2	1	3	0	naturalized
10981	69.98	40.35	0	0	0	0	native
10981	69.98	40.35	6	2	7	1	naturalized
10982	69.02	39.76	0	0	0	0	native
10982	69.02	39.76	4	1	5	0	naturalized
10983	70.64	40.05	0	0	0	0	native
10983	70.64	40.05	3	2	4	1	naturalized
10985	67.67	39.37	0	0	0	0	native
10985	67.67	39.37	5	2	6	1	naturalized
10986	68.21	39.25	0	0	0	0	native
10986	68.21	39.25	5	3	7	1	naturalized
10987	69.46	39.33	0	0	0	0	native
10987	69.46	39.33	4	1	5	0	naturalized
10989	68.66	38.78	0	0	0	0	native
10989	68.66	38.78	9	3	11	1	naturalized
10990	69.45	38.89	0	0	0	0	native
10990	69.45	38.89	3	1	4	0	naturalized
10991	70.13	39.02	0	0	0	0	native
10991	70.13	39.02	4	1	5	0	naturalized
10992	70.29	38.52	0	0	0	0	native
10992	70.29	38.52	2	1	3	0	naturalized
10993	70.81	38.43	0	0	0	0	native
10993	70.81	38.43	5	1	6	0	naturalized
10994	70.13	37.71	0	0	0	0	native
10994	70.13	37.71	3	2	4	1	naturalized
10996	68.58	38.33	0	0	0	0	native

10996	68.58	38.33	17	7	21	3	naturalized
10997	69.51	38.11	0	0	0	0	native
10997	69.51	38.11	7	1	8	0	naturalized
10998	68.39	37.55	0	0	0	0	native
10998	68.39	37.55	7	3	8	2	naturalized
10999	69.16	37.48	0	0	0	0	native
10999	69.16	37.48	2	1	3	0	naturalized
11001	71.31	39.18	0	0	0	0	native
11001	71.31	39.18	2	1	3	0	naturalized
11002	71.43	38.77	0	0	0	0	native
11002	71.43	38.77	2	0	2	0	naturalized
11003	71.81	38.43	0	0	0	0	native
11003	71.81	38.43	4	1	5	0	naturalized
11005	72.11	38.08	0	0	0	0	native
11005	72.11	38.08	4	2	5	1	naturalized
11006	72.16	37.48	0	0	0	0	native
11006	72.16	37.48	8	2	10	0	naturalized
11007	71.98	36.96	0	0	0	0	native
11007	71.98	36.96	2	1	3	0	naturalized
11009	73.54	38.28	0	0	0	0	native
11009	73.54	38.28	1	0	1	0	naturalized
11010	71.34	39.50	0	0	0	0	native
11010	71.34	39.50	2	0	2	0	naturalized
11011	131.50	-28.00	620	375	829	166	native
11011	131.50	-28.00	27	22	38	11	naturalized
11012	137.50	-28.00	622	467	879	210	native
11012	137.50	-28.00	59	41	81	19	naturalized
11013	130.98	-30.79	328	216	436	108	native
11013	130.98	-30.79	36	30	50	16	naturalized
11014	135.67	-30.92	536	363	739	160	native
11014	135.67	-30.92	61	42	84	19	naturalized
11015	138.61	-31.36	718	434	958	194	native
11015	138.61	-31.36	174	126	240	60	naturalized
11016	140.07	-31.59	409	258	555	112	native
11016	140.07	-31.59	67	53	96	24	naturalized
11017	135.51	-32.76	939	528	1223	244	native
11017	135.51	-32.76	221	158	303	76	naturalized
11018	138.52	-33.72	588	333	772	149	native
11018	138.52	-33.72	265	175	360	80	naturalized

11019	140.00	-34.23	808	492	1083	217	native
11019	140.00	-34.23	259	186	357	88	naturalized
11020	137.63	-34.44	464	266	608	122	native
11020	137.63	-34.44	155	135	226	64	naturalized
11021	138.68	-35.07	621	358	811	168	native
11021	138.68	-35.07	512	299	665	146	naturalized
11022	140.32	-36.56	728	407	948	187	native
11022	140.32	-36.56	288	199	390	97	naturalized
11025	82.99	25.27	237	91	289	39	native
11025	82.99	25.27	0	0	0	0	naturalized
11026	45.39	54.81	415	283	569	129	native
11026	45.39	54.81	0	0	0	0	naturalized
11029	7.39	45.73	1338	827	1780	385	native
11029	7.39	45.73	0	0	0	0	naturalized
11030	7.93	45.06	2122	1278	2794	606	native
11030	7.93	45.06	0	0	0	0	naturalized
11031	9.77	45.62	1966	1220	2608	578	native
11031	9.77	45.62	0	0	0	0	naturalized
11033	11.85	45.66	1977	1223	2628	572	native
11033	11.85	45.66	0	0	0	0	naturalized
11034	13.06	46.15	2014	1209	2661	562	native
11034	13.06	46.15	0	0	0	0	naturalized
11035	8.71	44.26	1823	1019	2368	474	native
11035	8.71	44.26	0	0	0	0	naturalized
11038	13.11	43.37	1658	931	2161	428	native
11038	13.11	43.37	0	0	0	0	naturalized
11039	12.49	42.97	1629	911	2123	417	native
11039	12.49	42.97	0	0	0	0	naturalized
11042	14.60	41.68	1604	901	2084	421	native
11042	14.60	41.68	0	0	0	0	naturalized
11043	14.84	40.86	1641	919	2129	431	native
11043	14.84	40.86	0	0	0	0	naturalized
11044	16.62	40.98	1445	786	1868	363	native
11044	16.62	40.98	0	0	0	0	naturalized
11045	16.08	40.50	1750	981	2267	464	native
11045	16.08	40.50	0	0	0	0	naturalized
11046	16.35	39.07	1684	927	2181	430	native
11046	16.35	39.07	0	0	0	0	naturalized
11047	9.81	54.19	1141	763	1568	336	native

11047	9.81	54.19	78	65	109	34	naturalized
11048	12.54	53.75	1331	822	1794	359	native
11048	12.54	53.75	80	66	113	33	naturalized
11049	9.17	52.77	1551	936	2075	412	native
11049	9.17	52.77	126	88	171	43	naturalized
11050	10.03	53.55	943	631	1297	277	native
11050	10.03	53.55	70	66	102	34	naturalized
11051	13.40	52.47	1306	817	1760	363	native
11051	13.40	52.47	114	98	165	47	naturalized
11052	11.71	52.01	1594	989	2144	439	native
11052	11.71	52.01	275	180	366	89	naturalized
11053	7.57	51.48	0	0	0	0	native
11053	7.57	51.48	159	131	224	66	naturalized
11054	9.04	50.60	1680	1000	2233	447	native
11054	9.04	50.60	158	115	215	58	naturalized
11055	11.03	50.90	1636	954	2166	424	native
11055	11.03	50.90	161	112	217	56	naturalized
11056	7.45	49.92	1724	999	2278	445	native
11056	7.45	49.92	160	136	228	68	naturalized
11057	11.42	48.95	2582	1415	3365	632	native
11057	11.42	48.95	185	139	255	69	naturalized
11058	9.05	48.54	1827	1085	2416	496	native
11058	9.05	48.54	135	110	192	53	naturalized
11059	6.96	49.39	1108	679	1487	300	native
11059	6.96	49.39	121	94	164	51	naturalized
11060	8.79	53.11	701	468	965	204	native
11060	8.79	53.11	44	45	64	25	naturalized
11061	13.41	52.50	911	570	1232	249	native
11061	13.41	52.50	73	71	110	34	naturalized
11062	38.74	-5.97	292	82	338	36	native
11062	38.74	-5.97	0	0	0	0	naturalized
11066	23.59	-15.29	644	201	741	104	native
11066	23.59	-15.29	0	0	0	0	naturalized
11067	26.64	-16.29	995	324	1140	179	native
11067	26.64	-16.29	0	0	0	0	naturalized
11068	26.11	-13.00	1168	357	1322	203	native
11068	26.11	-13.00	0	0	0	0	naturalized
11069	29.53	-14.07	1002	323	1144	181	native
11069	29.53	-14.07	0	0	0	0	naturalized

11070	30.66	-10.40	1542	500	1766	276	native
11070	30.66	-10.40	0	0	0	0	naturalized
11071	32.13	-13.09	895	318	1046	167	native
11071	32.13	-13.09	0	0	0	0	naturalized
11072	30.77	-20.90	1438	488	1657	269	native
11072	30.77	-20.90	39	28	57	10	naturalized
11073	27.73	-19.57	1332	477	1549	260	native
11073	27.73	-19.57	41	36	61	16	naturalized
11074	30.60	-18.66	1450	504	1685	269	native
11074	30.60	-18.66	78	51	105	24	naturalized
11075	32.60	-19.22	2037	693	2357	373	native
11075	32.60	-19.22	81	47	105	23	naturalized
11076	29.90	-17.15	1510	512	1738	284	native
11076	29.90	-17.15	44	29	60	13	naturalized
11078	32.45	-25.54	794	282	912	164	native
11078	32.45	-25.54	0	0	0	0	naturalized
11079	33.62	-23.08	701	250	804	147	native
11079	33.62	-23.08	0	0	0	0	naturalized
11081	32.76	-15.48	839	275	951	163	native
11081	32.76	-15.48	0	0	0	0	naturalized
11082	36.98	-16.66	1019	312	1158	173	native
11082	36.98	-16.66	0	0	0	0	naturalized
11083	38.02	-13.43	1242	372	1404	210	native
11083	38.02	-13.43	0	0	0	0	naturalized
11085	33.92	-13.57	807	259	920	146	native
11085	33.92	-13.57	0	0	0	0	naturalized
11086	33.93	-11.17	1203	397	1385	215	native
11086	33.93	-11.17	0	0	0	0	naturalized
11087	35.63	-15.93	504	161	582	83	native
11087	35.63	-15.93	0	0	0	0	naturalized
11088	NA	NA	313	102	365	50	native
11088	NA	NA	0	0	0	0	naturalized
11089	16.52	47.54	1155	721	1553	323	native
11089	16.52	47.54	72	56	99	29	naturalized
11090	16.38	48.22	1019	621	1362	278	native
11090	16.38	48.22	79	63	109	33	naturalized
11091	15.74	48.27	1580	999	2116	463	native
11091	15.74	48.27	95	70	130	35	naturalized
11092	13.95	48.13	1271	794	1706	359	native

11092	13.95	48.13	89	58	120	27	naturalized
11093	15.00	47.26	1484	941	1993	432	native
11093	15.00	47.26	83	65	114	34	naturalized
11094	13.91	46.77	1397	889	1871	415	native
11094	13.91	46.77	75	63	108	30	naturalized
11095	13.07	47.39	1202	774	1623	353	native
11095	13.07	47.39	67	51	94	24	naturalized
11096	11.50	47.20	1362	852	1823	391	native
11096	11.50	47.20	67	52	92	27	naturalized
11097	9.89	47.24	1119	692	1496	315	native
11097	9.89	47.24	53	41	74	20	naturalized
11098	11.42	46.70	1344	856	1809	391	native
11098	11.42	46.70	0	0	0	0	naturalized
11099	9.55	47.14	931	561	1237	255	native
11099	9.55	47.14	38	19	48	9	naturalized
11101	127.16	40.13	1134	702	1547	289	native
11101	127.16	40.13	43	36	63	16	naturalized
11102	127.79	36.35	0	0	0	0	native
11102	127.79	36.35	122	101	171	52	naturalized
11103	-102.38	21.98	778	278	935	121	native
11103	-102.38	21.98	100	54	133	21	naturalized
11104	-115.08	30.52	0	0	0	0	native
11104	-115.08	30.52	129	95	182	42	naturalized
11105	-112.03	25.88	0	0	0	0	native
11105	-112.03	25.88	90	54	120	24	naturalized
11106	-90.31	18.83	1979	670	2350	299	native
11106	-90.31	18.83	195	61	232	24	naturalized
11107	-92.48	16.49	6480	2321	7796	1005	native
11107	-92.48	16.49	379	160	470	69	naturalized
11108	-106.45	28.82	3410	1317	4121	606	native
11108	-106.45	28.82	185	93	243	35	naturalized
11109	-102.10	27.29	2566	1043	3107	502	native
11109	-102.10	27.29	188	103	248	43	naturalized
11110	-103.92	19.14	1608	532	1911	229	native
11110	-103.92	19.14	144	64	182	26	naturalized
11111	-99.09	19.24	1577	678	1945	310	native
11111	-99.09	19.24	169	129	236	62	naturalized
11112	-104.99	24.90	3208	1166	3847	527	native
11112	-104.99	24.90	129	71	173	27	naturalized

11113	-101.04	20.90	3952	1473	4743	682	native
11113	-101.04	20.90	314	188	412	90	naturalized
11114	-99.88	17.67	2105	785	2515	375	native
11114	-99.88	17.67	138	88	188	38	naturalized
11115	-98.86	20.48	4656	1494	5450	700	native
11115	-98.86	20.48	184	86	236	34	naturalized
11116	-103.63	20.56	2983	1170	3591	562	native
11116	-103.63	20.56	205	126	273	58	naturalized
11117	-99.63	19.34	4857	1615	5746	726	native
11117	-99.63	19.34	273	141	353	61	naturalized
11118	-101.88	19.20	4195	1468	4987	676	native
11118	-101.88	19.20	289	139	370	58	naturalized
11119	-99.01	18.73	2011	714	2403	322	native
11119	-99.01	18.73	264	114	331	47	naturalized
11120	-104.86	21.78	2705	815	3164	356	native
11120	-104.86	21.78	174	72	219	27	naturalized
11121	-100.00	25.58	2822	1098	3382	538	native
11121	-100.00	25.58	194	104	256	42	naturalized
11122	-96.44	16.96	7372	2494	8724	1142	native
11122	-96.44	16.96	303	134	381	56	naturalized
11123	-97.82	19.04	4510	1595	5373	732	native
11123	-97.82	19.04	260	128	329	59	naturalized
11124	-99.88	20.84	2961	1138	3539	560	native
11124	-99.88	20.84	203	105	261	47	naturalized
11125	-88.12	19.67	1781	592	2100	273	native
11125	-88.12	19.67	154	51	185	20	naturalized
11126	-100.45	22.61	4819	1659	5654	824	native
11126	-100.45	22.61	241	123	311	53	naturalized
11127	-107.49	24.99	2753	824	3187	390	native
11127	-107.49	24.99	191	83	242	32	naturalized
11128	-110.76	29.68	0	0	0	0	native
11128	-110.76	29.68	179	111	242	48	naturalized
11129	-92.62	17.93	2068	760	2504	324	native
11129	-92.62	17.93	183	63	222	24	naturalized
11130	-98.67	24.30	3313	1211	3946	578	native
11130	-98.67	24.30	203	106	266	43	naturalized
11131	-98.08	19.45	722	307	895	134	native
11131	-98.08	19.45	95	66	132	29	naturalized
11132	-96.38	19.35	6301	2358	7622	1037	native

11132	-96.38	19.35	411	194	518	87	naturalized
11133	-88.93	20.78	1806	614	2137	283	native
11133	-88.93	20.78	189	64	230	23	naturalized
11134	-102.71	23.27	2002	703	2370	335	native
11134	-102.71	23.27	133	66	175	24	naturalized
11135	-56.31	46.93	209	176	307	78	native
11135	-56.31	46.93	79	72	115	36	naturalized
11136	177.15	-7.25	22	9	27	4	native
11136	177.15	-7.25	0	0	0	0	naturalized
11156	-71.58	-29.03	21	17	28	10	native
11156	-71.58	-29.03	0	0	0	0	naturalized
11158	-71.54	-29.27	15	12	19	8	native
11158	-71.54	-29.27	0	0	0	0	naturalized
11270	15.67	43.69	140	56	174	22	native
11270	15.67	43.69	0	0	0	0	naturalized
11273	15.76	43.72	100	43	126	17	native
11273	15.76	43.72	0	0	0	0	naturalized
11274	15.76	43.69	105	43	131	17	native
11274	15.76	43.69	0	0	0	0	naturalized
11282	15.54	43.86	81	30	100	11	native
11282	15.54	43.86	0	0	0	0	naturalized
11314	15.66	43.65	286	132	360	58	native
11314	15.66	43.65	0	0	0	0	naturalized
11353	-179.16	-16.09	63	24	77	10	native
11353	-179.16	-16.09	0	0	0	0	naturalized
11390	102.16	4.04	0	0	0	0	native
11390	102.16	4.04	78	45	102	21	naturalized
11394	126.53	-17.33	948	399	1145	202	native
11394	126.53	-17.33	85	36	106	15	naturalized
11395	114.86	-28.33	1868	832	2149	551	native
11395	114.86	-28.33	255	215	362	108	naturalized
11396	126.26	-15.43	1244	543	1498	289	native
11396	126.26	-15.43	123	49	150	22	naturalized
11397	127.54	-18.36	672	283	816	139	native
11397	127.54	-18.36	67	26	83	10	naturalized
11398	118.52	-21.91	1043	540	1324	259	native
11398	118.52	-21.91	93	50	121	22	naturalized
11399	128.49	-15.85	815	377	1007	185	native
11399	128.49	-15.85	174	75	217	32	naturalized

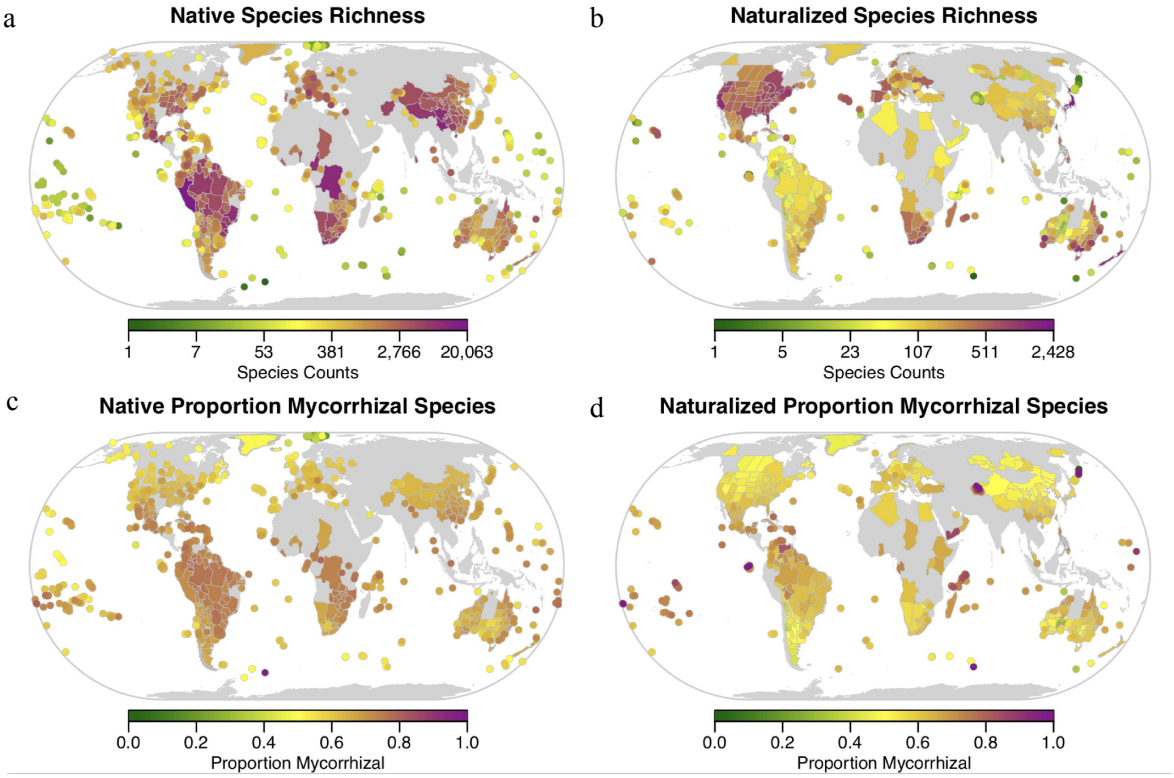
11400	117.33	-31.13	2724	1101	3095	730	native
11400	117.33	-31.13	385	291	534	142	naturalized
11401	121.13	-33.68	2160	811	2422	549	native
11401	121.13	-33.68	248	213	352	109	naturalized
11402	116.53	-33.34	2167	943	2457	653	native
11402	116.53	-33.34	668	400	886	182	naturalized
11403	120.29	-32.97	2151	792	2425	518	native
11403	120.29	-32.97	184	159	263	80	naturalized
11404	115.67	-31.18	1561	808	1823	546	native
11404	115.67	-31.18	787	473	1044	216	naturalized
11405	116.25	-34.55	954	452	1123	283	native
11405	116.25	-34.55	413	270	557	126	naturalized
11406	121.41	-31.29	1650	648	1934	364	native
11406	121.41	-31.29	148	127	211	64	naturalized
11407	119.51	-27.74	1258	672	1583	347	native
11407	119.51	-27.74	87	81	127	41	naturalized
11408	116.50	-28.36	1082	535	1325	292	native
11408	116.50	-28.36	59	80	96	43	naturalized
11409	125.55	-28.05	613	293	757	149	native
11409	125.55	-28.05	4	10	9	5	naturalized
11410	128.01	-25.24	472	240	596	116	native
11410	128.01	-25.24	10	9	14	5	naturalized
11411	114.58	-24.28	907	507	1164	250	native
11411	114.58	-24.28	106	85	149	42	naturalized
11412	125.96	-24.42	417	222	532	107	native
11412	125.96	-24.42	6	3	7	2	naturalized
11413	123.33	-18.16	885	428	1095	218	native
11413	123.33	-18.16	151	66	188	29	naturalized
11414	118.74	-24.71	835	496	1078	253	native
11414	118.74	-24.71	36	29	50	15	naturalized
11415	122.30	-24.00	624	329	793	160	native
11415	122.30	-24.00	12	10	18	4	naturalized
11416	128.28	-19.88	251	116	307	60	native
11416	128.28	-19.88	10	7	15	2	naturalized
11417	124.78	-20.99	549	266	683	132	native
11417	124.78	-20.99	14	8	20	2	naturalized
11418	127.44	-31.94	161	98	211	48	native
11418	127.44	-31.94	42	31	54	19	naturalized
11419	126.52	-30.60	285	163	361	87	native

11419	126.52	-30.60	50	36	66	20	naturalized
11420	152.44	-30.57	2006	726	2382	350	native
11420	152.44	-30.57	470	249	608	111	naturalized
11421	150.80	-33.68	1584	654	1911	327	native
11421	150.80	-33.68	532	336	717	151	naturalized
11422	149.96	-36.20	1281	499	1535	245	native
11422	149.96	-36.20	241	159	326	74	naturalized
11423	151.58	-30.46	1361	505	1628	238	native
11423	151.58	-30.46	246	160	335	71	naturalized
11424	149.79	-33.68	1449	535	1707	277	native
11424	149.79	-33.68	329	210	443	96	naturalized
11425	149.06	-35.73	1419	566	1715	270	native
11425	149.06	-35.73	307	209	425	91	naturalized
11426	150.31	-30.62	1216	496	1479	233	native
11426	150.31	-30.62	296	182	398	80	naturalized
11427	148.61	-33.03	1359	519	1638	240	native
11427	148.61	-33.03	308	201	419	90	naturalized
11428	147.31	-35.26	672	338	857	153	native
11428	147.31	-35.26	237	156	324	69	naturalized
11429	147.42	-30.32	1072	582	1384	270	native
11429	147.42	-30.32	197	132	268	61	naturalized
11430	145.36	-33.84	824	489	1094	219	native
11430	145.36	-33.84	209	157	296	70	naturalized
11431	142.98	-30.44	661	462	910	213	native
11431	142.98	-30.44	91	82	132	41	naturalized
11432	142.59	-33.16	545	354	741	158	native
11432	142.59	-33.16	74	70	111	33	naturalized
11438	44.94	40.29	0	0	0	0	native
11438	44.94	40.29	256	78	306	28	naturalized
11441	4.66	50.64	0	0	0	0	native
11441	4.66	50.64	237	169	330	76	naturalized
11452	29.78	26.57	0	0	0	0	native
11452	29.78	26.57	40	29	57	12	naturalized
11453	-8.16	53.18	0	0	0	0	native
11453	-8.16	53.18	176	125	245	56	naturalized
11456	25.55	58.67	0	0	0	0	native
11456	25.55	58.67	62	41	85	18	naturalized
11460	43.52	42.18	0	0	0	0	native
11460	43.52	42.18	64	45	89	20	naturalized

11465	22.98	39.04	0	0	0	0	native
11465	22.98	39.04	67	49	93	23	naturalized
11467	117.28	-2.23	0	0	0	0	native
11467	117.28	-2.23	258	130	329	59	naturalized
11469	24.93	56.85	0	0	0	0	native
11469	24.93	56.85	147	83	196	34	naturalized
11470	23.90	55.33	0	0	0	0	native
11470	23.90	55.33	126	82	168	40	naturalized
11471	6.09	49.77	0	0	0	0	native
11471	6.09	49.77	39	32	56	15	naturalized
11474	14.40	35.92	0	0	0	0	native
11474	14.40	35.92	39	32	56	15	naturalized
11476	28.47	47.20	0	0	0	0	native
11476	28.47	47.20	69	70	104	35	naturalized
11482	145.25	-6.48	0	0	0	0	native
11482	145.25	-6.48	52	31	69	14	naturalized
11494	35.18	39.06	0	0	0	0	native
11494	35.18	39.06	87	44	112	19	naturalized
11495	31.41	49.00	0	0	0	0	native
11495	31.41	49.00	329	243	456	116	naturalized
11506	19.48	48.71	0	0	0	0	native
11506	19.48	48.71	61	62	93	30	naturalized
11507	14.82	46.12	0	0	0	0	native
11507	14.82	46.12	111	62	148	25	naturalized

SI Figure 1. Locations of regions used in study with associated proportion M:NM.

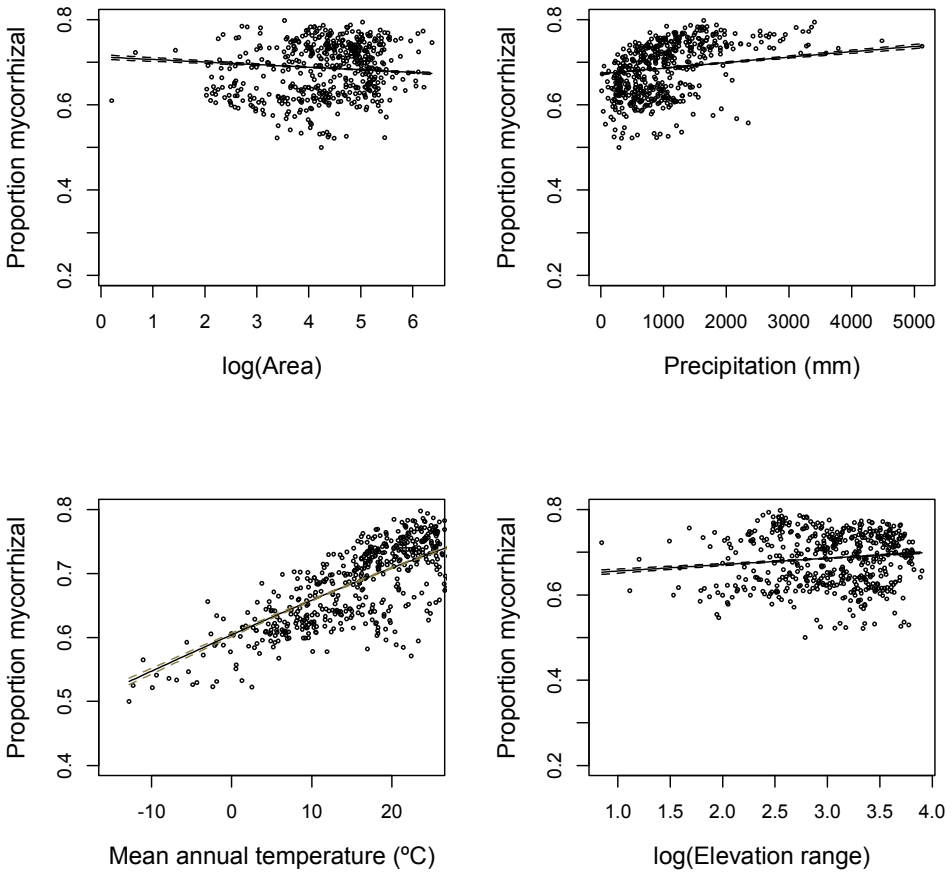
Maps of geographical regions showing the proportion of native (a) and naturalized (b) plant species richness and proportions of native (c) and naturalized (d) mycorrhizal plant species.



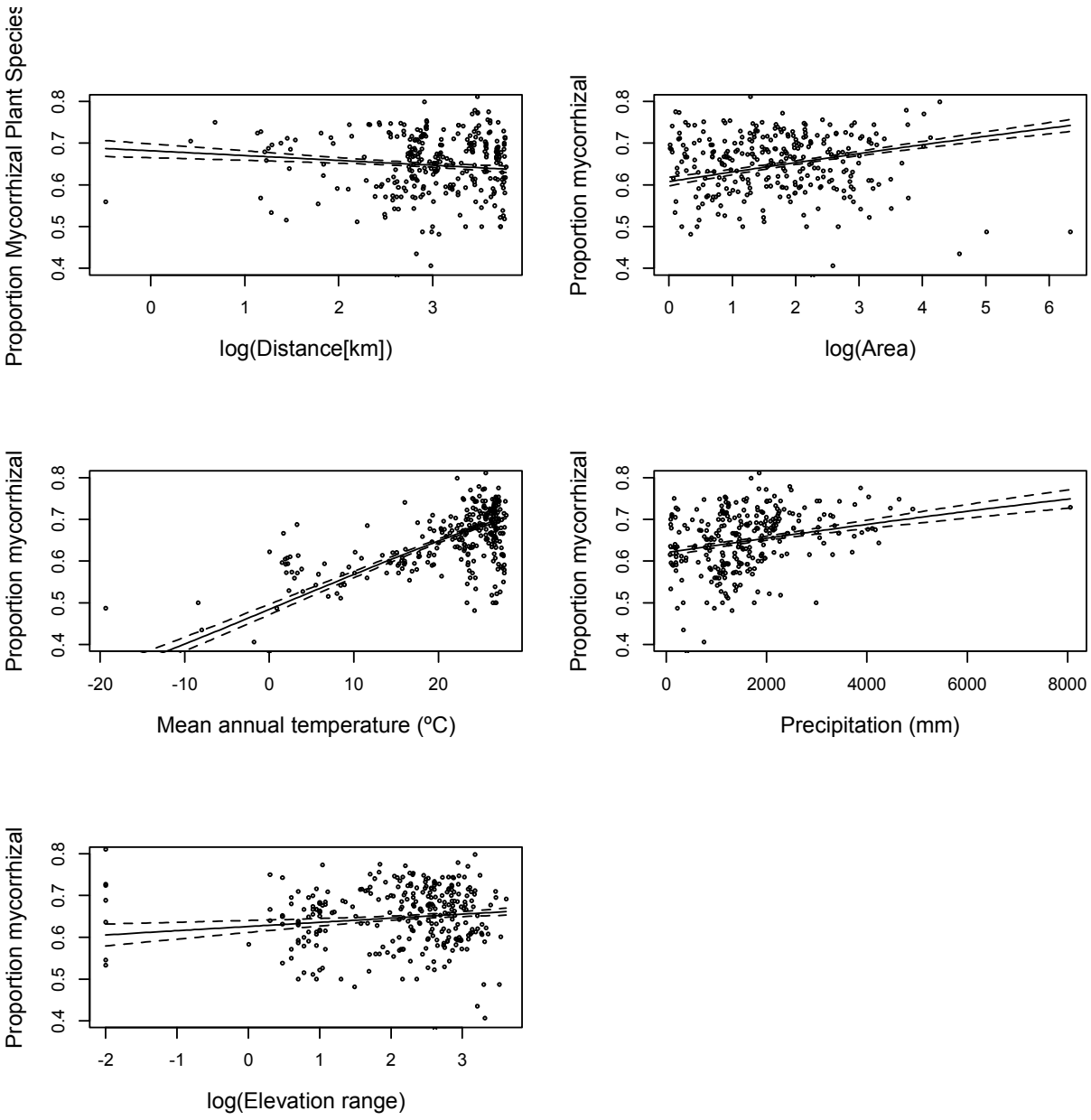
SI Figure 2. Native Models figures (M3, M4, M7).

Predicted probability graphs of each variable included in the respective model.

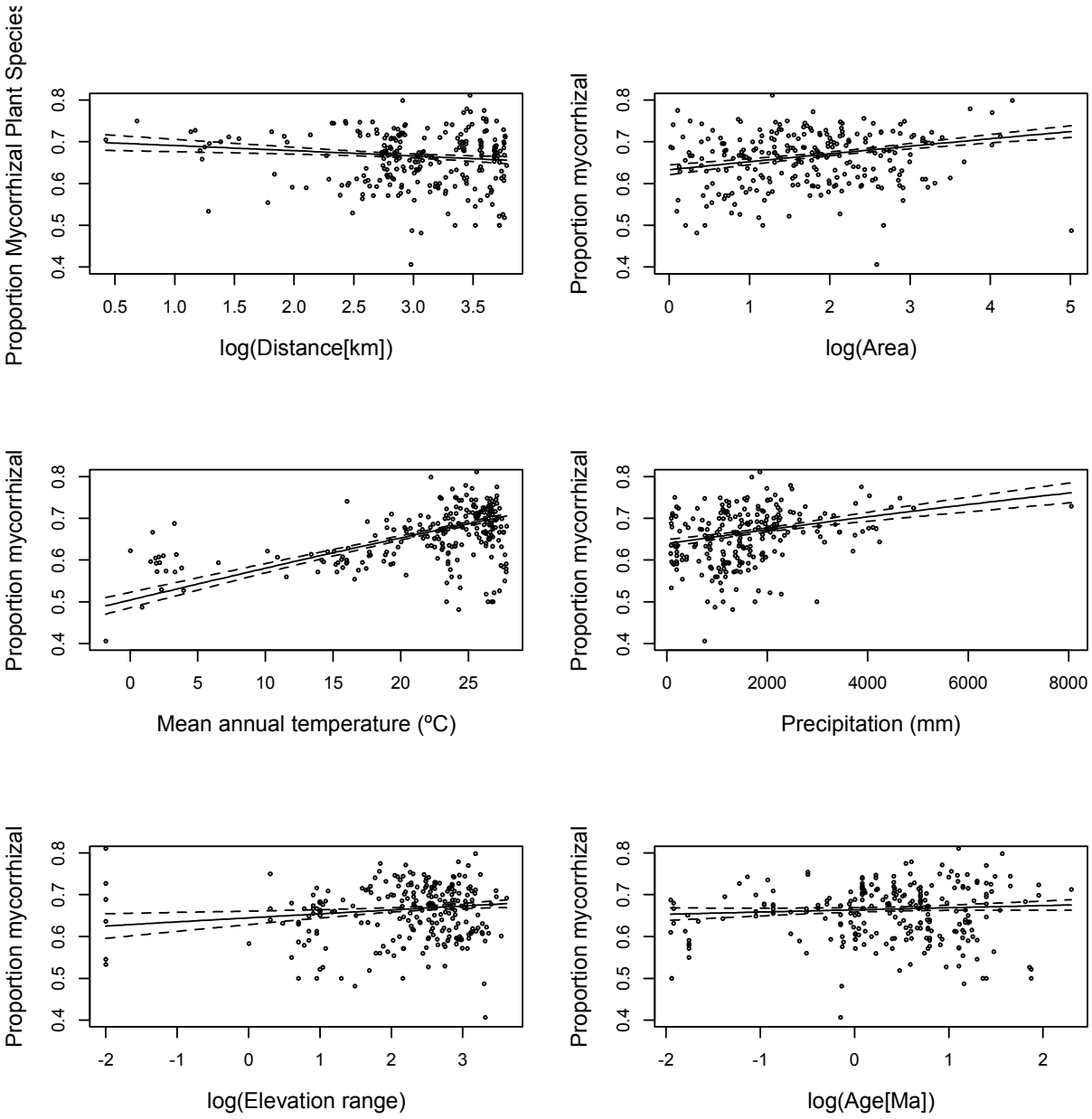
(a)



(b)


Proportion Mycorrhizal Plant Species
0.4 0.5 0.6 0.7 0.8
0 1 2 3
log(Distance[km])
Proportion mycorrhizal
0.4 0.5 0.6 0.7 0.8
0 1 2 3 4 5 6
log(Area)
Proportion mycorrhizal
0.4 0.5 0.6 0.7 0.8
-20 -10 0 10 20
Mean annual temperature (ºC)
Proportion mycorrhizal
0.4 0.5 0.6 0.7 0.8
0 2000 4000 6000 8000
Precipitation (mm)
Proportion mycorrhizal
0.4 0.5 0.6 0.7 0.8
-2 -1 0 1 2 3
log(Elevation range)

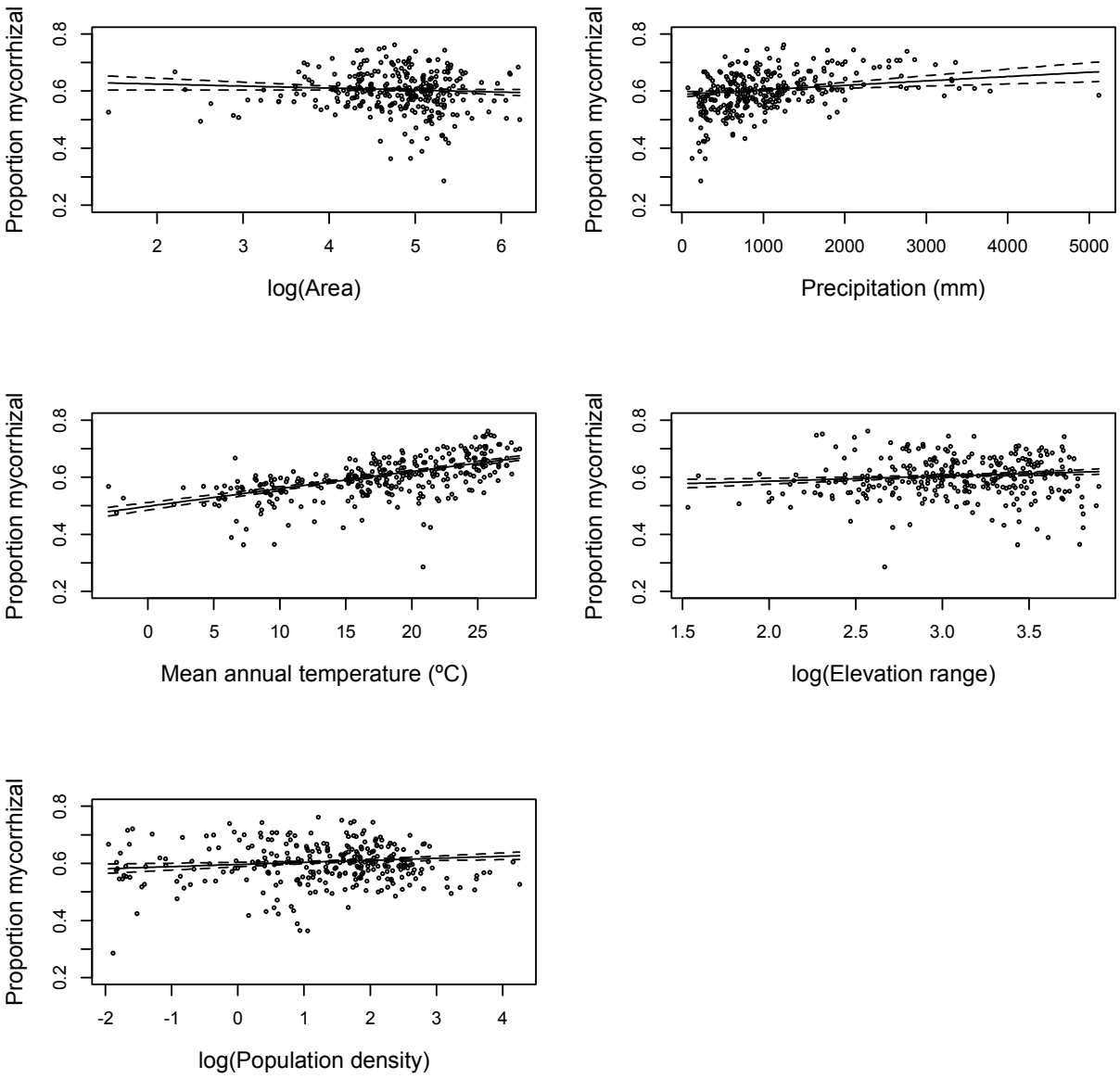
(c)



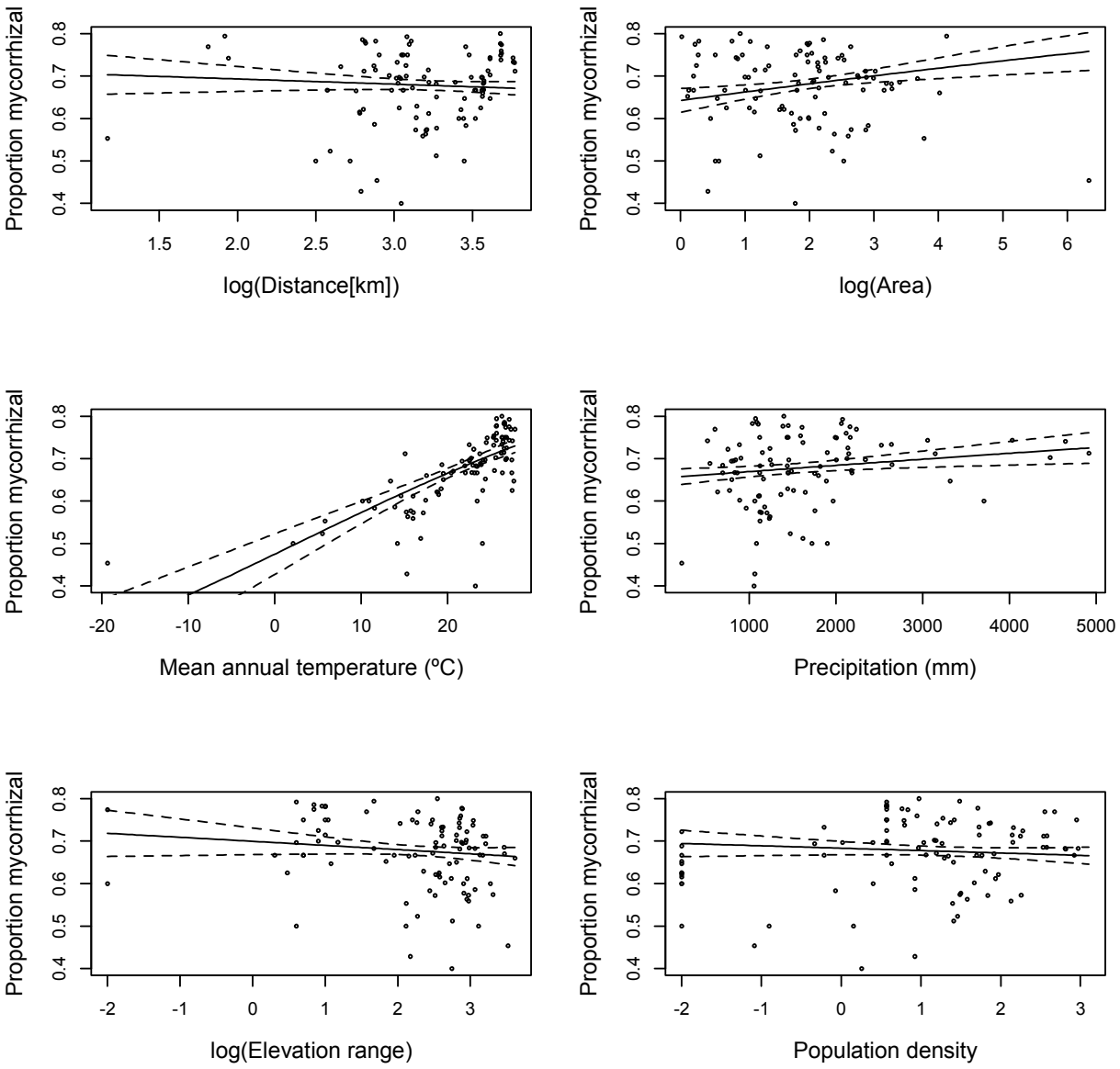
SI Figure 3. Naturalized Models Figures (M5, M6, M8).

Predicted probability graphs of each variable included in the respective model.

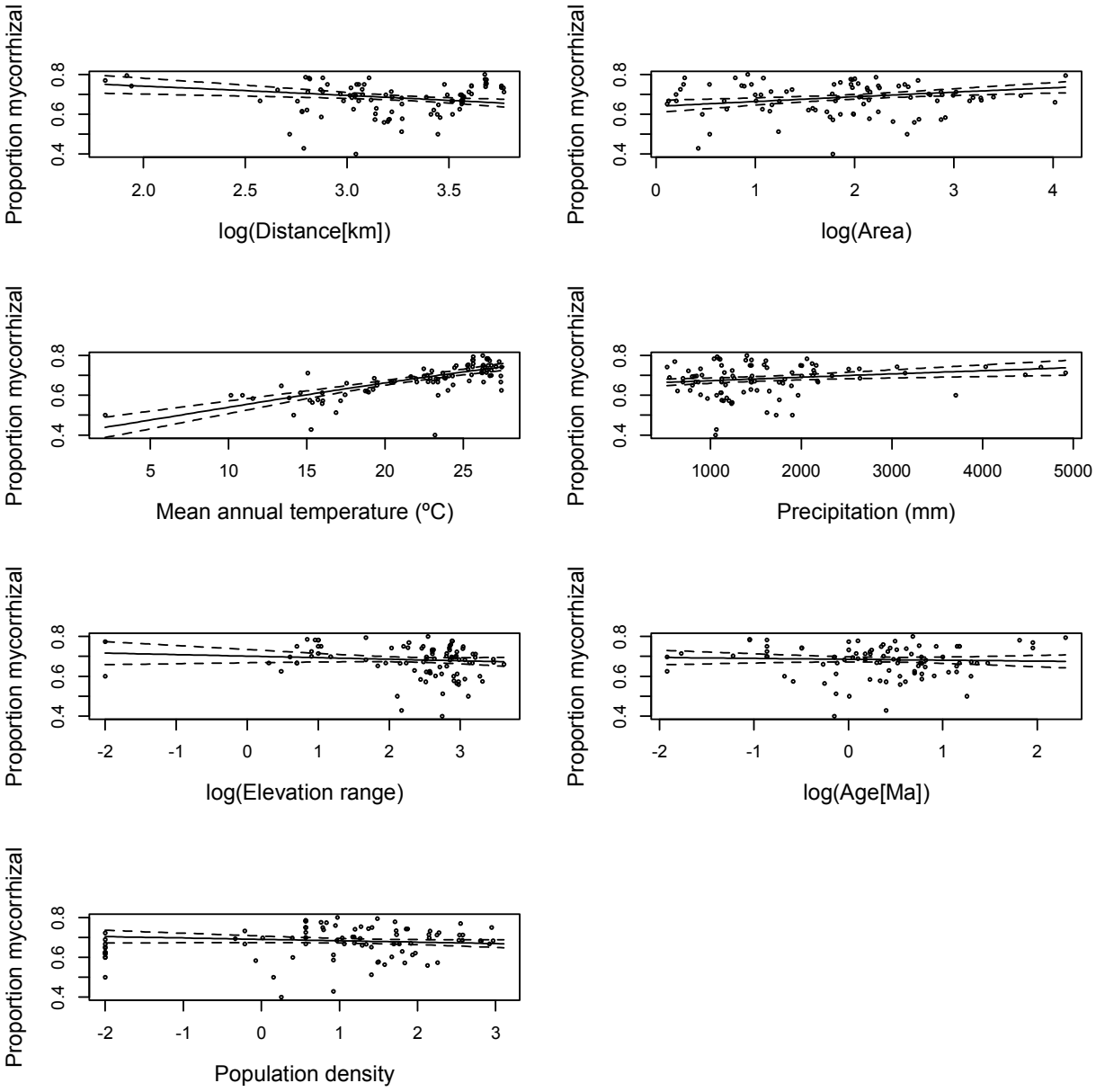
(a)


Proportion mycorrhizal
0.2
0.4
0.6
0.8
2
3
4
5
6
log(Area)
0
1000
2000
3000
4000
5000
Precipitation (mm)
0
5
10
15
20
25
Mean annual temperature (ºC)
1.5
2.0
2.5
3.0
3.5
log(Elevation range)
-2
-1
0
1
2
3
4
log(Population density)

(b)


Proportion mycorrhizal
0.4
0.5
0.6
0.7
0.8
1.5
2.0
2.5
3.0
3.5
log(Distance[km])
0
1
2
3
4
5
6
log(Area)
-20
-10
0
10
20
Mean annual temperature (ºC)
1000
2000
3000
4000
5000
Precipitation (mm)
-2
-1
0
1
2
3
log(Elevation range)
Population density

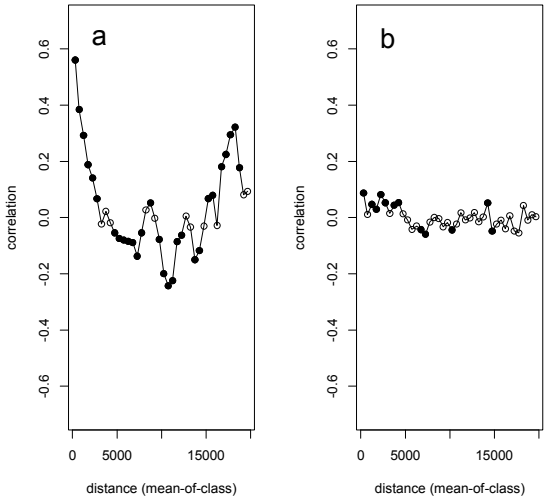
(c)


Proportion mycorrhizal
0.4
0.6
0.8
2.0
2.5
3.0
3.5
log(Distance[km])
0
1
2
3
4
log(Area)
5
10
15
20
25
Mean annual temperature (ºC)
1000
2000
3000
4000
5000
Precipitation (mm)
-2
-1
0
1
2
3
log(Elevation range)
log(Age[Ma])
Population density

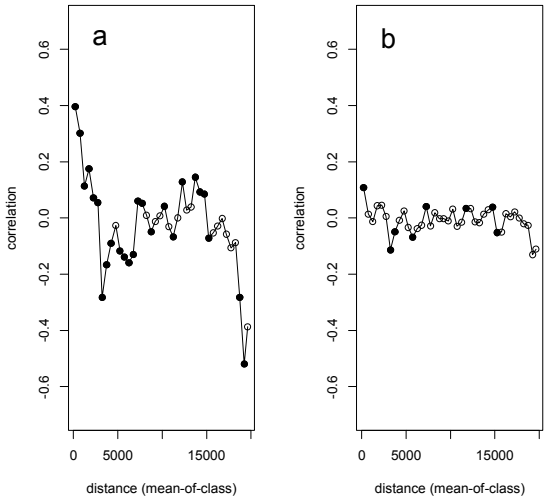
SI Figure 4. Moran's I correlograms testing for spatial autocorrelation.

Moran's I correlograms without (a) and including (b) spatial autocovariate.

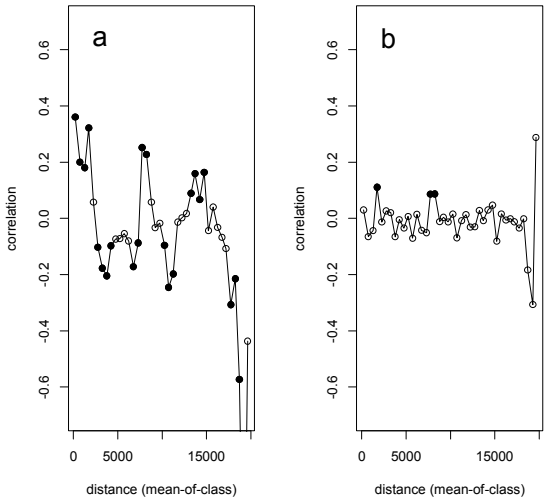
Native Mainland (M3)



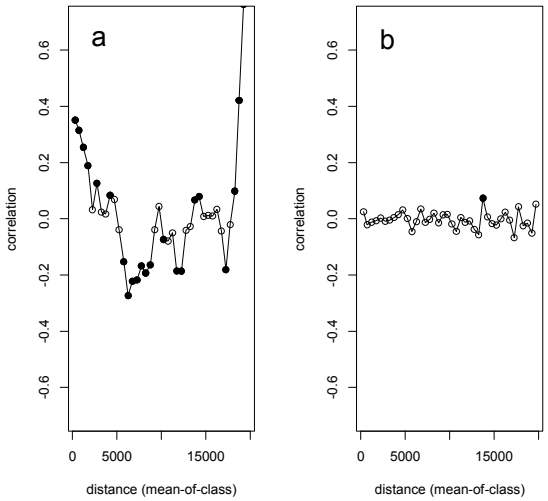
Native Island (M4)



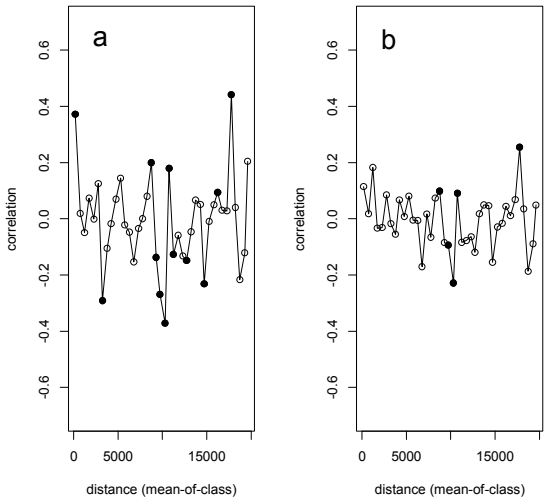
Native Island Age (M5)



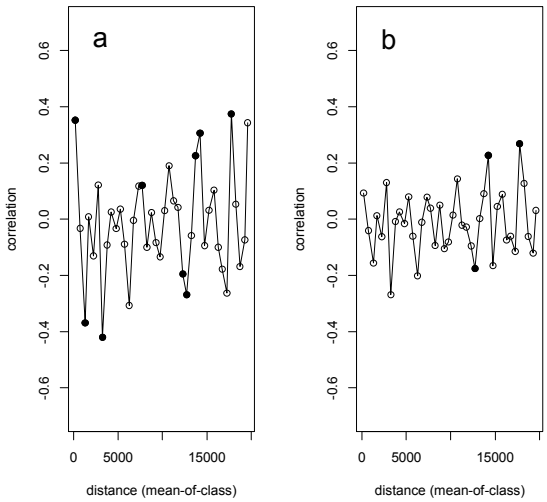
Naturalized Mainland (M6)



Naturalized Island (M7)



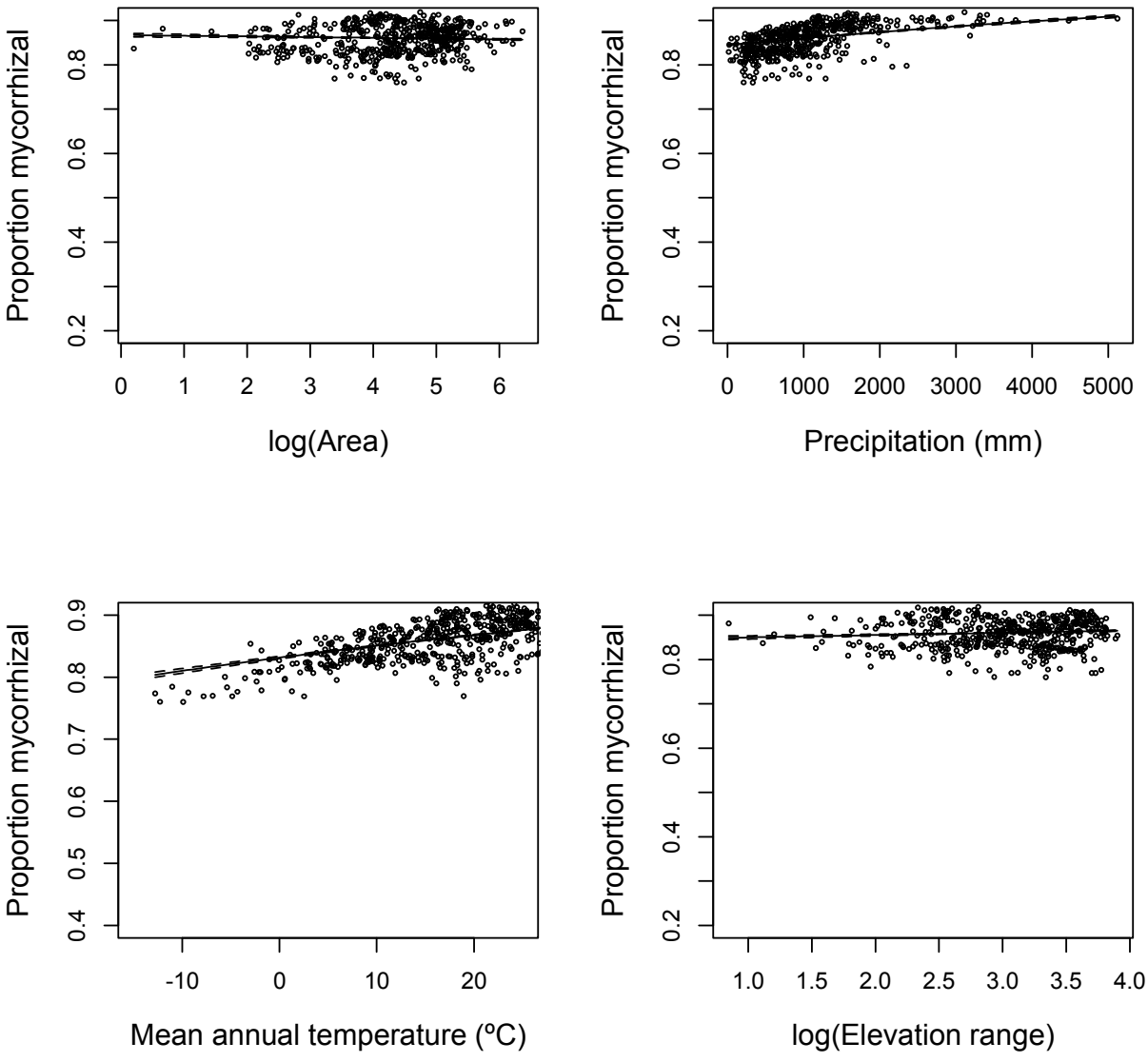
Naturalized Island Age (M8)



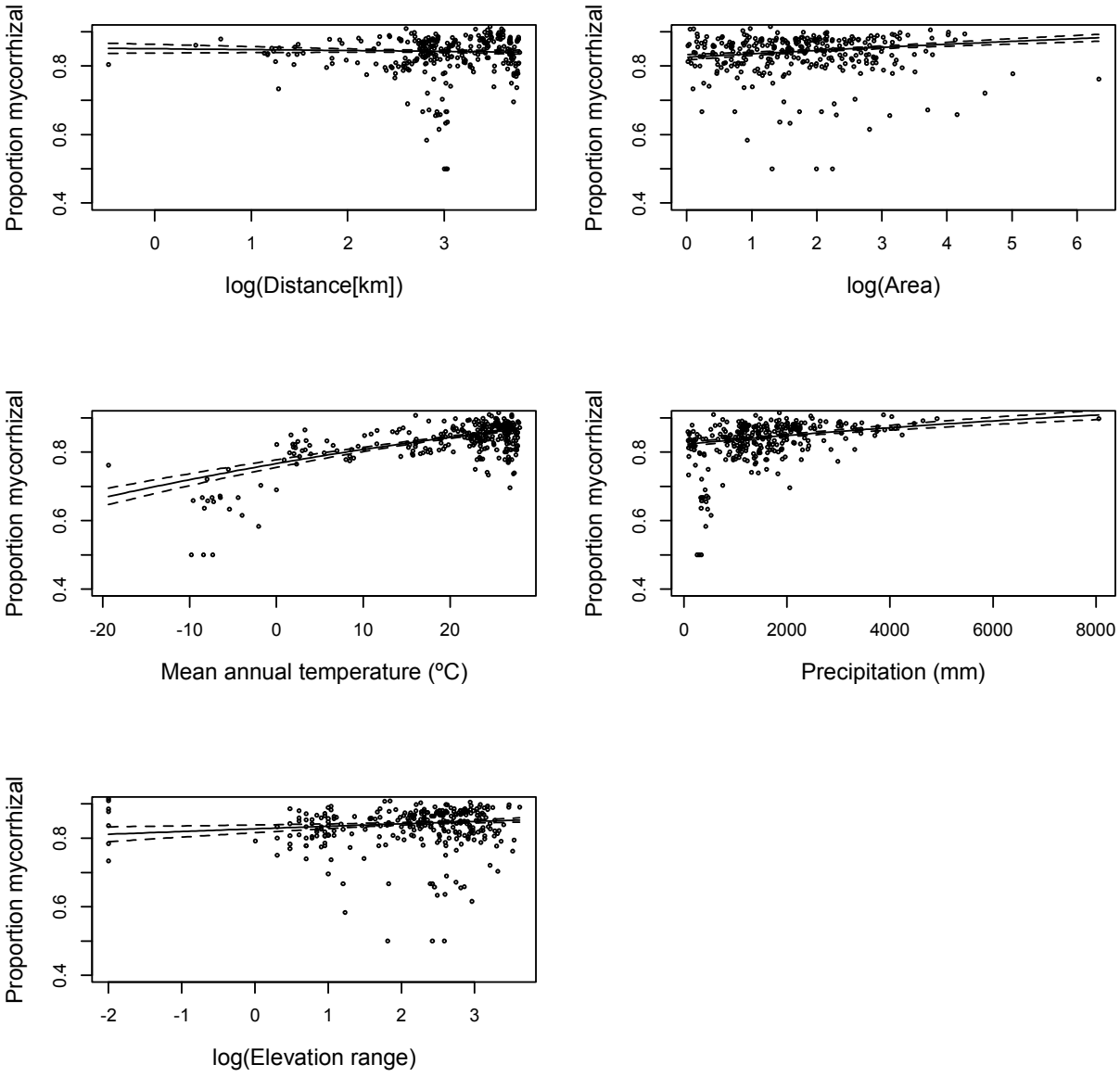
SI Figure 5. Native Models figures with AMNM plants were assigned to NM (M3.2, M4.2, M7.2).

Predicted probability graphs of each variable included in the respective model.

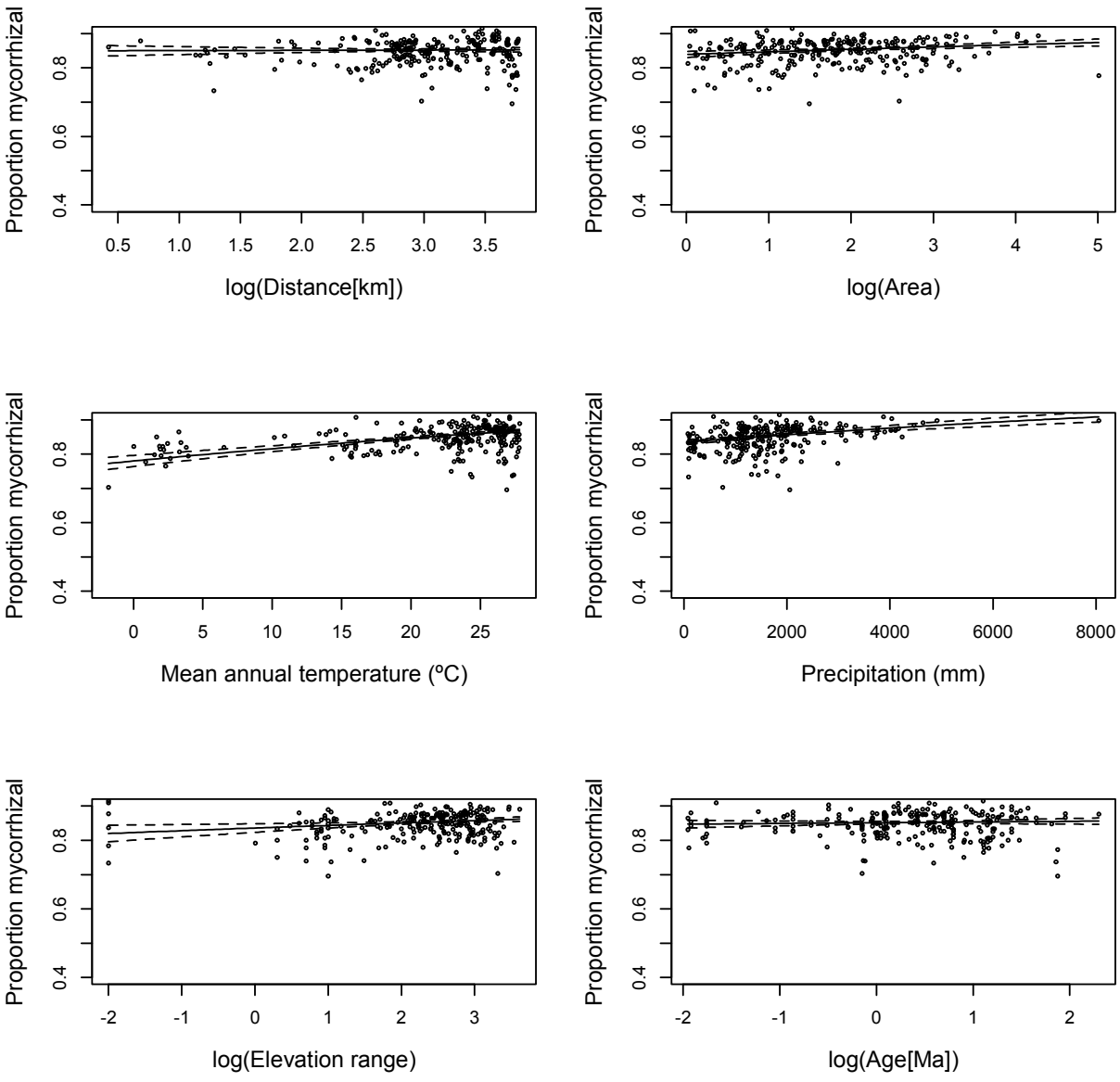
(a)



(b)


Proportion mycorrhizal
0.4
0.6
0.8
0
1
2
3
log(Distance[km])
Proportion mycorrhizal
0.4
0.6
0.8
0
1
2
3
4
5
6
log(Area)
Proportion mycorrhizal
0.4
0.6
0.8
-20
-10
0
10
20
Mean annual temperature (ºC)
Proportion mycorrhizal
0.4
0.6
0.8
0
2000
4000
6000
8000
Precipitation (mm)
Proportion mycorrhizal
0.4
0.6
0.8
-2
-1
0
1
2
3
log(Elevation range)

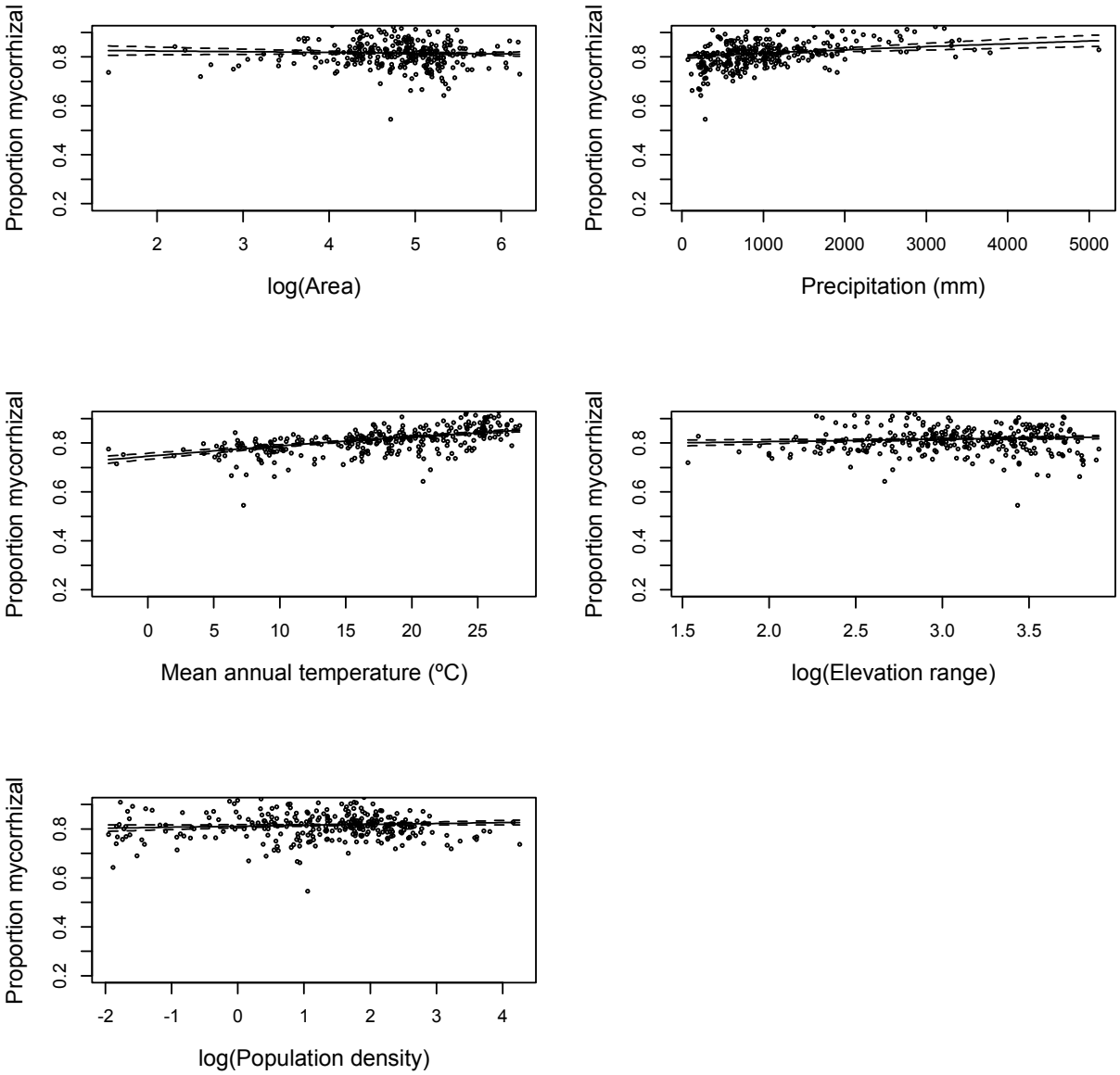
(c)


Proportion mycorrhizal
0.4
0.6
0.8
0.5 1.0 1.5 2.0 2.5 3.0 3.5
log(Distance[km])
0 1 2 3 4 5
log(Area)
0 5 10 15 20 25
Mean annual temperature (ºC)
0 2000 4000 6000 8000
Precipitation (mm)
-2 -1 0 1 2 3
log(Elevation range)
-2 -1 0 1 2
log(Age[Ma])

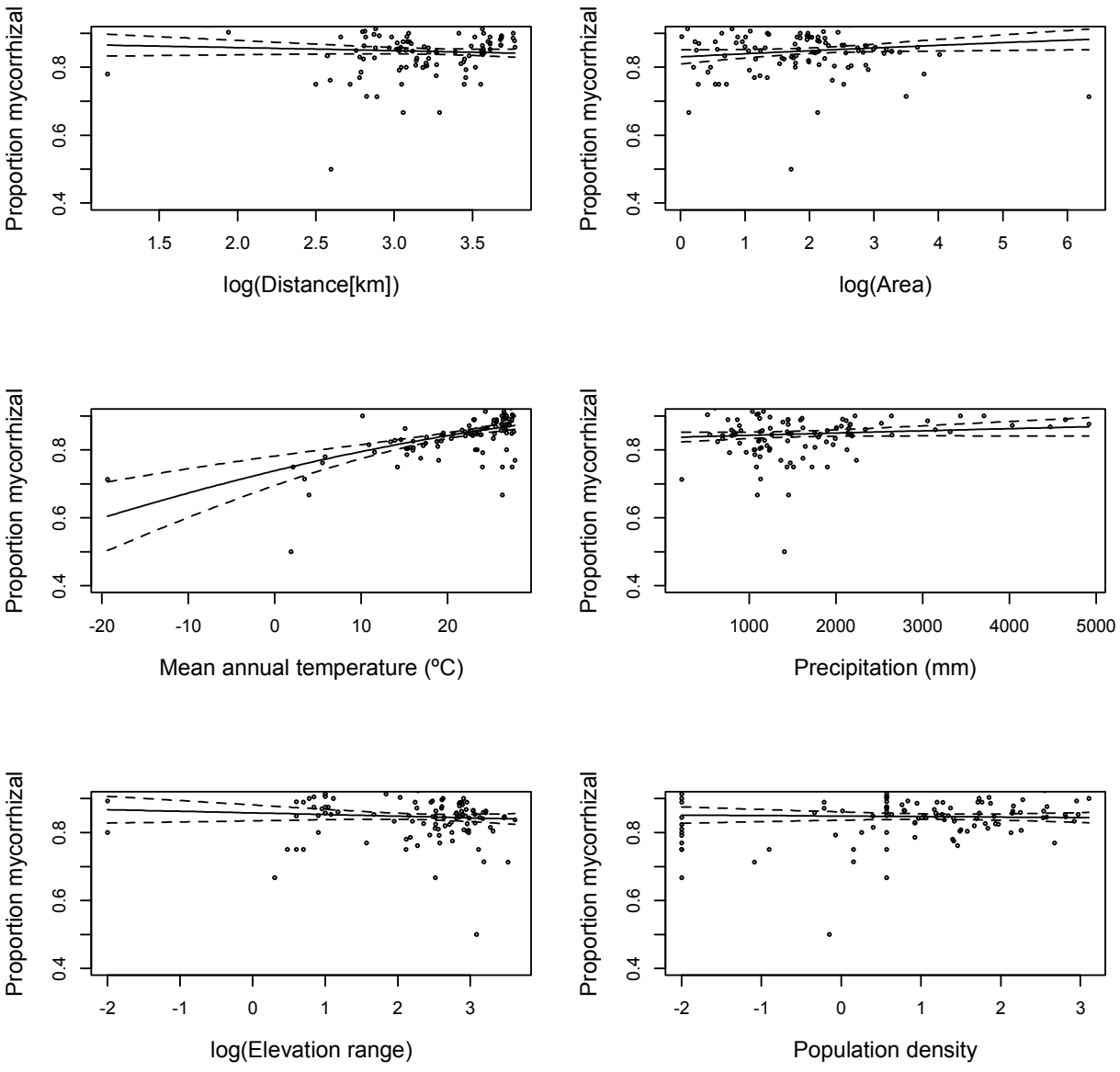
SI Figure 6. Naturalized Models Figures with AMNM plants assigned to NM (M5.2, M6.2, M8.2).

Predicted probability graphs of each variable included in the respective model.

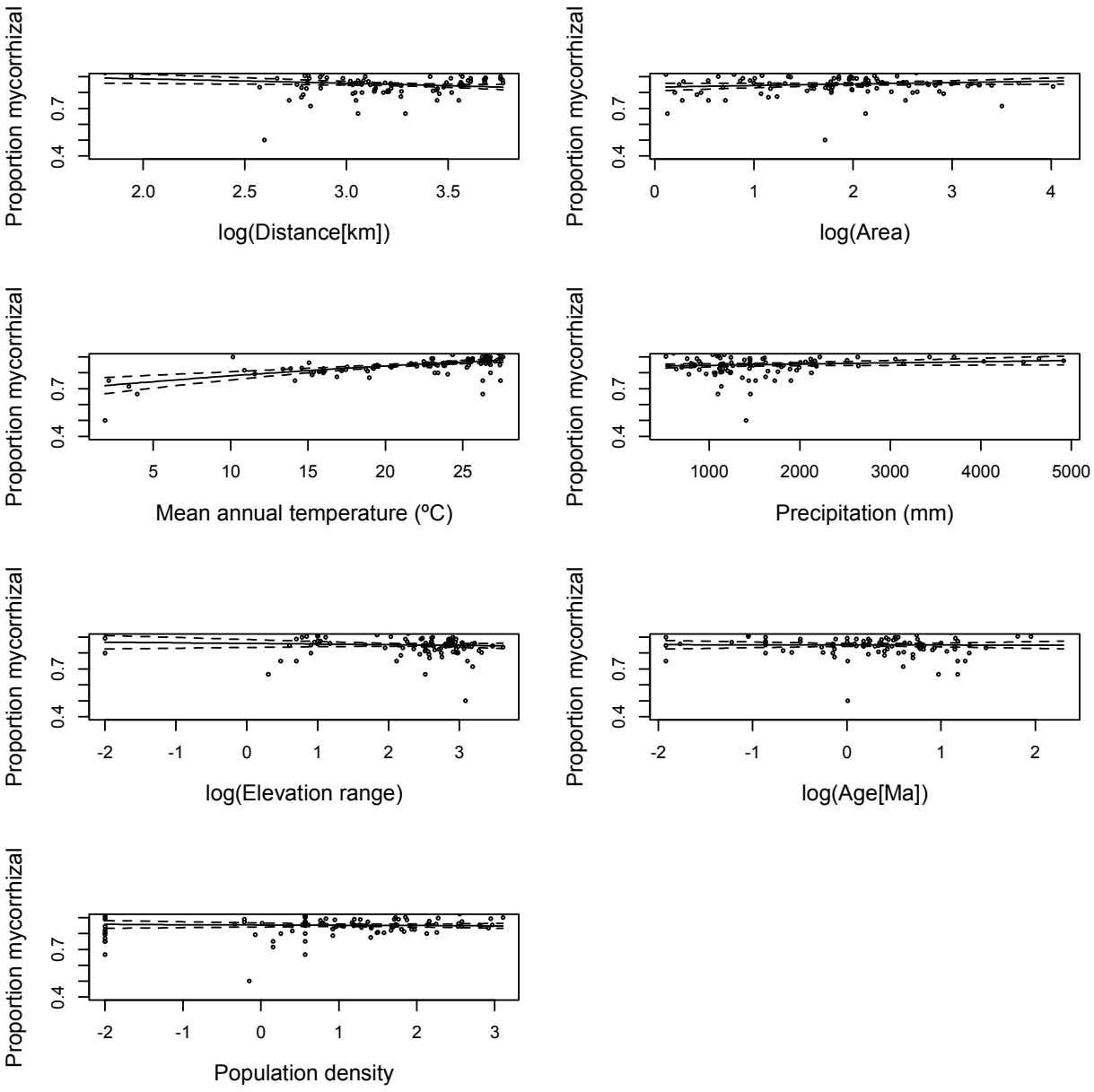
(a)



(b)


Proportion mycorrhizal
0.4
0.6
0.8
1.5
2.0
2.5
3.0
3.5
log(Distance[km])
Proportion mycorrhizal
0.4
0.6
0.8
0
1
2
3
4
5
6
log(Area)
Proportion mycorrhizal
0.4
0.6
0.8
-20
-10
0
10
20
Mean annual temperature (ºC)
Proportion mycorrhizal
0.4
0.6
0.8
1000
2000
3000
4000
5000
Precipitation (mm)
Proportion mycorrhizal
0.4
0.6
0.8
-2
-1
0
1
2
3
log(Elevation range)
Proportion mycorrhizal
0.4
0.6
0.8
-2
-1
0
1
2
3
Population density

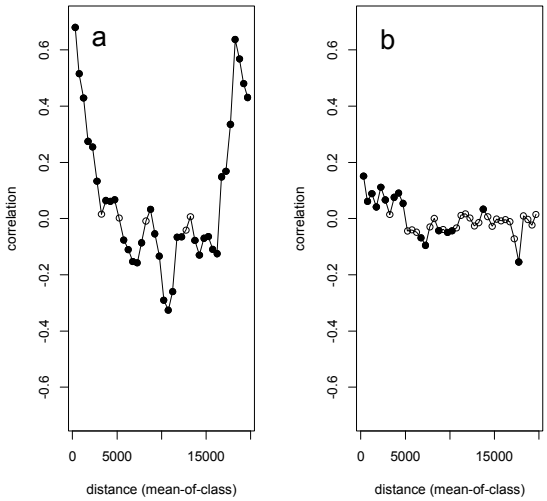
(c)


Proportion mycorrhizal
0.4
0.7
2.0
2.5
3.0
3.5
log(Distance[km])
Proportion mycorrhizal
0.4
0.7
0
1
2
3
4
log(Area)
Proportion mycorrhizal
0.4
0.7
5
10
15
20
25
Mean annual temperature (ºC)
Proportion mycorrhizal
0.4
0.7
1000
2000
3000
4000
5000
Precipitation (mm)
Proportion mycorrhizal
0.4
0.7
-2
-1
0
1
2
3
log(Elevation range)
Proportion mycorrhizal
0.4
0.7
-2
-1
0
1
2
log(Age[Ma])
Proportion mycorrhizal
0.4
0.7
-2
-1
0
1
2
3
Population density

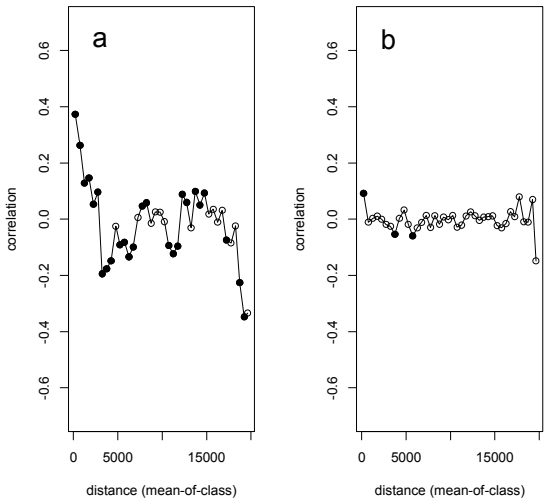
SI Figure 7. Moran's I correlograms testing for spatial autocorrelation with AMNM plants assigned to NM.

Moran's I correlograms of model residuals without (a) and including (b) spatial autocovariate.

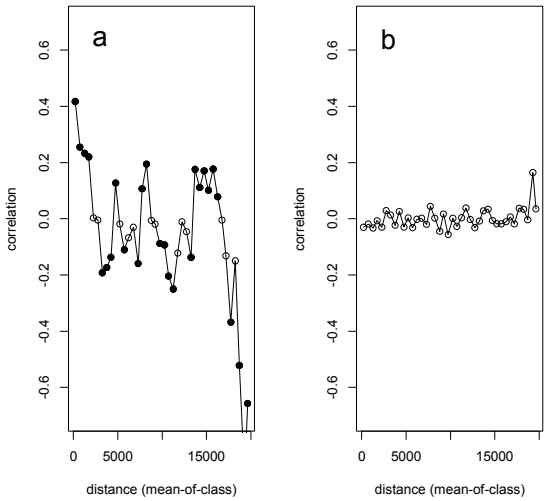
Native Mainland (M3.2)



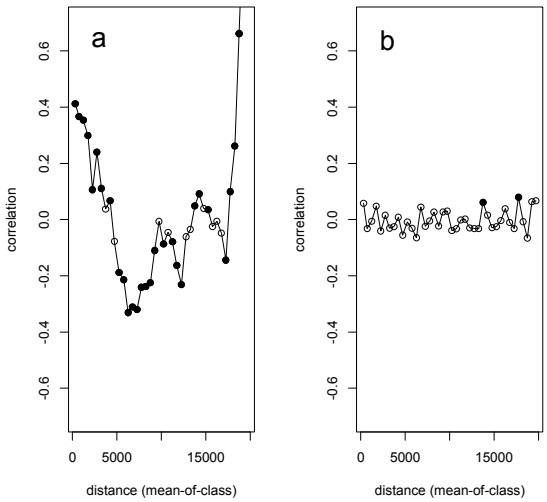
Native Island (M4.2)



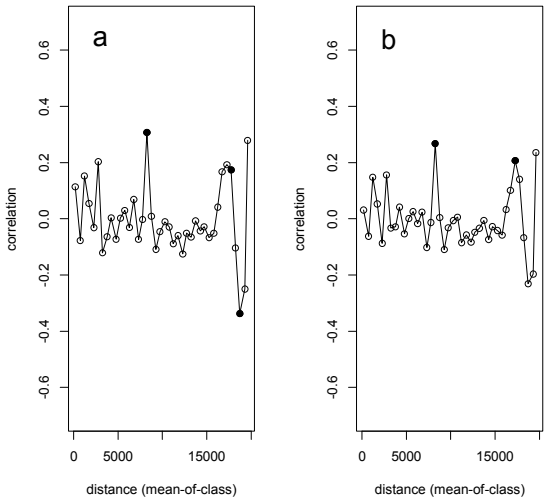
Native Island Age (M5.2)



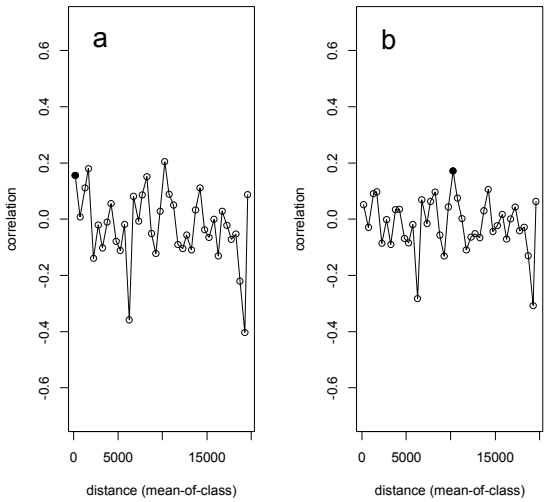
Naturalized Mainland (M6.2)



Naturalized Island (M7.2)



Naturalized Island Age (M8.2)



Appendix D: Supplementary Information for Mycorrhizal types influence island biogeography of plants

Materials and Methods

Global analysis

Plant distribution data, mycorrhizal status and explanatory variables

Plant species occurrence data (for mostly administratively defined regions such as countries and provinces or islands), native status (native versus naturalized) and explanatory variables with regional characteristics were extracted from the Global Naturalized Alien Flora, GloNAF (Pysek et al. 2017, van Kleunen et al. 2019), and from the Global Inventory of Floras and Traits v 1.0, GIFT (Weigelt et al. 2019), databases. From the GloNAF database, we included only well-documented regions for which it was estimated that at least 50% of the naturalized species occurring in the given region were recorded. From the GIFT database, we used all regions for which checklists of native angiosperms were available. When there were overlapping regions, the smaller regions were kept if larger than 100 km^2 for mainland regions; for islands, the smaller units were always preferred. Finally, we removed islands for which island geology (i.e. volcanic, floor, shelf, fragment, etc.) was undetermined.

The mycorrhizal status of plant species included in this study was determined by assigning each species to its plant family according to The Plant List (Kalwij 2012), incorporating the classification from APG IV (Byng et al. 2016). Following methods from

Delavaux et al. (2019), we relied on published family proportions of plant species' mycorrhizal status to assign mycorrhizal status proportions to the regional plant assemblages. We used three review papers to determine a consensus proportion of mycorrhizal status per plant family (Gerdemann 1968, Brundrett 2009, Maherali et al. 2016). Different classifications and proportions between the reference papers were accounted for by using the average proportion for each mycorrhizal type across the three references. While concerns have been raised over incorrect classification in these reviews(Brundrett and Tedersoo 2018a, Bueno et al. 2018), they cannot be addressed at this time due to the lack of species-specific corrections. Errors are mostly associated with EM plant species, while most of our database is composed of AM plant species. We classified plant mycorrhizal status into four major types: arbuscular mycorrhizal (AM), ectomycorrhizal (EM, which includes ecto- and ericoid-mycorrhizae), orchid mycorrhizal (ORC; occurs in Orchidaceae) and non-mycorrhizal (NM). We do not have *a priori* reason to expect differential colonization ability of ectomycorrhizal or ericoid mycorrhizal fungi because they share a common clade (Vrålstad et al. 2000). Therefore, we combine these mycorrhizal groups into one functional group in our analyses (EM). We incorporated ambiguous classifications of mycorrhizal status (AMEM and AMNM), representing species found with both specified statuses, by running separate analyses, assigning species to either potential type. The full table of families and corresponding consensus proportions of mycorrhizal status can be found in Supplementary Information (Data S1).

Explanatory variables for each region were extracted from the GIFT database. For details of environmental data collection, see Weigelt et al. (Weigelt et al. 2019). Explanatory variables included land type (mainland or oceanic island), absolute latitude and longitude of the region's centroid, area (km²), mean annual temperature (°C) and mean annual precipitation (mm;(Karger

et al. 2017)), elevational range (difference between lowest and highest elevation in m; (Danielson and Gesch 2011)), human population density (n/km²;(Center for International Earth Science Information Network - CIESIN - Columbia University 2005)) and human land use. Human land use was calculated by combining two land-use metrics, cultivated and managed vegetation and urban land use area, as a sum followed by natural log transformation (km^2 (Tuanmu and Jetz 2014)). When elevation range was unknown or reported as zero from aerial elevation maps, we assigned an elevation of 1m as a minimum necessary elevation. For islands, we also included island distance to the nearest mainland (km) as a measure of geographical isolation (Weigelt and Kreft 2013) and island geological age. Data for endemism analyses represent a subset from the dataset for which endemism data is known.

We considered non-oceanic islands as oceanic islands if they were covered with ice (at least 80%) during the last glacial maximum(Tuanmu and Jetz 2014), because they resemble newly formed oceanic islands after the plant and fungal communities were exterminated by glaciation. Before running models, we removed regions where there was a zero in total calculated species counts within any mycorrhizal type in a particular region. We removed these regions because these zero values may result from limited knowledge of mycorrhizal status of locally abundant plant families. We did not remove regions with a zero total for ORC as the orchid mycorrhizal association occurs only in Orchidaceae, which are likely to be correctly enumerated; therefore, we can reasonably assume that false zeros were unlikely for this mutualism.

Statistical analysis

To investigate patterns of mycorrhizal plant distributions, we first used a multinomial logistic regression analogous to those described below to test how land type predicted mycorrhizal type of plant species; we found that proportion AM relative to NM plant species was reduced, while proportion EM relative to NM increased on oceanic islands compared to mainlands ($p < 0.001$, Table S1). While this analysis has the advantage of incorporating all mycorrhizal types simultaneously, it cannot account for non-independence of nearby islands (i.e., cannot include random effects). In order to account appropriately for the non-independence of geographically proximal islands, we used mixed models that correct for non-independence due to spatial proximity for a series of bivariate response variables (relative species number of pairs of mycorrhizal groupings) corresponding to the orthogonal comparisons: ectomycorrhizal to arbuscular mycorrhizal plant species (EM:AM) and orchid mycorrhizal to all other types of mycorrhizal plant species (M), including arbuscular, ectomycorrhizal and ambiguous (AMEM) mycorrhizal plant species (ORC:M). Next, to understand how these mycorrhizal types compare to non-mycorrhizal plant species (NM), we used additional comparisons of each mycorrhizal type compared to NM (EM:NM, AM:NM and ORC:NM).

In our first set of models, we compared the species-richness patterns of plants with differing mycorrhizal associations. We ran models comparing i) EM to AM plant species richness ii) ORC to M plant species richness and iii) each of the three mycorrhizal types (AM, EM, ORC) compared to NM species richness. For each comparison, separate models were run for native and naturalized plants to predict plant species richness. In these generalized linear mixed effects models (GLMMs), we used a Poisson distribution because the response variable, species richness, was count data. The fixed effects were mycorrhizal status, land type (mainland non-oceanic island and oceanic islands) and their interaction; we also included the covariates of

absolute latitude, the natural logarithm of area, the natural logarithm of elevation, and the natural logarithm of plant species richness. The random effects were region, nested within land type, and the interaction of region nested within land type with mycorrhizal status. These random terms control for the non-independence of individual plant species records within floras, thereby providing general tests for differences in the proportion of mycorrhizal species across the floras of the different land types. The sample size (n) in these models represents a unique regional combination of native status (native or naturalized) and mycorrhizal status (reported in corresponding model tables). To create Fig. 1, we converted these count estimates to proportions.

Next, to investigate geographical and environmental drivers of mycorrhizal status for native and naturalized plants in mainland and oceanic island floras, we ran models comparing the proportion of: i) EM to AM plant species, ii) ORC to M plant species, and iii) each of the three mycorrhizal types (AM, EM, ORC) compared to NM plant species. We used a composite response variable with species richness of each of the two mycorrhizal groupings of interest to account for differences in species richness. For these analyses, we used generalized linear models (GLMs) with a logit link function, assuming a binomial distribution of the response variable. For the native mainland models, we included the natural logarithm of area, the natural logarithm of elevation range, mean annual precipitation, mean annual temperature, absolute latitude and squared latitude. For the native island models, we included the same six variables with the addition of island distance to the mainland. We initially explored models with island age, however, as 1) this effect was not significant, 2) inclusion of this predictor substantially reduced the number of regions in the model and 3) it did not meaningfully change distance effects, we include results of models without island age in the manuscript. The choice of variables was informed by prior studies of their effects on this dataset (Kreft et al. 2008,

Delavaux et al. 2019) as well as other island biogeographic studies (Kueffer et al. 2010, Triantis et al. 2015). As the presence of naturalized species is likely to be driven by human activities, the naturalized mainland models and the naturalized island models included human population density in addition to the explanatory variables included in the corresponding models for native species. Results of these models are presented in Table S2 through Table S6. Sample size (n) in all models excluding M1 and M2 are true n representing unique regions. Next, we ran models testing for the proportion of endemic species in native oceanic island floras, using a composite response variable with counts of endemic species and non-endemic species. For these analyses, we used generalized linear models (GLMs) with a logit link function, assuming a binomial distribution of the response variable. As covariates, we included the natural logarithm of area, the natural logarithm of elevation range, the natural logarithm of age, absolute latitude and squared latitude. All predictor variables in models were mean scaled prior to analysis.

To explore linear and non-linear latitudinal relationships, we reran all models comparing the five proportions described above including only absolute latitude and absolute latitude squared as the independent predictor variables, mean-scaled prior to analysis. We also ran models to investigate anthropogenic drivers of mycorrhizal status in naturalized plants only. For these models, we included a combined variable of urban land-use area and cultivated and managed vegetation, 'human land use' (sum of both variables). To assess the robustness of our results given the uncertainty in mycorrhizal status assignment, we reran all models testing for all combinations of ambiguous mycorrhizal status. In the main manuscript, we report statistics from models specified in Tables designated *a* (e.g. Table S2a) in Tables S2-S6.

Generally, overdispersion in GLMs was adequately corrected using a quasi-binomial or quasi-Poisson family model. However, for latitude models, a negative binomial GLM was

necessary to correct for overdispersion. In addition, most of our model residuals showed spatial autocorrelation as tested using Moran's I, which is expected in global scale models with spatially clustered geographic regions. We corrected for this spatial autocorrelation by including a spatial autocovariate that incorporates a matrix of longitude and latitude coordinates of the regions (Crase et al. 2012)in the *spdep* package in R (Bivand and Piras 2015). After checking for spatial autocorrelation in our corrected models, some models still showed some spatial autocorrelation (as determined through Moran's I), but all spatial autocorrelation was reduced substantially. Because the naturalized ORC models (ORC:M and ORC:NM) were highly zero-inflated, caution should be taken in interpretation of results. All analyses were done in R 3.4.1 (Team 2019) in the *lme4* package(Bates et al. 2015).

Recent island colonization test

Data for the analyses of the two short-term time series on Rakata Island (Krakatau islands, Indonesia) and Surtsey island (Iceland) were obtained from surveys conducted between 1886 and 1994 and between 1965 and 2008, respectively (Bush and Whittaker 1991, Magnússon et al. 2009). The same mycorrhizal status data described above was used to determine mycorrhizal status of each flora. The only predictor in these models was year since eruption, and this was determined from the associated literature. To analyze patterns in these two recently denuded/emerged and newly colonized islands (108 years and 43 years, respectively), we ran models predicting the species counts of each plant species mycorrhizal type, including AM, EM, ORC and NM. For these analyses, we used generalized linear models (GLMs), assuming a

Poisson distribution of the response variable. For these models, we took the natural logarithm of year since eruption/emergence to normalize distributions.

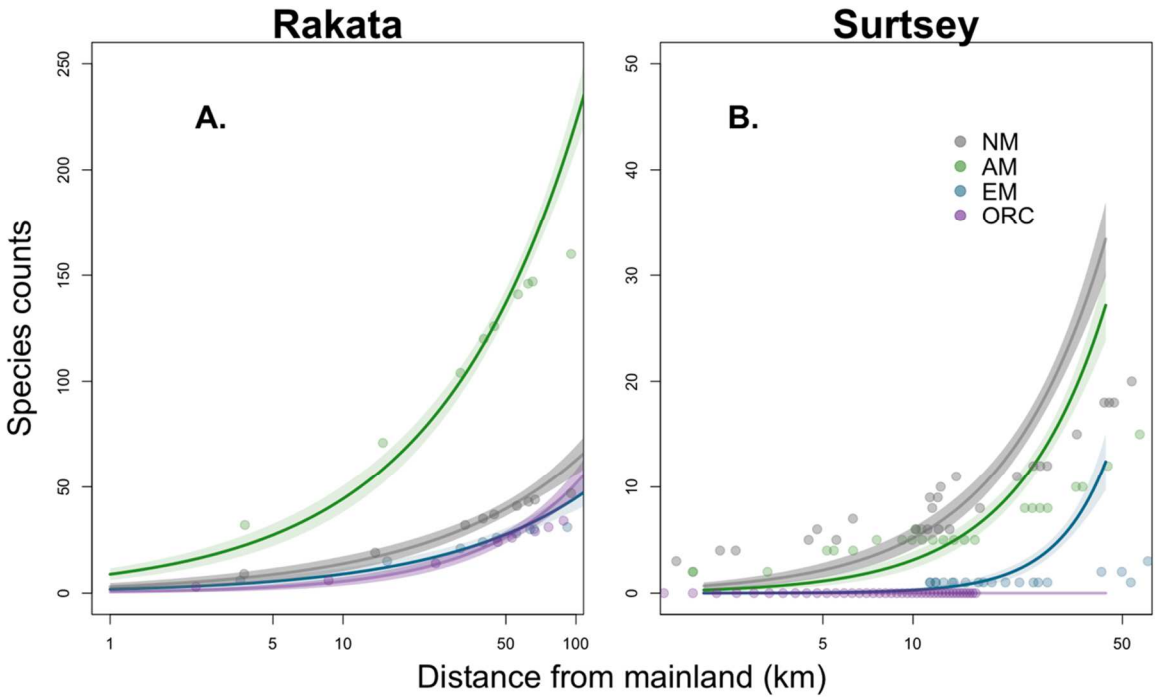


Figure S1. Cumulative species counts of AM, EM, ORC and NM plants in the native island flora in Rakata and Surtsey.

Cumulative species counts of AM, EM, ORC and NM plants in the native island flora increase with year since eruption over a short time scale (respectively 108 and 43 years) for Rakata (A, $p < 0.001$, n = 14; GLM) and for AM, EM and NM plants for Surtsey (B; $p < 0.001$, n = 44; GLM).

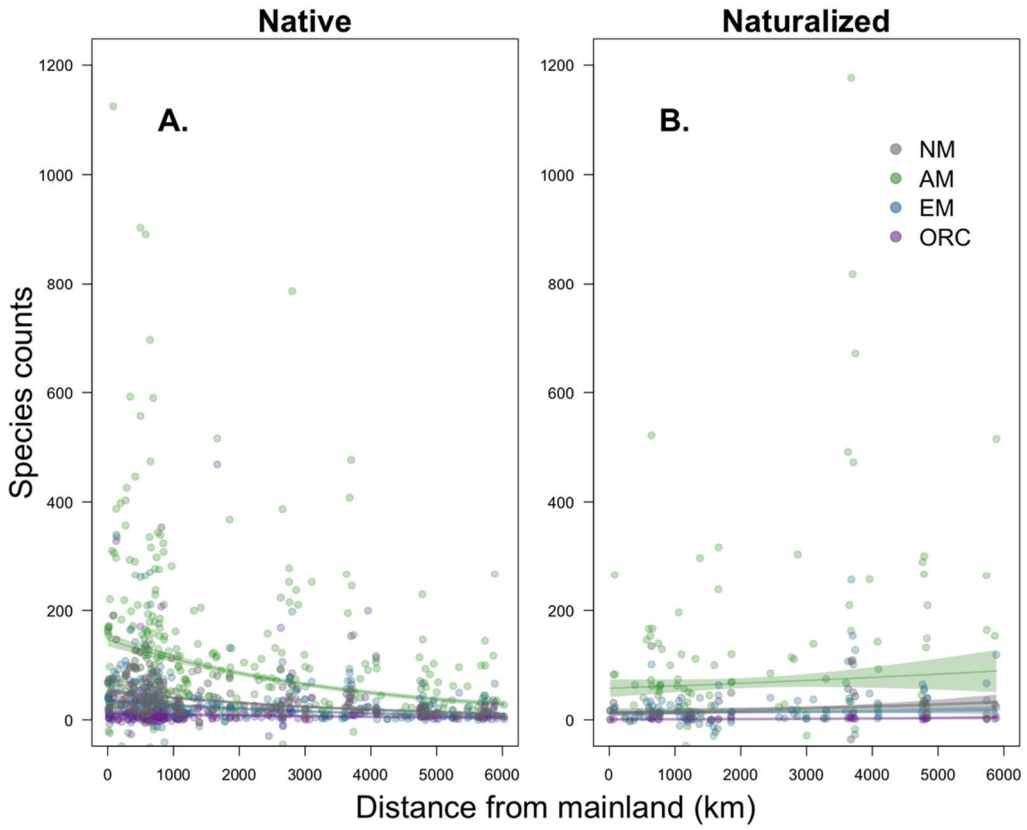


Figure S2. Plant species counts in native and naturalized oceanic island flora with distance. The proportion of AM:NM plants in the native island flora decreases with oceanic island distance from the mainland (A, estimate = -0.034 ± -0.006, $p < 0.01$, n = 325; GLM), consistent with AM plants being differentially limited in colonization of far islands. In contrast, no patterns with distance are detectable in naturalized oceanic island floras (B, estimate = 0.034 ± 0.005, $p = 0.25$, n = 105; GLM).

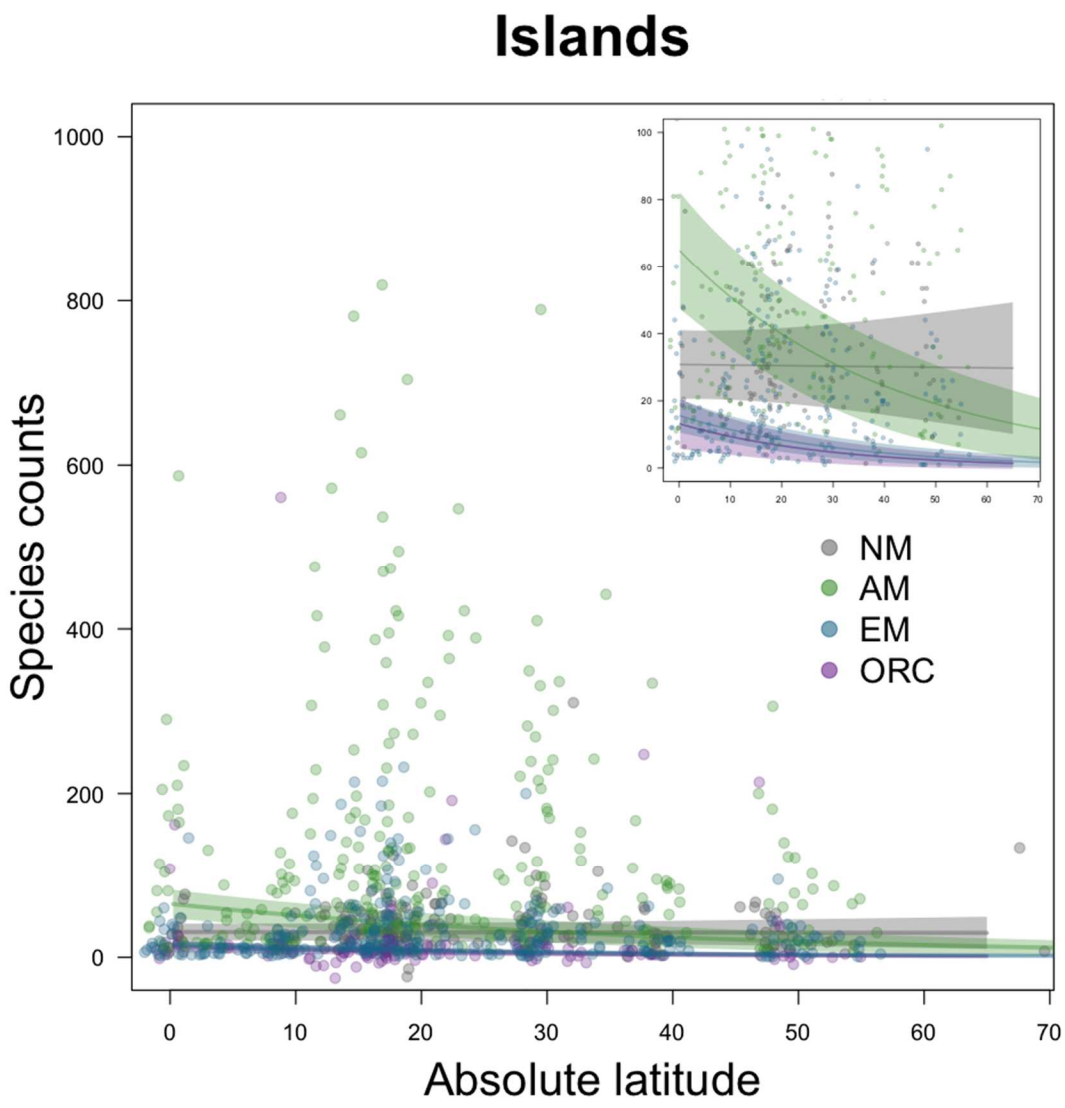


Figure S3. Latitudinal pattern in native oceanic island floras.

The latitudinal plant species gradient is not as strongly influenced by type of mycorrhizal plant species as in mainland plant species. In island regions, the proportion of mycorrhizal plant species decreases with absolute latitude (º from equator) more strongly for arbuscular mycorrhizal (AM; green line: absolute latitude estimate = 0.067 ± 0.012, $p < 0.001$, n = 264; squared latitude estimate -0.001 ± 0.000, $p < 0.001$, n = 264; GLM), than for ectomycorrhizal plant species (EM; blue line: absolute latitude estimate = 0.080 ± 0.014, $p < 0.01$, n = 264;

squared latitude estimate = -0.002 ± 0.000, $p <$ 0.001, n = 264; GLM) and orchid mycorrhizal plant species (ORC; purple line: absolute latitude estimate = 0.025 ± 0.024, $p = 0.28$, n = 158; squared latitude estimate = -0.001 ± 0.000, $p = 0.03$, n = 158). Non-mycorrhizal species counts plotted for reference (NM; grey line: absolute latitude estimate = 0.014 ± 0.014, $p = 0.30$, n = 158; squared latitude estimate = -0.000 ± 0.000, $p = 0.34$, n = 158; GLM). The Insert shows the relationship for a limited span of the y axis (0 to 100 species) for clarity.

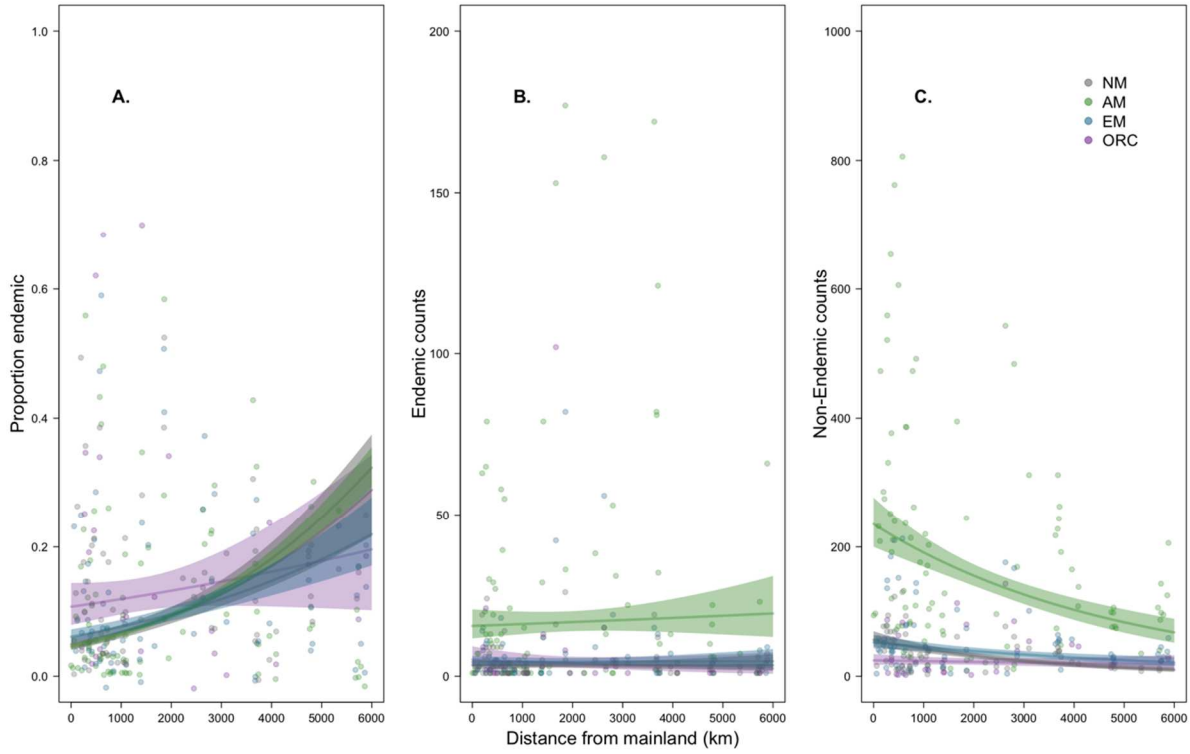


Figure S4. Variation in mycorrhizal types in oceanic island floras with distance from mainland source regions.

The proportion of oceanic island plant species endemic to non-endemic increases most rapidly with distance for AM plant species (A, estimate = 0.432 ± 0.063, $p < 0.001$, n = 254; GLM). The

number of endemic AM species does not change with distance (B, estimate 0.048 ± 0.147, p = 0.74, n = 254; GLM). The non-endemic species for AM decreases most strongly compared to other mycorrhizal types and to NM plants (C, estimate = -0.265 ± 0.067, $p < 0.001$, n = 254; GLM).

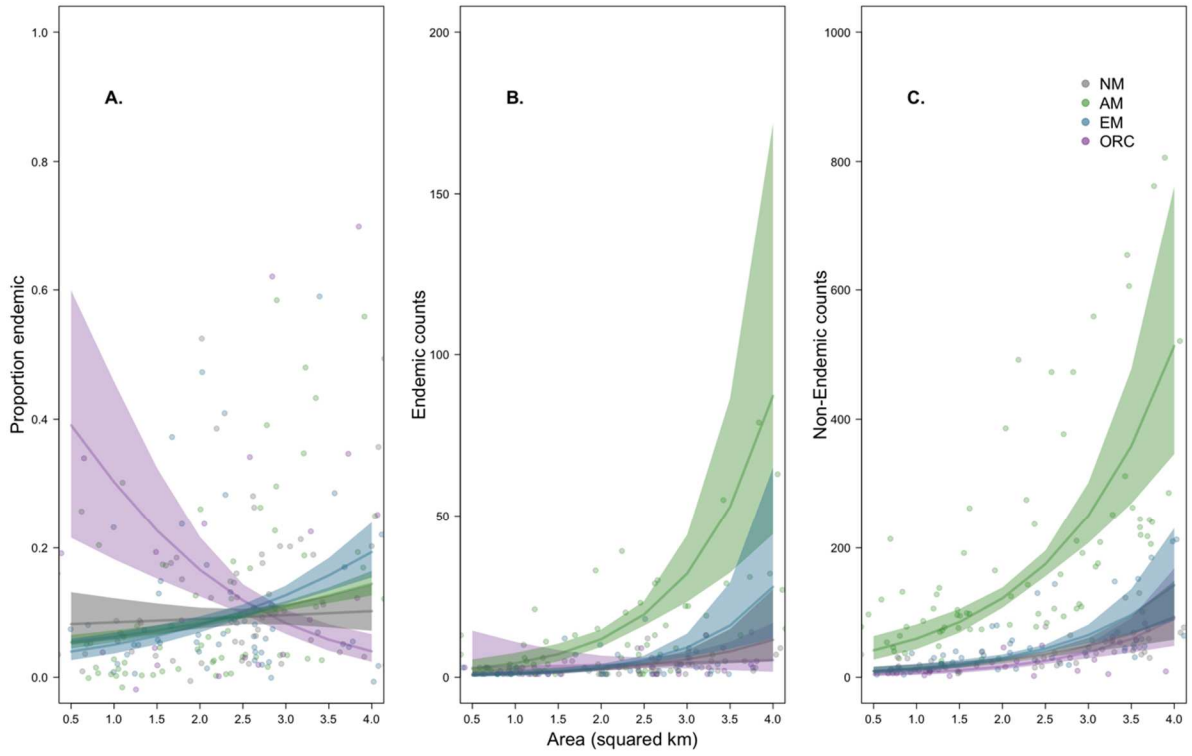


Figure S5 Variation in mycorrhizal types in oceanic island floras with area.

The proportion of oceanic island plant species endemic to non-endemic increases least with island area for NM plant species (A, estimate = -0.338 ± 0.122, p < 0.01, n = 254; GLM) and decreases for ORC plant species (estimate = -0.998 ± 0.165, p < 0.001, n=254, GLM). Both the number of endemic species and non-endemic species increase with area (B, C; endemic: estimate 0.866 ± 0.200, p < 0.001, n = 254, GLM; non-endemic: estimate 0.608 ± 0.113, p < 0.001, n =

254, GLM) for all mycorrhizal types except ORC endemic species. For endemic species, ORC plant species richness decreases with area (estimate -0.734 ± 0.355, p = 0.04, n = 254, GLM).

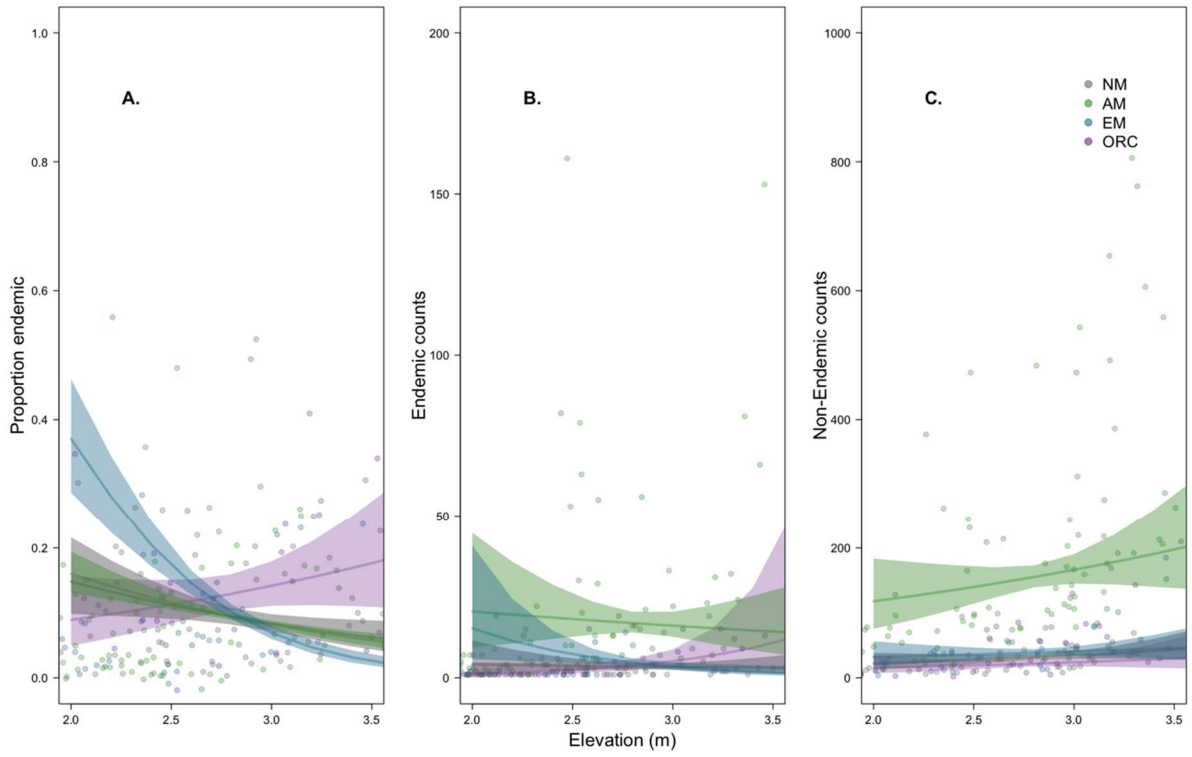


Figure S6. Variation in mycorrhizal types in oceanic island floras with elevation.

The proportion of oceanic island of plant species endemic to non-endemic decreases most strongly with elevation for EM plant species (A, estimate = -0.705 ± 0.080, p < 0.001, n = 254; GLM). The number of endemic species decreases with elevation (B, estimate -0.503± 0.200, p = 0.01, n = 254) for all mycorrhizal types except ORC plants, which increase in species richness with elevation (estimate 1.007 ± 0.346, p < 0.01, n = 254, GLM). The number of non-endemic species shows no relationship with elevation across mycorrhizal types (estimate 0.082 ± 0.113, p = 0.47, n = 254, GLM).

Table S1. Multinomial model results.

Multinomial model explaining the probability of mycorrhizal types as a function of land type (mainlands and oceanic islands), absolute latitude, area, elevation range, and species richness. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

SI Table 1						
M1: Native Model (N = 1,273,566)						
	(Intercept)	land type (oceanic)	absolute latitude	area	elevation range	species richness
AM	0.901***	-0.088***	-0.23***	-0.003	-0.017***	0.028***
EM	-0.927***	0.221***	-0.16***	0.049***	-0.145***	0.099***
ORC	-1.872***	1.152***	-0.709***	-0.238***	0.124***	0.529***
M2: Naturalized Model (N = 121,886)						
AM	0.505***	0.07**	-0.196***	-0.06***	-0.016	0.046**
EM	-1.753***	0.295***	-0.137***	-0.157***	0.017	0.148***
ORC	-6.279***	2.093***	-1.081***	0.387**	-0.2*	0.339*

Tables S2-S6.

In our first set of models (M1 and M2 in each table), we compared the species richness patterns of plants with differing mycorrhizal associations. For each comparison, separate models were run for native and naturalized plants to predict plant species richness. In these generalized linear mixed effects models (GLMMs), we used a Poisson distribution because the response variable, species richness, is count data. The fixed effects were mycorrhizal status, land type (mainland, non-oceanic island or oceanic island) and their interaction; we also included the covariates of log-transformed absolute latitude, area, elevation, and plant species richness. The random effects were region, nested within land type and its interaction with mycorrhizal status. These random terms control for the non-independence of individual plant species records within floras, thereby providing general tests for differences in proportion of mycorrhizal species across island and mainland floras.

In our second set of models (M3, M4, M5, M6 in each table), we investigated geographical and environmental drivers of mycorrhizal status for native and naturalized plants in mainland and oceanic island floras. For each model, we used a composite response variable with species richness of each of the two mycorrhizal categories of interest to account for differences in species richness. For these analyses, we used generalized linear models (GLMs) with a logit link function, assuming a binomial distribution of the response variable. For these models, we took the natural logarithm of area, human population density, distance to the nearest mainland, elevation range and island age to normalize distributions. For the native mainland models, we included area, mean annual precipitation, mean annual temperature and elevation range. For the

native island models, we included the same four variables with the addition of island age, island age squared and distance to the mainland.

Below we report the results from each of these sets of models (M1-M6) for analyses of the proportion of plant species in the floras that are ectomycorrhizal versus arbuscular mycorrhizal (EM:AM) plants (Table S2), orchid mycorrhizal versus AM or EM (ORC:M) plants (Table S3), AM versus non-mycorrhizal (AM:NM) plants (Table S4), EM versus NM (EM:NM) plants (Table S5), and ORC versus NM (ORC:NM) plants (Table S6). To ensure robust interpretation of patterns in the floras in the face of species with ambiguous mycorrhizal status, we repeated these models (M1-M6) for each combination of assigning ambiguous AMEM plants to AM or EM, and the ambiguous AMNM plants as AM or NM. Four combinations of these ambiguous designations are arranged as follows: (a) AMEM plants assigned to EM and AMNM plants assigned to NM, (b) AMEM plants assigned to EM and AMNM plants assigned to AM, (c) AMEM plants assigned to AM and AMNM plants assigned to NM, and (d) AMEM plants assigned to AM and AMNM plants assigned to AM. In all cases, the results reported in the main text correspond to models that assign AMEM plants to EM and AMNM plants to NM.

For models M1 and M2 in each table, the table lists the reported estimate relative to the reference category along with a p value range of significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Note that these estimates are on the logit scale, reflecting the appropriate transformation for statistical analysis (see figures for back-transformed representations of the results). The reference for M1 and M2 for land type is always mainland, the reference for M1 and M2 for mycorrhizal status depends on the model, but is the mycorrhizal status not listed in parentheses. We are particularly interested in whether the proportion of mycorrhizal plant species in the island floras differ from that in the mainland floras. This effect is tested in the

'mycorrhizal status*land type' interaction term. In Table1 M1 A, the estimate for the 'mycorrhizal status*land type (oceanic)' is 0.147 and is significant at p = 1.73e-10. This represents how much greater the proportion of EM compared to AM plant species are in oceanic island floras relative to their proportion in mainland floras.

Table S2. GLM explaining relative proportion EM and AM (EM:AM) plant species.

(1A) AMEM plants assigned to EM and AMNM plants assigned to NM, (1B) AMEM plants assigned to EM and AMNM plants assigned to AM, (1C) AMEM plants assigned to AM and AMNM plants assigned to NM, and (1D) AMEM plants assigned to AM and AMNM plants assigned to AM. The models in (1A) are those reported in the manuscript. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

SI Table 2				
M1: EM:AM Native Model (GLMM), N = 1925				
	A	**B**	**C**	**D**
(intercept)	5.436***	5.796***	5.546***	5.83***
mycorrhizal status (EM)	-1.506***	-1.779***	-1.649***	-1.915***
land type (non-oceanic)	1.199***	1.051***	1.027***	1.013***
land type (oceanic)	0.619***	0.512***	0.471***	0.478***
absolute latitude	-0.226***	1.371***	1.414***	1.358***
area	0.346***	0.000	-0.001	0.001
elevation range	0.161***	0.35***	0.309***	0.343***
species richness	1.448***	0.138***	0.145***	0.145***
mycorrhizal status*land type (non-oceanic)	0.013	-0.021	0.075**	0.038
mycorrhizal status*land type (oceanic)	0.147***	0.086***	0.177***	0.115***
M2: EM:AM Naturalized Model (GLMM), N = 1148				
(intercept)	3.805***	4.186***	3.817***	4.192***
mycorrhizal status (EM)	-1.9***	-2.28***	-2.012***	-2.382***

land type (non-oceanic)	1.048***	1.015***	1.076***	1.043***
land type (oceanic)	1.474***	1.382***	1.506***	1.414***
absolute latitude	1.052***	1.01***	1.053***	1.009***
area	-0.001	-0.001	-0.002	-0.001
elevation range	0.173	0.218.	0.183	0.232.
species richness	0.092	0.093	0.087	0.088
mycorrhizal status*land type (non-oceanic)	0.214**	0.31***	0.115.	0.206**
mycorrhizal status*land type (oceanic)	0.282***	0.356***	0.17***	0.233***
M3: EM:AM Mainland Native Model (GLM), N = 515				
(intercept)	-1.426***	-1.715***	-1.583***	-1.864***
area	0.049***	0.049***	0.036***	0.037***
absolute latitude	0.115***	0.123***	0.049***	0.059***
squared latitude	0.027.	-0.042**	0.096***	0.027.
precipitation	0.004	0.008*	-0.005	-0.001
temperature	0.05***	0.067***	0.058***	0.075***
elevation range	-0.073***	-0.075***	-0.054***	-0.057***
spatial autocovariate	0.062***	0.06***	0.067***	0.067***
M4: EM:AM Oceanic Island Native Model (GLM), N = 313				
(intercept)	-1.427***	-1.737***	-1.516***	-1.821***
area	0.171***	0.188***	0.097***	0.118***
distance	0.000	-0.012	0.002	-0.009
precipitation	0.036**	0.047***	0.019	0.03*
absolute latitude	0.135***	0.154***	0.052	0.075.
squared latitude	-0.146**	-0.226***	-0.118*	-0.198***
temperature	0.019	0.061.	-0.071*	-0.028
elevation range	-0.084***	-0.079***	-0.092***	-0.089***
spatial autocovariate	0.098***	0.097***	0.124***	0.12***
M5: EM:AM Mainland Naturalized Model (GLM), N = 287				
(intercept)	-1.825***	-2.195***	-1.977***	-2.341***
area	-0.122***	-0.133***	-0.075**	-0.088***
population density	0.000*	0.000**	0.000	0.000
absolute latitude	0.229*	0.252**	0.186.	0.21*
squared latitude	-0.107	-0.139.	-0.049	-0.083
precipitation	0.025	0.045*	0.027	0.046*
temperature	-0.016	0.041	0.001	0.056
elevation range	0.053**	0.062***	0.044*	0.053**
spatial autocovariate	0.153***	0.151***	0.169***	0.167***

M6: EM:AM Oceanic Island Naturalized Model (GLM), N = 100				
(intercept)	-1.668***	-1.96***	-1.836***	-2.121***
area	0.193***	0.193***	0.095.	0.1.
distance	-0.052	-0.041	-0.158***	-0.15***
precipitation	0.037	0.049	0.078*	0.091**
population density	0.000	0.000	0.000	0.000
absolute latitude	0.115	0.056	-0.049	-0.105
squared latitude	-0.453*	-0.476*	-0.161	-0.188
temperature	0.051	0.071	0.053	0.073
elevation range	-0.141**	-0.153***	-0.119*	-0.13*
spatial autocovariate	0.115*	0.11*	0.189**	0.185**

Table S3. GLM explaining proportion Orchid versus EM and AM mycorrhizal (ORC:M) plant species.

(2A) AMNM plants assigned to NM and (2B) AMNM plants assigned to M. The models in (2A) are those reported in the manuscript. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

SI Table 3		
M1: ORC:M Native Model (GLMM), N = 2212		
	A	**B**
(intercept)	5.356***	5.625***
mycorrhizal status (ORC)	-3.634***	-3.863***
land type (non-oceanic)	1.614***	1.586***
land type (oceanic)	1.07***	1.068***
absolute latitude	-0.37***	1.668***
area	1.719***	-0.319***
elevation range	0.255***	0.269***
species richness	0.348***	0.353***
mycorrhizal status*land type (non-oceanic)	-0.087	-0.122
mycorrhizal status*land type (oceanic)	-0.143.	-0.202**
M2: ORC:M Naturalized Model (GLMM), N = 2212		
(intercept)	-0.391	-0.335
mycorrhizal status (ORC)	-6.905***	-7.291***
land type (non-oceanic)	0.271	0.208
land type (oceanic)	0.415	0.287
absolute latitude	-0.826*	-0.961*
area	-1.135***	-1.167***

elevation range	3.927***	4.185***
species richness	-0.822***	-0.872***
mycorrhizal status*land type (non-oceanic)	1.372**	1.484***
mycorrhizal status*land type (oceanic)	0.954***	1.038***
M3: ORC:M Mainland Native Model (GLM), N = 486		
(intercept)	-3.211***	-3.446***
area	-0.068***	-0.483***
absolute latitude	-0.504***	-0.183***
squared latitude	-0.115***	0.147***
precipitation	0.147***	-0.301***
temperature	-0.32***	0.203***
elevation range	0.215***	0.077***
spatial autocovariate	0.077***	0.077***
M4: ORC:M Oceanic Island Native Model (GLM), N = 177		
(intercept)	-3.169***	-3.414***
area	0.293***	0.325***
distance	0.098***	0.093***
absolute latitude	-0.156*	-0.13*
squared latitude	-0.08	-0.135
precipitation	0.404***	0.408***
temperature	-0.146*	-0.101
elevation range	0.039	0.029
spatial autocovariate	0.154***	0.157***
M5: ORC:M Mainland Naturalized Model (GLM), N = 71		
(intercept)	-5.341***	-5.655***
area	-0.058	-0.071
population density	-0.001	-0.001
absolute latitude	-1.974***	-1.966***
squared latitude	1.584	1.574
precipitation	0.101	0.103
temperature	0.229	0.284
elevation range	-0.138	-0.134
spatial autocovariate	1.013***	1.02***
M6: ORC:M Oceanic Island Naturalized Model (GLM), N = 27		
(intercept)	-4.701***	-4.924***
area	-0.442	-0.485
distance	0.302	0.32
absolute latitude	0.096	0.125
squared latitude	-0.09	-0.245
precipitation	0.013	0.022
population density	0.001	0.002.
temperature	0.137	0.063
elevation range	0.057	0.051

spatial autocovariate	0.839.	0.875.

Table S4. GLM explaining proportion of AM versus Non-mycorrhizal (AM:NM) plant species.

(3A) AMEM plants assigned to AM and AMNM plants assigned to NM, (3B) AMEM plants assigned to AM and AMNM plants assigned to AM, (3C) AMEM plants assigned to EM and AMNM plants assigned to NM, and (3D) AMEM plants assigned to EM and AMNM plants assigned to AM. The models in (3A) are those reported in the manuscript. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

SI Table 4				
M1: AM:NM Native Model (GLMM), N = 1941 (A,C,D); N = 1938 (B)				
	A	**B**	**C**	**D**
(intercept)	5.532***	5.53***	5.51***	5.51***
mycorrhizal status (AM)	-0.756***	-1.757***	-0.728***	-0.728***
land type (non-oceanic)	1.016***	1.034***	1.018***	1.018***
land type (oceanic)	0.44***	0.458***	0.434***	0.434***
absolute latitude	-0.117***	1.359***	1.345***	1.345***
area	1.35***	-0.149***	-0.119***	-0.119***
elevation range	0.327***	0.323***	0.323***	0.323***
species richness	0.213***	0.208***	0.216***	0.216***
mycorrhizal status*land type (non-oceanic)	0.155***	0.143***	0.143***	0.143***
mycorrhizal status*land type (oceanic)	0.229***	0.212***	0.224***	0.224***
M2: AM:NM Naturalized Model (GLMM), N = 1181 (A,C,D); N = 1160 (B)				
(intercept)	3.79***	3.896***	3.893***	3.893***
mycorrhizal status (AM)	-0.356***	-1.348***	-0.342***	-0.342***
land type (non-oceanic)	1.171***	0.967***	0.968***	0.968***
land type (oceanic)	1.5***	1.333***	1.277***	1.277***
absolute latitude	0.976***	0.949***	0.912***	0.912***

area	-0.001	0.228***	0.277***	0.277***
elevation range	0.297*	0.203	0.22.	0.22.
species richness	0.1	0.083	0.093	0.093
mycorrhizal status*land type (non-oceanic)	-0.298***	-0.243***	-0.284***	-0.284***
mycorrhizal status*land type (oceanic)	-0.232***	-0.16***	-0.214***	-0.214***
M3: AM:NM Mainland Native Model (GLM), N = 515				
(intercept)	0.705***	1.71***	0.674***	0.674***
area	0.004.	0.000	0.001	0.001
absolute latitude	-0.018.	-0.013	-0.029**	-0.029**
squared latitude	-0.189***	-0.171***	-0.179***	-0.179***
precipitation	0.037***	0.071***	0.037***	0.037***
temperature	0.06***	0.026***	0.061***	0.061***
elevation range	-0.021***	-0.019***	-0.016***	-0.016***
spatial autocovariate	0.051***	0.073***	0.051***	0.051***
M4: AM:NM Oceanic Island Native Model (GLM), N = 325 (A,C, D); N = 323 (B)				
(intercept)	0.576***	1.587***	0.56***	0.56***
area	0.08***	0.06***	0.062***	0.062***
distance	-0.034**	-0.006	-0.032**	-0.032**
absolute latitude	0.036	0.067	0.02	0.02
squared latitude	-0.209***	-0.263***	-0.206***	-0.206***
precipitation	0.057***	0.074***	0.052***	0.052***
temperature	0.194***	0.125***	0.172***	0.172***
elevation range	0.032*	0.056*	0.032*	0.032*
spatial autocovariate	0.105***	0.148***	0.11***	0.11***
M5: AM:NM Mainland Naturalized Model (GLM), N = 294				
(intercept)	0.383***	1.379***	0.362***	0.362***
area	-0.051***	-0.062***	-0.041***	-0.041***
population density	0.000***	0.000**	0.000**	0.000**
absolute latitude	0.074	0.105	0.064	0.064
squared latitude	-0.102*	-0.133*	-0.092.	-0.092.
precipitation	0.068***	0.088***	0.067***	0.067***
temperature	0.185***	0.18***	0.187***	0.187***
elevation range	0.026*	0.027.	0.025*	0.025*
spatial autocovariate	0.125***	0.17***	0.127***	0.127***
M6: AM:NM Oceanic Island Naturalized Model (GLM), N = 109 (A,C,D); N = 105 (B)				
(intercept)	0.649***	1.591***	0.627***	0.627***
area	0.035	0.041	0.016	0.016
distance	0.034	0.005	0.022	0.022

absolute latitude	-0.36***	-0.255*	-0.379***	-0.379***
squared latitude	0.156	0.101	0.196	0.196
precipitation	0.055*	0.046	0.059*	0.059*
population density	0.000	0.000	0.000	0.000
temperature	0.138	0.171	0.13	0.13
elevation range	-0.052	-0.042	-0.047	-0.047
spatial autocovariate	0.091**	0.092	0.096**	0.096**

Table S5. GLM explaining proportion of EM versus non-mycorrhizal (EM:NM) plant species.

(4A) AMEM plants assigned to EM and AMNM plants assigned to NM, (4B) AMEM plants assigned to EM and AMNM plants assigned to AM, (4C) AMEM plants assigned to AM and AMNM plants assigned to NM, and (4D) AMEM plants assigned to AM and AMNM plants assigned to AM. The models in (4A) are those reported in the manuscript. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

SI Table 5				
M1: EM:NM Native Model (GLMM), N = 1924 (A,C); N = 1921(B,D)				
	A	**B**	**C**	**D**
(intercept)	4.022***	4.033***	3.924***	3.937***
mycorrhizal status (EM)	0.779***	-0.223***	0.894***	-0.108***
land type (non-oceanic)	1.057***	1.059***	1.077***	1.077***
land type (oceanic)	0.614***	0.617***	0.611***	0.613***
absolute latitude	-0.061***	1.312***	1.307***	1.298***
area	1.32***	-0.079***	-0.061***	-0.082***
elevation range	0.384***	0.386***	0.372***	0.374***
species richness	0.16***	0.15***	0.167***	0.156***
mycorrhizal status*land type (non-oceanic)	0.124**	0.112**	0.075.	0.062.
mycorrhizal status*land type (oceanic)	0.074*	0.053.	0.047.	0.028
M2: EM:NM Naturalized Model (GLMM), N = 1141 (A,C); N = 1120 (B,D)				
(intercept)	2.074***	2.107***	1.995***	2.037***

mycorrhizal status (EM)	1.584***	0.583***	1.674***	0.674***
land type (non-oceanic)	1.143***	1.057***	1.047***	0.952***
land type (oceanic)	1.535***	1.536***	1.43***	1.423***
absolute latitude	0.803***	0.801***	0.787***	0.78***
area	0.375***	0.356***	0.388***	0.368***
elevation range	0.145	0.099	0.158	0.111
species richness	0.164*	0.158.	0.154.	0.143.
mycorrhizal status*land type (non-oceanic)	-0.585***	-0.504***	-0.496***	-0.411***
mycorrhizal status*land type (oceanic)	-0.568***	-0.481***	-0.462***	-0.375***
M3: EM:NM Mainland Native Model (GLM), N = 515				
(intercept)	-0.753***	0.254***	-0.881***	0.125***
area	0.05***	0.049***	0.04***	0.038***
absolute latitude	0.083***	0.094***	0.033*	0.049**
squared latitude	-0.156***	-0.142***	-0.099***	-0.093***
precipitation	0.044***	0.078***	0.034***	0.07***
temperature	0.1***	0.065***	0.109***	0.073***
elevation range	-0.09***	-0.094***	-0.075***	-0.079***
spatial autocovariate	0.07***	0.082***	0.075***	0.082***
M4: EM:NM Oceanic Island Native Model (GLM), N = 244				
(intercept)	-0.878***	0.15***	-0.935***	0.088***
area	0.241***	0.207***	0.178***	0.147***
distance	-0.04*	-0.004	-0.033.	0.002
absolute latitude	0.203***	0.177**	0.111.	0.084
squared latitude	-0.317***	-0.269*	-0.228*	-0.178
precipitation	0.096***	0.111***	0.075***	0.09***
temperature	0.307***	0.26***	0.245***	0.203***
elevation range	-0.038	-0.008	-0.041	-0.009
spatial autocovariate	0.138***	0.187***	0.155***	0.201***
M5: EM:NM Mainland Naturalized Model (GLM), N = 287				
(intercept)	-1.462***	-0.463***	-1.595***	-0.596***
area	-0.172***	-0.194***	-0.129***	-0.149***
population density	0.000***	0.000***	0.000**	0.000***
absolute latitude	0.28**	0.319**	0.241*	0.278*
squared latitude	-0.184*	-0.227*	-0.132	-0.175.
precipitation	0.094***	0.111***	0.092***	0.109***
temperature	0.175***	0.168***	0.189***	0.18***
elevation range	0.081***	0.083***	0.074***	0.076**
spatial autocovariate	0.167***	0.195***	0.183***	0.21***
M6: EM:NM Oceanic Island Naturalized Model (GLM), N = 90 (A,C); N = 89 (B,D)				

(intercept)	-1.111***	-0.164**	-1.261***	-0.313***
area	0.161**	0.173*	0.108.	0.122
distance	-0.035	-0.072	-0.133*	-0.167**
absolute latitude	0.37*	0.424.	0.274	0.317
squared latitude	-1.222***	55.**	-1.068**	-1.038**
precipitation	0.064.	0.064	0.102*	0.1*
population density	0.000	0.000***	0.000***	0.000***
temperature	0.242	0.262	0.215	0.243
elevation range	-0.162**	-0.157*	-0.161*	-0.156*
spatial autocovariate	0.078.	0.087	0.116.	0.123

Table S6. GLM explaining the proportion orchid versus non-mycorrhizal (ORC:NM) plant species.

GLM explaining the proportion orchid versus non-mycorrhizal (ORC:NM) plant species.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

SI Table 6	
M1: ORC:NM Native Model (GLMM), N = 2212	
(intercept)	3.517***
mycorrhizal status (ORC)	-1.704***
land type (non-oceanic)	1.593***
land type (oceanic)	1.11***
absolute latitude	-0.216***
area	1.588***
elevation range	0.28***
species richness	0.381***
mycorrhizal status*land type (non-oceanic)	-0.241*
mycorrhizal status*land type (oceanic)	-0.359***
M2: ORC:NM Naturalized Model (GLMM), N = 2212	
(intercept)	-0.581*
mycorrhizal status (ORC)	-5.468***
land type (non-oceanic)	0.426
land type (oceanic)	0.548
absolute latitude	-0.24
area	-0.634***
elevation range	2.774***
species richness	-0.565**
mycorrhizal status*land type (non-oceanic)	1.88***
mycorrhizal status*land type (oceanic)	1.406***
M3: ORC:NM Mainland Native Model (GLM), N = 486	

(intercept)	-1.283***
area	-0.037***
absolute latitude	-0.446***
squared latitude	-0.327***
precipitation	0.253***
temperature	-0.279***
elevation range	0.161***
spatial autocovariate	0.105***
M4: ORC:NM Oceanic Island Native Model (GLM), N = 177	
(intercept)	-1.26***
area	0.401***
distance	0.088**
absolute latitude	-0.109
squared latitude	-0.279**
precipitation	0.53***
temperature	-0.067
elevation range	-0.027
spatial autocovariate	0.213***
M5: ORC:NM Mainland Naturalized Model (GLM), N = 71	
(intercept)	-3.782***
area	-0.099
population density	-0.001
absolute latitude	-2.02***
squared latitude	1.604
precipitation	0.169
temperature	0.352
elevation range	-0.168
spatial autocovariate	1.106***
M6: ORC:NM Oceanic Island Naturalized Model (GLM), N = 27	
(intercept)	-2.854***
area	-0.596
distance	0.353
absolute latitude	0.37
squared latitude	-0.863
precipitation	0.009
population density	0.002.
temperature	-0.147
elevation range	-0.011
spatial autocovariate	1.002*

Table S7. GLM explaining the proportion ectomycorrhizal versus arbuscular (EM:AM) and versus non-mycorrhizal (EM:NM) plant species with latitude.

GLM explaining the proportion ectomycorrhizal versus arbuscular (EM:AM) and versus non-mycorrhizal (EM:NM) plant species as a function of latitude and squared latitude in mainlands and oceanic islands.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

SI Table 7	
M1: EM:AM Mainland Native Model (GLMM), N = 515	
(intercept)	-1.442***
absolute latitude	0.138***
squared latitude	-0.043***
spatial autocovariate	0.057***
M2: EM:AM Oceanic Island Native Model (GLMM), N = 264	
(intercept)	-1.334***
absolute latitude	0.129**
squared latitude	-0.165***
spatial autocovariate	0.103***
M3: EM:NM Mainland Native Model (GLMM), N = 515	
(intercept)	-0.776***
absolute latitude	0.031**
squared latitude	-0.213***
spatial autocovariate	0.066***
M4: EM:NM Native Model (GLMM), N = 264	
(intercept)	-0.621***
absolute latitude	0.245***
squared latitude	-0.473***
spatial autocovariate	0.131***

Data S1. Table of families and corresponding consensus proportions of mycorrhizal status.

Consensus proportions were calculated by averaging each mycorrhizal status proportion within each family across the three status references.

Family	AVG_AMP	AVG_EM/ERP	AVG_AMEMP	AVG_AMNMP	AVG_NMP	AVG_ORCP
Acanthaceae	1.000	NA	NA	NA	0.400	0.000
Acoraceae	NA	NA	NA	1.000	0.000	0.000
Actinidiaceae	1.000	NA	NA	NA	0.667	0.000
Adiantaceae	NA	NA	NA	NA	NA	0.000
Adoxaceae	0.500	NA	NA	0.750	0.045	0.000
Agavaceae	NA	NA	NA	NA	NA	0.000
Aizoaceae	0.667	NA	NA	0.667	0.417	0.000
Alismataceae	0.571	NA	NA	0.714	0.063	0.000
Alliaceae	NA	NA	NA	NA	NA	0.000
Alstroemeriaceae	1.000	NA	NA	NA	0.000	0.000
Altingiaceae	1.000	NA	NA	NA	0.000	0.000
Amaranthaceae	0.548	NA	NA	0.726	0.322	0.000
Amaryllidaceae	0.955	NA	NA	0.045	0.022	0.000
Anacardiaceae	0.952	NA	NA	0.048	0.045	0.000
Aneuraceae	NA	NA	NA	NA	NA	0.000
Anisophylleaceae	1.000	NA	NA	NA	0.500	0.000
Annonaceae	1.000	NA	NA	NA	0.091	0.000
Anarthriaceae	1.000	NA	NA	NA	0.000	0.000
Anisophylleaceae	1.000	NA	NA	NA	0.500	0.000
Anthocerotaceae	NA	NA	NA	NA	NA	0.000
Apiaceae	0.804	NA	NA	0.196	0.089	0.000
Apocynaceae	0.905	NA	NA	0.095	0.067	0.000
Apodanthaceae	NA	NA	NA	NA	1.000	0.000
Aponogetonaceae	NA	NA	NA	1.000	0.000	0.000
Aquifoliaceae	1.000	NA	NA	NA	0.000	0.000
Araceae	0.957	NA	NA	0.522	0.117	0.000
Araliaceae	0.733	NA	NA	0.267	0.167	0.000
Araucariaceae	1.000	NA	NA	NA	0.000	0.000
Arecaceae	0.941	NA	NA	0.059	0.000	0.000
Aristolochiaceae	NA	NA	NA	1.000	0.000	0.000
Arnelliaceae	NA	NA	NA	NA	NA	0.000
Asparagaceae	0.878	NA	NA	0.122	0.058	0.000

Asphodelaceae	NA	NA	NA	NA	NA	0.000
Aspleniaceae	NA	NA	NA	NA	NA	0.000
Asteliaceae	1.000	NA	NA	NA	0.000	0.000
Asteraceae	0.861	NA	NA	0.139	0.037	0.000
Asteropeiceae	NA	1.000	NA	NA	0.000	0.000
Avicenniaceae	NA	NA	NA	1.000	0.000	0.000
Aytoniaceae	NA	NA	NA	NA	NA	0.000
Azollaceae	NA	NA	NA	NA	NA	0.000
Balanophoraceae	NA	NA	NA	NA	1.000	0.000
Balsaminaceae	0.429	NA	NA	0.571	0.000	0.000
Bataceae	NA	NA	NA	NA	1.000	0.000
Begoniaceae	1.000	NA	NA	NA	0.100	0.000
Berberidaceae	0.769	NA	NA	0.231	0.071	0.000
Betulaceae	0.030	0.990	NA	NA	0.000	0.000
Biebersteiniaceae	1.000	NA	NA	NA	0.000	0.000
Bignoniaceae	0.909	NA	NA	0.091	0.000	0.000
Bixaceae	1.000	NA	NA	NA	0.000	0.000
Blasiaceae	NA	NA	NA	NA	NA	0.000
Blechnaceae	NA	NA	NA	NA	NA	0.000
Boraginaceae	0.708	NA	NA	0.292	0.213	0.000
Brassicaceae	0.250	NA	NA	0.750	0.872	0.000
Bromeliaceae	1.000	NA	NA	1.000	0.125	0.000
Bruniaceae	1.000	NA	NA	NA	0.000	0.000
Burmanniaceae	NA	NA	NA	NA	NA	0.000
Burseraceae	0.857	NA	NA	0.143	0.000	0.000
Butomaceae	NA	NA	NA	1.000	0.500	0.000
Buxaceae	1.000	NA	NA	NA	0.000	0.000
Byblidaceae	NA	NA	NA	NA	1.000	0.000
Cactaceae	1.000	NA	NA	NA	0.310	0.000
Calceolariaceae	1.000	NA	NA	NA	0.000	0.000
Callitrichaceae	NA	NA	NA	1.000	0.000	0.000
Calophyllaceae	1.000	NA	NA	NA	0.000	0.000
Calypogeiaceae	NA	NA	NA	NA	NA	0.000
Campanulaceae	0.767	NA	NA	0.233	0.044	0.000
Cannabaceae	0.833	NA	NA	0.167	0.143	0.000
Cannaceae	NA	NA	NA	NA	1.000	0.000
Capparaceae	1.000	NA	NA	NA	0.750	0.000
Capparidaceae	NA	NA	NA	NA	NA	0.000
Caprifoliaceae	0.767	NA	NA	0.233	0.085	0.000

Caricaceae	1.000	NA	NA	NA	0.000	0.000
Carlemanniaceae	1.000	NA	NA	NA	0.000	0.000
Caryocaraceae	1.000	NA	NA	NA	0.000	0.000
Caryophyllaceae	0.267	NA	NA	0.867	0.382	0.000
Casuarinaceae	NA	1.000	NA	NA	0.000	0.000
Ceasalpiniaceae	NA	NA	NA	NA	NA	0.000
Celastraceae	0.667	NA	NA	0.333	0.077	0.000
Centrolepidaceae	NA	NA	NA	NA	1.000	0.000
Cephaloziaceae	NA	NA	NA	NA	NA	0.000
Cephaloziellaceae	NA	NA	NA	NA	NA	0.000
Ceratophyllaceae	NA	NA	NA	NA	1.000	0.000
Chenopodiaceae	NA	NA	NA	1.000	0.000	0.000
Chrysobalanaceae	1.000	NA	NA	NA	0.000	0.000
Cistaceae	0.071	0.964	NA	NA	0.000	0.000
Cleomaceae	1.000	NA	NA	NA	0.000	0.000
Clethraceae	1.000	NA	NA	NA	0.000	0.000
Clusiaceae	1.000	NA	NA	NA	0.100	0.000
Codoniaceae	NA	NA	NA	NA	NA	0.000
Colchicaceae	1.000	NA	NA	NA	0.000	0.000
Combretaceae	1.000	NA	NA	NA	0.000	0.000
Commelinaceae	0.500	NA	NA	0.750	0.556	0.000
Conocephalaceae	NA	NA	NA	NA	NA	0.000
Convolvulaceae	0.929	NA	NA	0.071	0.563	0.000
Coriariaceae	1.000	NA	NA	NA	0.000	0.000
Cornaceae	0.750	NA	NA	0.250	0.000	0.000
Corsiaceae	NA	NA	NA	NA	NA	0.000
Costaceae	1.000	NA	NA	NA	0.000	0.000
Crassulaceae	0.615	NA	NA	0.385	0.833	0.000
Cruciferae	NA	NA	NA	1.000	0.000	0.000
Cucurbitaceae	1.000	NA	NA	NA	0.000	0.000
Cunoniaceae	NA	1.000	NA	NA	0.000	0.000
Cupressaceae	1.000	NA	NA	NA	0.000	0.000
Cyatheaceae	NA	NA	NA	NA	NA	0.000
Cycadaceae	1.000	NA	NA	NA	0.000	0.000
Cyclanthaceae	1.000	NA	NA	NA	0.500	0.000
Cymodoceaceae	NA	NA	NA	NA	1.000	0.000
Cynomoriaceae	NA	NA	NA	NA	1.000	0.000
Cyperaceae	0.721	0.504	NA	0.636	0.188	0.000
Cytinaceae	NA	NA	NA	NA	1.000	0.000

Dasypogonaceae	NA	NA	NA	NA	1.000	0.000
Davalliaceae	NA	NA	NA	NA	NA	0.000
Dennstaedtiaceae	NA	NA	NA	NA	NA	0.000
Diapensiaceae	NA	NA	NA	NA	NA	0.000
Dichapetalaceae	1.000	NA	NA	NA	0.000	0.000
Dicksoniaceae	NA	NA	NA	NA	NA	0.000
Dilleniaceae	1.000	NA	NA	NA	0.000	0.000
Dioscoreaceae	1.000	NA	NA	NA	0.500	0.000
Dipsacaceae	NA	NA	NA	NA	NA	0.000
Dipterocarpaceae	0.158	0.921	NA	NA	0.025	0.000
Droseraceae	NA	NA	NA	1.000	0.750	0.000
Drosophyllaceae	NA	NA	NA	NA	1.000	0.000
Dryopteridaceae	NA	NA	NA	NA	NA	0.000
Ebenaceae	1.000	NA	NA	NA	0.000	0.000
Elaeagnaceae	0.600	NA	NA	0.400	0.000	0.000
Elaeocarpaceae	1.000	NA	NA	NA	0.000	0.000
Elatinaceae	1.000	NA	NA	NA	0.000	0.000
Ephedraceae	1.000	NA	NA	NA	0.000	0.000
Equisetaceae	NA	NA	NA	NA	NA	0.000
Eremolepidaceae	NA	NA	NA	NA	1.000	0.000
Ericaceae	0.615	0.692	NA	NA	0.139	0.000
Erythroxylaceae	1.000	NA	NA	NA	0.500	0.000
Escalloniaceae	1.000	NA	NA	NA	0.000	0.000
Euphorbiaceae	0.940	1.000	NA	0.060	0.014	0.000
Fabaceae	0.834	0.551	NA	0.064	0.018	0.000
Fagaceae	NA	0.986	NA	0.043	0.014	0.000
Fouquieriaceae	1.000	NA	NA	NA	0.000	0.000
Frankeniaceae	1.000	NA	NA	NA	0.833	0.000
Fumariaceae	NA	NA	NA	1.000	0.000	0.000
Garryaceae	1.000	NA	NA	NA	0.000	0.000
Gentianaceae	0.800	NA	NA	0.200	0.063	0.000
Geocalycaceae	NA	NA	NA	NA	NA	0.000
Geraniaceae	0.781	NA	NA	0.219	0.000	0.000
Gesneriaceae	1.000	NA	NA	NA	0.000	0.000
Ginkgoaceae	1.000	NA	NA	NA	0.000	0.000
Gleicheniaceae	NA	NA	NA	NA	NA	0.000
Gnetaceae	0.333	0.833	NA	NA	0.000	0.000
Goodeniaceae	1.000	NA	NA	NA	0.000	0.000
Grammitidaceae	NA	NA	NA	NA	NA	0.000

Griseliniaceae	1.000	NA	NA	NA	0.000	0.000
Grossulariaceae	0.833	NA	NA	0.167	0.000	0.000
Gunneraceae	1.000	NA	NA	NA	0.000	0.000
Gymnomitriaceae	NA	NA	NA	NA	NA	0.000
Haemodoraceae	NA	NA	NA	NA	1.000	0.000
Haloragaceae	0.500	NA	NA	0.750	0.167	0.000
Hamamelidaceae	1.000	NA	NA	NA	0.000	0.000
Haplomitriaceae	NA	NA	NA	NA	NA	0.000
Heliconiaceae	1.000	NA	NA	NA	0.000	0.000
Hemerocallidaceae	NA	NA	NA	NA	NA	0.000
Herbertaceae	NA	NA	NA	NA	NA	0.000
Hippuridaceae	NA	NA	NA	NA	1.000	0.000
Humiriaceae	1.000	NA	NA	NA	0.000	0.000
Hyacinthaceae	NA	NA	NA	NA	NA	0.000
Hydatellaceae	NA	NA	NA	NA	1.000	0.000
Hydnoraceae	NA	NA	NA	NA	1.000	0.000
Hydrangeaceae	0.933	NA	NA	0.067	0.063	0.000
Hydrocharitaceae	1.000	NA	NA	1.000	0.333	0.000
Hydrophyllaceae	NA	NA	NA	1.000	0.000	0.000
Hymenophyllaceae	NA	NA	NA	NA	NA	0.000
Hypericaceae	0.625	NA	NA	0.375	0.333	0.000
Hypoxidaceae	1.000	NA	NA	NA	0.000	0.000
Icacinaceae	1.000	NA	NA	NA	0.000	0.000
Iridaceae	0.950	NA	NA	0.050	0.000	0.000
Irvingiaceae	1.000	NA	NA	NA	0.000	0.000
Isoetaceae	NA	NA	NA	NA	NA	0.000
Ixioliriaceae	NA	NA	NA	1.000	0.000	0.000
Jubulaceae	NA	NA	NA	NA	NA	0.000
Juglandaceae	0.125	0.625	1.000	0.250	0.000	0.000
Junglandaceae	NA	1.000	NA	NA	0.000	0.000
Juncaceae	0.217	NA	NA	0.891	0.475	0.000
Juncaginaceae	NA	NA	NA	1.000	0.375	0.000
Jungermanniaceae	NA	NA	NA	NA	NA	0.000
Krameriaceae	1.000	NA	NA	NA	0.000	0.000
Lamiaceae	0.879	NA	NA	0.121	0.043	0.000
Lauraceae	1.000	NA	NA	NA	0.500	0.000
Lecythidaceae	1.000	NA	NA	NA	0.000	0.000
Lejeuneaceae	NA	NA	NA	NA	NA	0.000
Lennoaceae	NA	NA	NA	NA	1.000	0.000

Lentibulariaceae	NA	NA	NA	NA	1.000	0.000
Lepidoziaceae	NA	NA	NA	NA	NA	0.000
Liliaceae	0.923	NA	NA	0.077	0.037	0.000
Limnocharitaceae	NA	NA	NA	1.000	0.000	0.000
Linaceae	0.769	NA	NA	0.231	0.000	0.000
Linderniaceae	1.000	NA	NA	NA	0.000	0.000
Loasaceae	NA	NA	NA	NA	1.000	0.000
Loganiaceae	1.000	NA	NA	NA	0.000	0.000
Loranthaceae	NA	NA	NA	NA	1.000	0.000
Lunulariaceae	NA	NA	NA	NA	NA	0.000
Lycopodiaceae	NA	NA	NA	NA	NA	0.000
Lythraceae	0.833	NA	NA	0.167	0.143	0.000
Magnoliaceae	0.889	NA	NA	0.111	0.000	0.000
Malpighiaceae	1.000	NA	NA	NA	0.000	0.000
Malvaceae	0.889	0.111	NA	NA	0.018	0.000
Bombacoideae	NA	NA	NA	NA	NA	0.000
Sterculioideae	NA	NA	NA	NA	NA	0.000
Tilioideae	NA	NA	NA	NA	NA	0.000
Marantaceae	1.000	NA	NA	NA	0.200	0.000
Marattiaceae	NA	NA	NA	NA	NA	0.000
Marchantiaceae	NA	NA	NA	NA	NA	0.000
Marsileaceae	NA	NA	NA	NA	NA	0.000
Melanthiaceae	0.750	NA	NA	0.250	0.000	0.000
Melastomataceae	0.750	0.125	NA	0.125	0.000	0.000
Meliaceae	0.950	1.000	NA	0.050	0.000	0.000
Menispermaceae	1.000	NA	NA	NA	0.000	0.000
Menyanthaceae	NA	NA	NA	1.000	0.250	0.000
Mesembranthaceae	NA	NA	NA	NA	1.000	0.000
Metzgeriaceae	NA	NA	NA	NA	NA	0.000
Mimosaceae	NA	1.000	NA	NA	0.000	0.000
Misodendraceae	NA	NA	NA	NA	1.000	0.000
Mitrastemonaceae	NA	NA	NA	NA	1.000	0.000
Molluginaceae	NA	NA	NA	NA	1.000	0.000
Montiaceae	0.500	NA	NA	0.500	0.500	0.000
Moraceae	0.875	NA	NA	0.125	0.111	0.000
Moringaceae	1.000	NA	NA	NA	0.000	0.000
Musaceae	1.000	NA	NA	NA	0.000	0.000
Myricaceae	0.600	NA	NA	0.400	0.500	0.000
Myristicaceae	1.000	NA	NA	NA	0.000	0.000

Myrsinaceae	NA	NA	NA	NA	NA	0.000
Myrtaceae	0.418	0.784	1.000	0.015	0.005	0.000
Najadaceae	NA	NA	NA	1.000	0.000	0.000
Nartheciaceae	NA	NA	NA	1.000	0.000	0.000
Nelumbonaceae	NA	NA	NA	1.000	0.000	0.000
Nepenthaceae	NA	NA	NA	NA	1.000	0.000
Nephrolepidaceae	NA	NA	NA	NA	NA	0.000
Nitrariaceae	1.000	NA	NA	NA	0.000	0.000
Nothofagaceae	NA	1.000	NA	NA	0.000	0.000
Nyctaginaceae	0.667	1.000	NA	0.667	0.167	0.000
Nymphaeaceae	NA	NA	NA	1.000	0.500	0.000
Ochnaceae	1.000	NA	NA	NA	0.000	0.000
Olacaceae	1.000	NA	NA	NA	0.500	0.000
Oleaceae	0.731	NA	NA	0.269	0.103	0.000
Onagraceae	0.778	NA	NA	0.222	0.069	0.000
Ophioglossaceae	NA	NA	NA	NA	NA	0.000
Opiliaceae	NA	NA	NA	NA	1.000	0.000
Orobanchaceae	0.400	NA	NA	0.600	0.896	0.000
Osmundaceae	NA	NA	NA	NA	NA	0.000
Oxalidaceae	0.857	NA	NA	0.143	0.000	0.000
Paeoniaceae	1.000	NA	NA	NA	0.000	0.000
Pandaceae	1.000	NA	NA	NA	0.000	0.000
Pandanaceae	1.000	NA	NA	NA	0.667	0.000
Papaveraceae	0.571	NA	NA	0.714	0.316	0.000
Papilionaceae	NA	1.000	NA	NA	0.000	0.000
Parnassiaceae	NA	NA	NA	NA	NA	0.000
Passifloraceae	1.000	NA	NA	NA	0.000	0.000
Paulowniaceae	1.000	NA	NA	NA	0.000	0.000
Pedaliaceae	1.000	NA	NA	NA	0.000	0.000
Pelliaceae	NA	NA	NA	NA	NA	0.000
Petrosaviaceae	NA	NA	NA	NA	NA	0.000
Phrymaceae	0.500	NA	NA	0.500	0.000	0.000
Phyllanthaceae	0.625	0.656	NA	0.063	0.079	0.000
Phytolaccaceae	NA	NA	NA	1.000	0.500	0.000
Picrodendraceae	1.000	NA	NA	NA	0.000	0.000
Pinaceae	NA	1.000	NA	NA	0.000	0.000
Piperaceae	0.833	NA	NA	0.583	0.071	0.000
Pittosporaceae	1.000	NA	NA	NA	0.000	0.000
Plagiochilaceae	NA	NA	NA	NA	NA	0.000

Plagiogyriaceae	NA	NA	NA	NA	NA	0.000
Plantaginaceae	0.810	NA	NA	0.190	0.137	0.000
Plantanaceae	1.000	NA	NA	NA	0.000	0.000
Plumbaginaceae	0.667	NA	NA	0.667	0.200	0.000
Poaceae	0.794	NA	NA	0.206	0.075	0.000
Podocarpaceae	1.000	NA	NA	NA	0.000	0.000
Podostemaceae	NA	NA	NA	1.000	0.000	0.000
Polemoniaceae	0.800	NA	NA	0.200	0.167	0.000
Polygalaceae	0.750	NA	NA	0.250	0.000	0.000
Polygonaceae	0.226	0.581	NA	0.806	0.142	0.000
Polypodiaceae	NA	NA	NA	NA	NA	0.000
Pontederiaceae	NA	NA	NA	1.000	0.000	0.000
Porellaceae	NA	NA	NA	NA	NA	0.000
Portulacaceae	NA	NA	NA	1.000	0.267	0.000
Posidoniaceae	NA	NA	NA	NA	1.000	0.000
Potamogetonaceae	0.500	NA	NA	0.750	0.389	0.000
Primulaceae	0.667	NA	NA	0.333	0.160	0.000
Proteaceae	1.000	NA	NA	NA	0.667	0.000
Pseudolepicoleaceae	NA	NA	NA	NA	NA	0.000
Psilotaceae	NA	NA	NA	NA	NA	0.000
Pteridaceae	NA	NA	NA	NA	NA	0.000
Putranjivaceae	1.000	NA	NA	NA	0.500	0.000
Quiinaceae	NA	NA	NA	1.000	0.000	0.000
Radulaceae	NA	NA	NA	NA	NA	0.000
Rafflesiaceae	NA	NA	NA	NA	1.000	0.000
Ranunculaceae	0.827	NA	NA	0.173	0.067	0.000
Resedaceae	NA	NA	NA	1.000	0.000	0.000
Restoniaceae	1.000	NA	NA	NA	0.500	0.000
Rhamnaceae	0.818	1.000	NA	0.182	0.022	0.000
Rhizophoraceae	NA	NA	NA	1.000	0.000	0.000
Ricciaceae	NA	NA	NA	NA	NA	0.000
Roridulaceae	NA	NA	NA	NA	1.000	0.000
Rosaceae	0.707	0.512	NA	0.269	0.020	0.000
Rubiaceae	0.857	NA	NA	0.143	0.113	0.000
Ruppiaceae	NA	NA	NA	1.000	0.000	0.000
Ruscaceae	NA	NA	NA	NA	NA	0.000
Rutaceae	0.963	NA	NA	0.037	0.000	0.000
Salicaceae	0.152	0.917	1.000	0.015	0.005	0.000

Santalaceae	1.000	NA	NA	1.000	0.400	0.000
Sapindaceae	0.710	NA	NA	0.290	0.000	0.000
Sapotaceae	1.000	1.000	NA	NA	0.000	0.000
Sarcolaenaceae	NA	1.000	NA	NA	0.000	0.000
Sarraceniaceae	NA	NA	NA	NA	1.000	0.000
Saxifragaceae	0.300	NA	NA	0.850	0.333	0.000
Scapaniaceae	NA	NA	NA	NA	NA	0.000
Scheuchzeriaceae	NA	NA	NA	NA	1.000	0.000
Schisandraceae	0.750	NA	NA	0.250	0.000	0.000
Schizaeaceae	NA	NA	NA	NA	NA	0.000
Scrophulariaceae	0.813	NA	NA	0.188	0.600	0.000
Selaginellaceae	NA	NA	NA	NA	NA	0.000
Simaroubaceae	0.667	NA	NA	0.333	0.000	0.000
Smilacaceae	1.000	NA	NA	NA	0.000	0.000
Solanaceae	0.933	NA	NA	0.067	0.032	0.000
Sparganiaceae	NA	NA	NA	NA	NA	0.000
Staphyleaceae	NA	NA	NA	1.000	0.500	0.000
Stegnospermataceae	1.000	NA	NA	NA	0.000	0.000
Styracaceae	NA	NA	NA	NA	NA	0.000
Tamaricaceae	0.800	NA	NA	0.600	0.000	0.000
Taxaceae	1.000	NA	NA	NA	0.000	0.000
Taxodiaceae	1.000	NA	NA	NA	0.000	0.000
Tetramelaceae	1.000	NA	NA	NA	0.000	0.000
Tetrameristaceae	1.000	NA	NA	NA	0.000	0.000
Theaceae	1.000	NA	NA	NA	0.000	0.000
Thelypteridaceae	NA	NA	NA	NA	NA	0.000
Themidaceae	NA	NA	NA	NA	NA	0.000
Theophrastaceae	NA	NA	NA	NA	NA	0.000
Thymelaeaceae	0.875	NA	NA	0.125	0.000	0.000
Tiliaceae	NA	NA	1.000	1.000	0.000	0.000
Tofieldiaceae	0.500	NA	NA	0.500	0.333	0.000
Triuridaceae	NA	NA	NA	NA	NA	0.000
Tropaeolaceae	NA	NA	NA	NA	NA	0.000
Turneraceae	NA	NA	NA	NA	NA	0.000
Typhaceae	0.333	NA	NA	0.667	0.625	0.000
Ulmaceae	0.333	NA	NA	0.667	0.000	0.000
Urticaceae	0.833	NA	NA	0.722	0.048	0.000
Valerianaceae	NA	NA	NA	NA	NA	0.000

Verbenaceae	1.000	NA	NA	NA	0.000	0.000
Violaceae	0.741	NA	NA	0.259	0.069	0.000
Viscaceae	NA	NA	NA	NA	1.000	0.000
Vitaceae	1.000	NA	NA	NA	0.000	0.000
Vittariaceae	NA	NA	NA	NA	NA	0.000
Welwitschiaceae	1.000	NA	NA	NA	0.000	0.000
Xanthorrhoeaceae	1.000	NA	NA	NA	0.000	0.000
Xyridaceae	NA	NA	NA	NA	1.000	0.000
Zamiaceae	1.000	NA	NA	NA	0.000	0.000
Zingiberaceae	1.000	NA	NA	NA	0.000	0.000
Zosteraceae	NA	NA	NA	NA	1.000	0.000
Zygophyllaceae	0.875	NA	NA	0.563	0.136	0.000
Orchidaceae	0.000	0.000	0.000	0.000	0.000	1.000

Appendix E: Supplementary Information for Root pathogen diversity and composition varies with climate in undisturbed grasslands, but less so in anthropogenically-disturbed grasslands

Material from: Delavaux, C.S., Schemanski, J.L., House, G.L. *et al.* (2021). Root pathogen diversity and composition varies with climate in undisturbed grasslands, but less so in anthropogenically disturbed grasslands. *ISME J*ournal. DOI: https://doi.org/10.1038/s41396-020-00783-z

Table S1. Soil chemical analyses results and climate variables for this study.

Values for soil properties (Bray 2 phosphorus, potassium, and pH), and climate variable (mean annual temperature and mean annual precipitation) across study sites.

Site	State	Disturbance	Lat	Long	Mean Temp	Mean Precip	P	K	pH
Klemme	OK	Disturbed	35.40	-99.06	15.60	77.18	NA	NA	NA
Klemme	OK	Disturbed	35.40	-99.06	15.60	77.18	NA	NA	NA
Klemme	OK	Disturbed	35.40	-99.06	15.60	77.18	NA	NA	NA
Hays	KS	Disturbed	38.86	-99.39	12.08	60.00	20	317	7.5
Hays	KS	Disturbed	38.86	-99.39	12.08	60.00	20	317	7.5
Hays	KS	Disturbed	38.86	-99.39	12.08	60.00	20	317	7.5
Hays	KS	Disturbed	38.86	-99.39	12.08	60.00	20	317	7.5
Welda	KS	Disturbed	38.18	-95.27	13.16	104.11	NA	NA	NA
Welda	KS	Disturbed	38.18	-95.27	13.16	104.11	NA	NA	NA
Konza	KS	Disturbed	39.10	-96.61	12.70	85.81	19	296	6.2
Konza	KS	Disturbed	39.10	-96.61	12.70	85.81	19	296	6.2
Klemme	OK	Disturbed	35.40	-99.06	15.60	77.18	NA	NA	NA
Konza	KS	Disturbed	39.10	-96.61	12.70	85.81	19	296	6.2
Osage	MO	Disturbed	37.75	-94.33	13.39	115.91	10	61	7.1

Osage	MO	Disturbed	37.75	-94.33	13.39	115.91	10	61	7.1
Taberville	MO	Disturbed	38.04	-93.97	13.14	112.46	10	61	7.1
WahKonTah	MO	Disturbed	37.92	-94.01	13.28	117.53	10	61	7.1
Morris	MO	Disturbed	40.38	-92.94	10.58	101.80	13	132	5.8
Morris	MO	Disturbed	40.38	-92.94	10.58	101.80	13	132	5.8
Rockefeller	KS	Disturbed	39.05	-95.19	12.73	99.16	NA	NA	NA
Rockefeller	KS	Disturbed	39.05	-95.19	12.73	99.16	NA	NA	NA
Klemme	OK	Remnant	35.42	-99.06	15.54	77.66	52	148	7.7
Klemme	OK	Remnant	35.42	-99.06	15.54	77.66	52	148	7.7
Klemme	OK	Remnant	35.42	-99.06	15.54	77.66	52	148	7.7
Klemme	OK	Remnant	35.42	-99.06	15.54	77.66	52	148	7.7
Klemme	OK	Remnant	35.42	-99.06	15.54	77.66	52	148	7.7
Klemme	OK	Remnant	35.42	-99.06	15.54	77.66	52	148	7.7
Klemme	OK	Remnant	35.42	-99.06	15.54	77.66	52	148	7.7
Klemme	OK	Remnant	35.42	-99.06	15.54	77.66	52	148	7.7
Hays	KS	Remnant	38.86	-99.38	12.10	60.07	9	248	7.7
Hays	KS	Remnant	38.86	-99.38	12.10	60.07	9	248	7.7
Hays	KS	Remnant	38.86	-99.38	12.10	60.07	9	248	7.7
Hays	KS	Remnant	38.86	-99.38	12.10	60.07	9	248	7.7
Hays	KS	Remnant	38.86	-99.38	12.10	60.07	9	248	7.7
Konza	KS	Remnant	39.07	-96.57	12.28	87.51	9	299	6.3
Konza	KS	Remnant	39.07	-96.57	12.28	87.51	9	299	6.3
Konza	KS	Remnant	39.07	-96.57	12.28	87.51	9	299	6.3
Konza	KS	Remnant	39.07	-96.57	12.28	87.51	9	299	6.3
Rockefeller	KS	Remnant	39.05	-95.21	12.67	99.07	8	141	6.2
Rockefeller	KS	Remnant	39.05	-95.21	12.67	99.07	8	141	6.2
Rockefeller	KS	Remnant	39.05	-95.21	12.67	99.07	8	141	6.2
Welda	KS	Remnant	38.18	-95.27	13.14	104.08	2	76	6.1
Welda	KS	Remnant	38.18	-95.27	13.14	104.08	2	76	6.1
Rockefeller	KS	Remnant	39.05	-95.21	12.67	99.07	8	141	6.2
Welda	KS	Remnant	38.18	-95.27	13.14	104.08	2	76	6.1
Weston	IL	Remnant	40.75	-88.61	10.49	91.34	NA	NA	NA
Weston	IL	Remnant	40.75	-88.61	10.49	91.34	NA	NA	NA
Sunbury	IL	Remnant	41.08	-88.60	10.21	93.75	NA	NA	NA
Sunbury	IL	Remnant	41.08	-88.60	10.21	93.75	NA	NA	NA
Goose_Lake	IL	Remnant	41.36	-88.31	9.92	92.80	NA	NA	NA
Goose_Lake	IL	Remnant	41.36	-88.31	9.92	92.80	NA	NA	NA
Konza	KS	Remnant	39.07	-96.57	12.28	87.51	9	299	6.3
Konza	KS	Remnant	39.07	-96.57	12.28	87.51	9	299	6.3

Konza	KS	Remnant	39.07	-96.57	12.28	87.51	9	299	6.3
Konza	KS	Remnant	39.07	-96.57	12.28	87.51	9	299	6.3
Welda	KS	Remnant	38.18	-95.27	13.14	104.08	2	76	6.1
Big_Osage	MO	Remnant	37.74	-94.33	13.38	115.81	2	28	5.7
Big_Osage	MO	Remnant	37.74	-94.33	13.38	115.81	2	28	5.7
Little_Osage	MO	Remnant	37.77	-94.34	13.39	116.19	2	28	5.7
Little_Osage	MO	Remnant	37.77	-94.34	13.39	116.19	2	28	5.7
WahKonTah	MO	Remnant	37.92	-94.01	13.28	117.53	8	78	5.9
WahKonTah	MO	Remnant	37.92	-94.01	13.28	117.53	8	78	5.9
Taberville	MO	Remnant	38.06	-93.97	13.12	112.09	8	78	5.9
Taberville	MO	Remnant	38.06	-93.97	13.12	112.09	8	78	5.9
Morris	MO	Remnant	40.38	-92.94	10.58	101.80	3	74	6.3
Morris	MO	Remnant	40.38	-92.94	10.58	101.80	3	74	6.3
IL_Rte54	IL	Disturbed	40.40	-88.46	10.60	97.10	19	223	6.1
IL_Rte54	IL	Disturbed	40.40	-88.46	10.60	97.10	19	223	6.1
IL_Rte54	IL	Disturbed	40.40	-88.46	10.60	97.10	19	223	6.1
Paxton_East	IL	Disturbed	40.46	-88.06	10.48	96.87	25	230	7.3
Paxton_East	IL	Disturbed	40.46	-88.06	10.48	96.87	25	230	7.3
Paxton_East	IL	Disturbed	40.46	-88.06	10.48	96.87	25	230	7.3
Stillwater	OK	Remnant	36.07	-97.19	15.88	92.51	NA	NA	NA
Stillwater	OK	Remnant	36.07	-97.19	15.88	92.51	NA	NA	NA
Stillwater	OK	Remnant	36.07	-97.19	15.88	92.51	NA	NA	NA
Stillwater	OK	Remnant	36.07	-97.19	15.88	92.51	NA	NA	NA
Stillwater	OK	Disturbed	36.06	-97.24	15.90	92.20	NA	NA	NA
Stillwater	OK	Disturbed	36.06	-97.24	15.90	92.20	NA	NA	NA

Table S2. PSR richness GLM results for BLAST oomycetes.

These tests are univariate tests including either the interaction of the predictor variable and disturbance (if across all sites), or only the predictor variable in determining PSR richness. The all sample test includes a random effect of disturbance nested within site; tests of remnant or disturbed samples include a random effect of plot. The model distribution is poisson. All significant predictors are in bold.

TABLE S2

Subset of samples	Predictor variables	Estimate	*p* value
All samples	Disturbance	-0.048	0.647
	Disturbance × Mean Annual Precipitation	0.094	0.321
	Disturbance × Mean Annual Temperature	-0.154	0.165
Remnant samples only	Mean Annual Precipitation	0.163	**0.016**
	Mean Annual Temperature	-0.058	0.421
	Precipitation × Temperature	-0.100	0.357
Disturbed samples only	Mean Annual Precipitation	0.072	0.345
	Mean Annual Temperature	0.096	0.236
	Precipitation × Temperature	-0.018	0.893

Table S3. PERMANOVA results for BLAST oomycetes.

Separate tests were run for all, and again within remnant and disturbed sites; a second set was run for remnant and disturbed sites to test for the interaction between temperature and precipitation. We stratified the PERMANOVA by each combination of disturbance and site to account for random effects due to spatial proximity of paired disturbed and remnant plots within any one site. *P < 0.05, **P < 0.01, ***P < 0.001.

TABLE S3			
Subset of samples	Predictor variables	R^2 value	*p* value
All samples	Disturbance	0.06182	***
	Mean Annual Precipitation	0.09192	***
	Mean Annual Temperature	0.04450	**
	Bray 2 Phosphorus	0.05432	***
	Potassium	0.03397	**
	Calcium	0.02885	*
	Soil pH	0.01168	
	Disturbance × Mean Annual Precipitation	0.03820	**
	Disturbance × Mean Annual Temperature	0.01787	
	Disturbance × Bray 2 Phosphorus	0.02282	
	Disturbance × Potassium	0.02194	
	Disturbance × Calcium	0.02223	
	Disturbance × Soil pH	0.01982	
	Sequence number	0.06689	***

Remnant samples only	Mean Annual Precipitation	0.03030	
	Mean Annual Temperature	0.05460	*
	Bray 2 Phosphorus	0.04809	
	Potassium	0.03817	
	Calcium	0.04965	*
	Soil pH	0.04305	
	Sequence number	0.06206	*
	Mean Annual Precipitation × Temperature	0.02373	
Disturbed samples only	Mean Annual Precipitation	0.04849	
	Mean Annual Temperature	0.04656	
	Bray 2 Phosphorus	0.04582	
	Potassium	0.04651	
	Calcium	0.04713	
	Soil pH	0.04587	
	Sequence number	0.03609	
	Mean Annual Precipitation × Temperature	0.04884	

Table S4. FUNGuild results.

The proportion of fungi that were identified with FUNGuild from all fungal OTUs, the proportion of fungi identified in remnant versus disturbed sites with FUNGuild, and the proportion of FUNGuild fungi identified as either pathotroph or saprotroph.

TABLE S4	
Proportion identified	
Proportion Identified FUNGuild	0.154
Proportion FUNGuild Identified in Remnant Samples	0.114
Proportion FUNGuild Identified in Disturbed Samples	0.136
Proportion FUNGuild Fungi Identified as Pathotroph	0.178
Proportion FUNGuild Fungi Identified as Saprotroph	0.704

Figure S1. Venn diagrams across land use for pathogen groups and saprotrophs.

Venn diagrams for oomycetes (a), fungal pathogens (b) and saprotrophic fungi (c). Venn diagrams showed considerable overlap with respect to fungal pathogen (46%), oomycete (BLAST; 38%) and saprotrophic fungi (46%) OTUs. These diagrams show that there are more unique OTUs found in remnant sites than in disturbed sites. Blue indicates remnant, while red indicates disturbed sites.

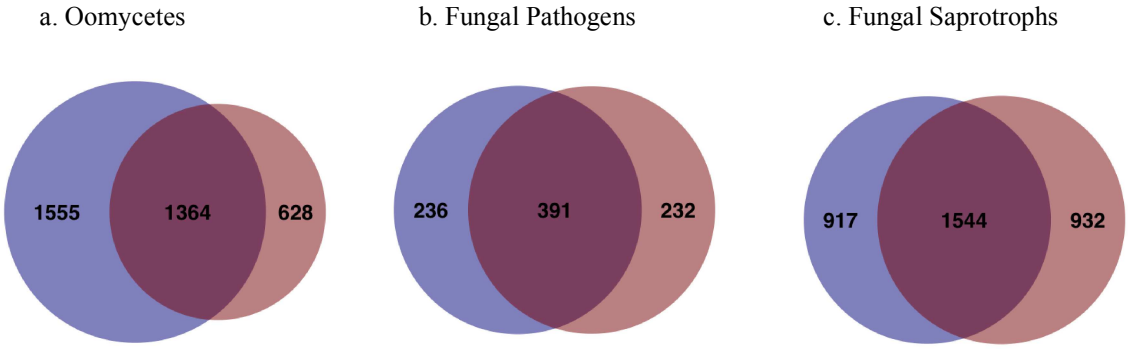


Figure S2. Species saturation curves.

Saturation Curves for phylogenetic oomycetes (a), BLAST oomycetes (b), fungal pathogens (c), and saprotrophic fungi (d). The bands represent a 95% confidence interval.

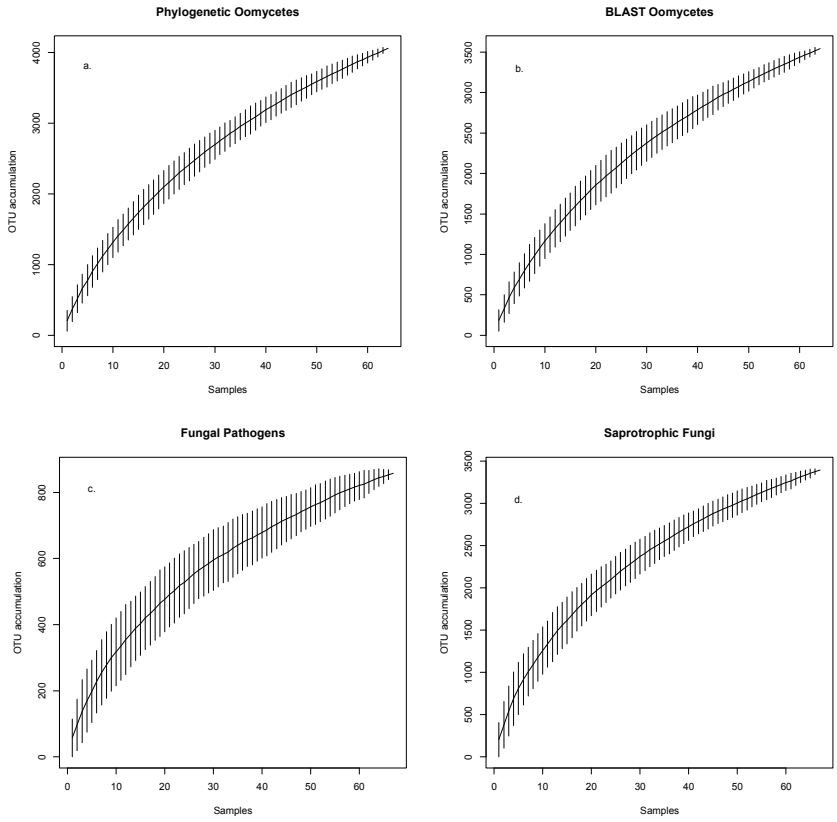
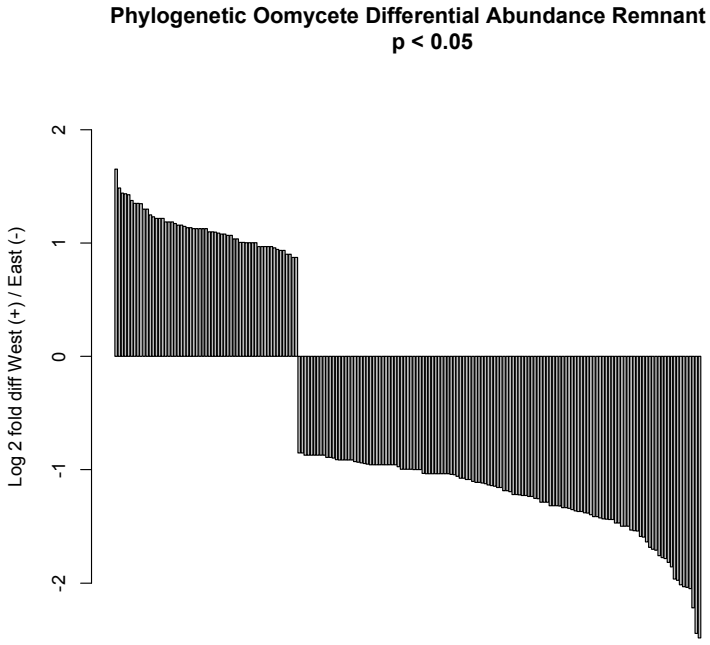


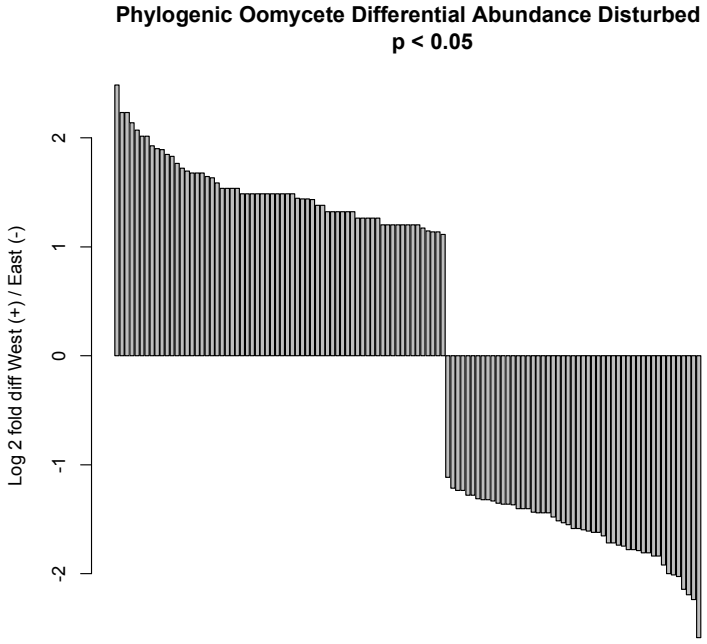
Figure S3. Differential OTU abundance between Western and Eastern grasslands.

Differential OTU abundance between Western and Eastern grasslands phylogenetic oomycetes (a), BLAST oomycetes (b), fungal pathogens (c) and saprotrophic fungi (d) in remnant (i) and disturbed (ii) grasslands. Overall, greater turnover is seen in remnant versus disturbed grasslands in all groups. Only significant OTUs are shown; each bar represents one OTU.

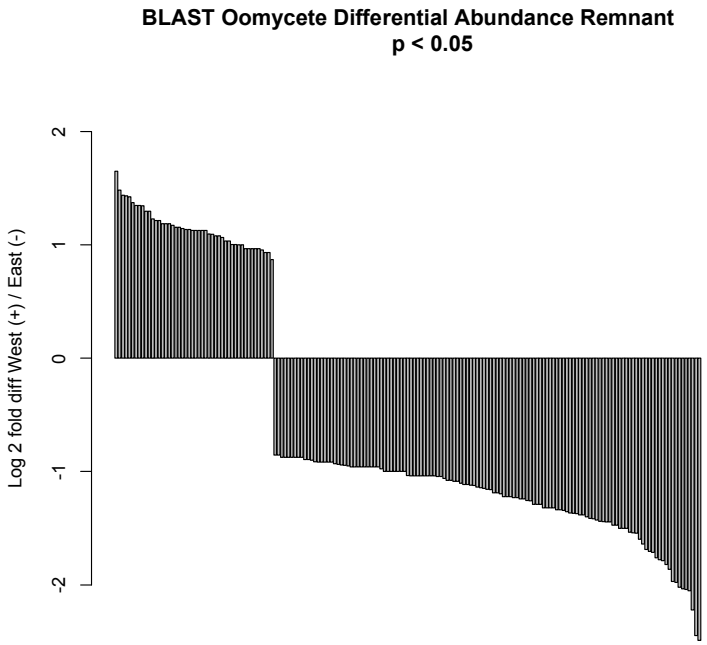
a. i.



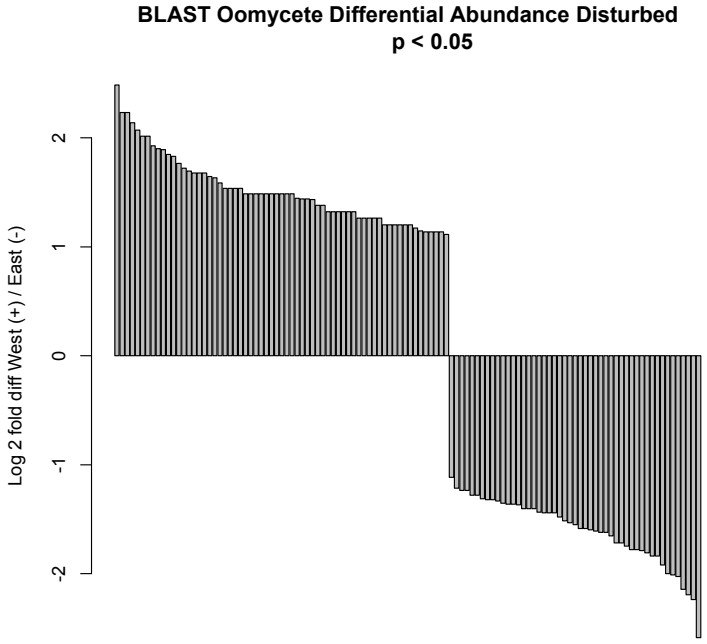
a ii.


Phylogenic Oomycete Differential Abundance Disturbed
p < 0.05
Log 2 fold diff West (+) / East (-)
2
1
0
-1
-2

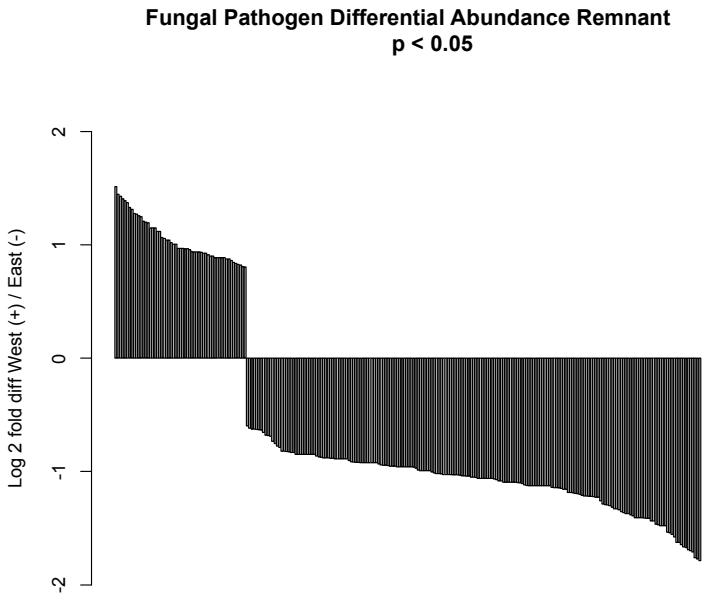
b. i.


BLAST Oomycete Differential Abundance Remnant
p < 0.05
Log 2 fold diff West (+) / East (-)
2
1
0
-1
-2

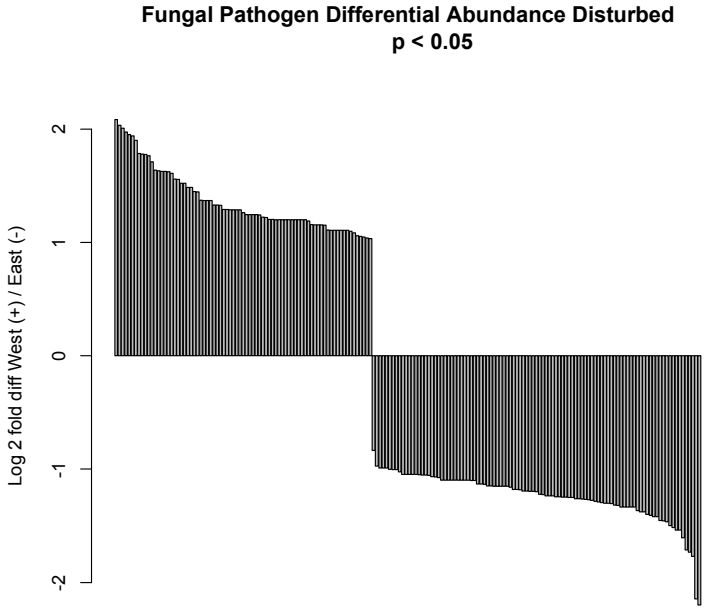
b. ii.


BLAST Oomycete Differential Abundance Disturbed
p < 0.05
Log 2 fold diff West (+) / East (-)
2
1
0
-1
-2

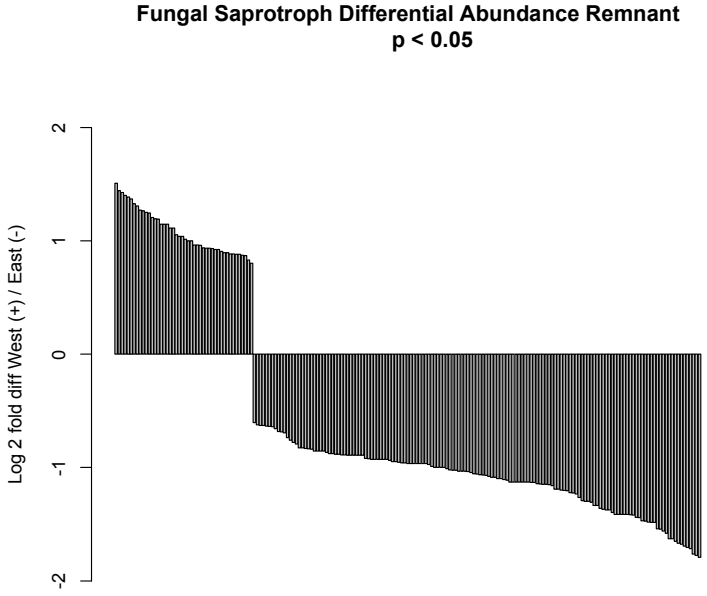
c. i.


Fungal Pathogen Differential Abundance Remnant
p < 0.05
Log 2 fold diff West (+) / East (-)
2
1
0
-1
-2

c. ii.


Fungal Pathogen Differential Abundance Disturbed
p < 0.05
Log 2 fold diff West (+) / East (-)
2
1
0
-1
-2

d. i


Fungal Saprotroph Differential Abundance Remnant
p < 0.05
Log 2 fold diff West (+) / East (-)
2
1
0
-1
-2

d. ii.

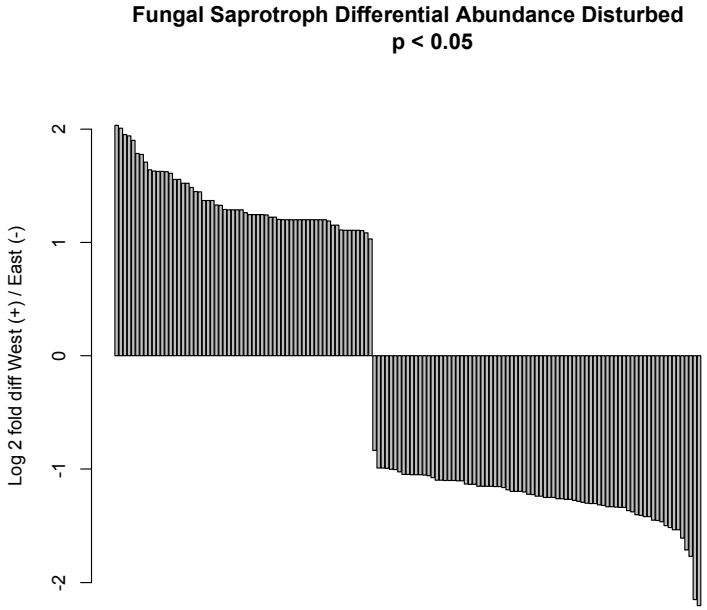
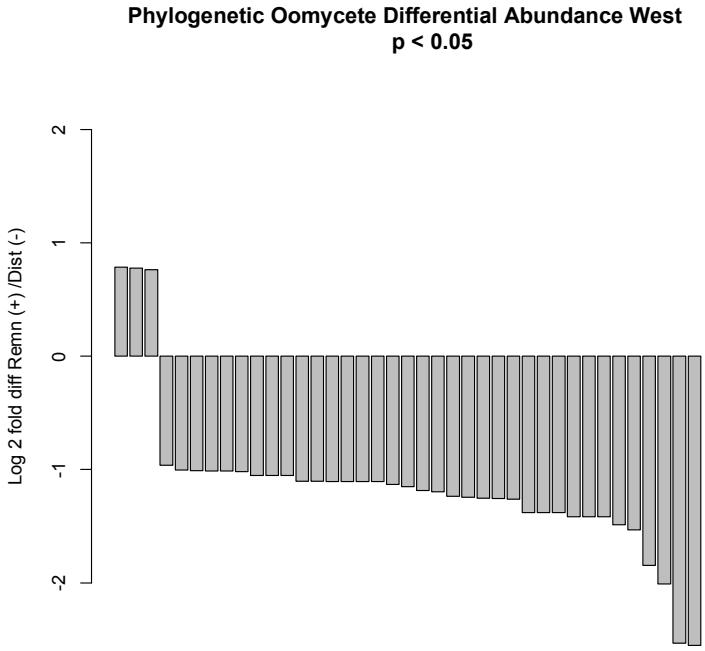
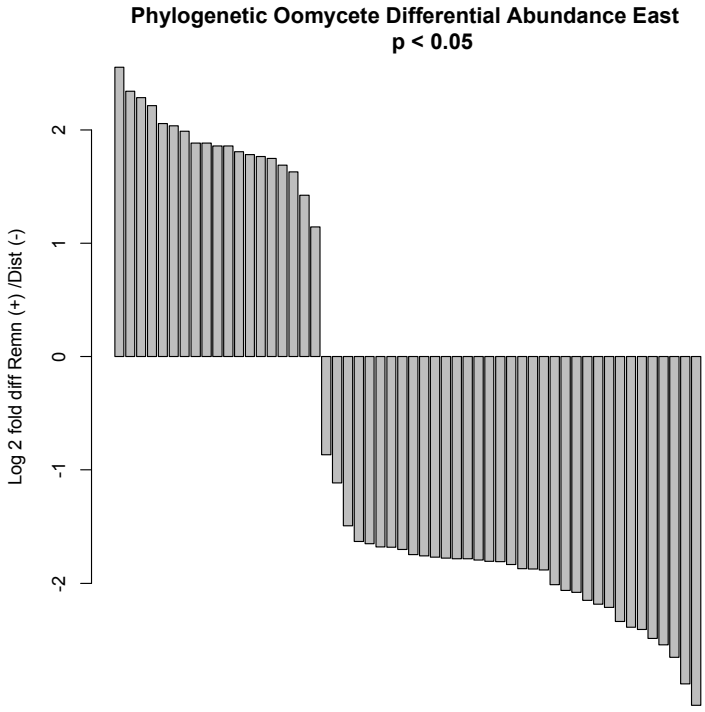


Figure S4. Differential OTU abundance between remnant and disturbed grasslands. Differential OTU abundance between remnant and disturbed grasslands for fungal pathogens (a), phylogenetic oomycetes (a), BLAST oomycetes (b), fungal pathogens (c) and fungal saprotrophs (d) in Western (i) and Eastern (ii) grasslands. Only significant OTUs shown; each bar represents an OTU.

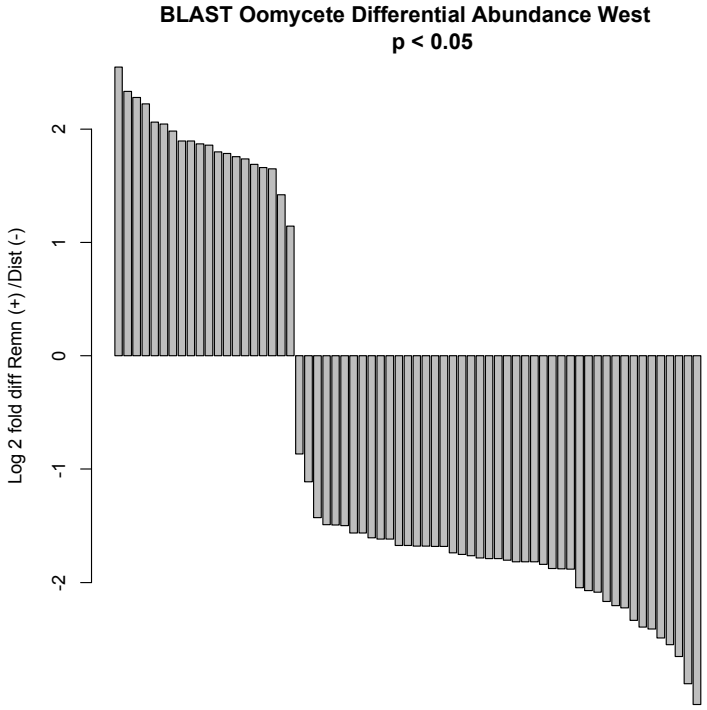
a. i.


Phylogenetic Oomycete Differential Abundance West
p < 0.05
Log 2 fold diff Remn (+) /Dist (-)
2
1
0
-1
-2

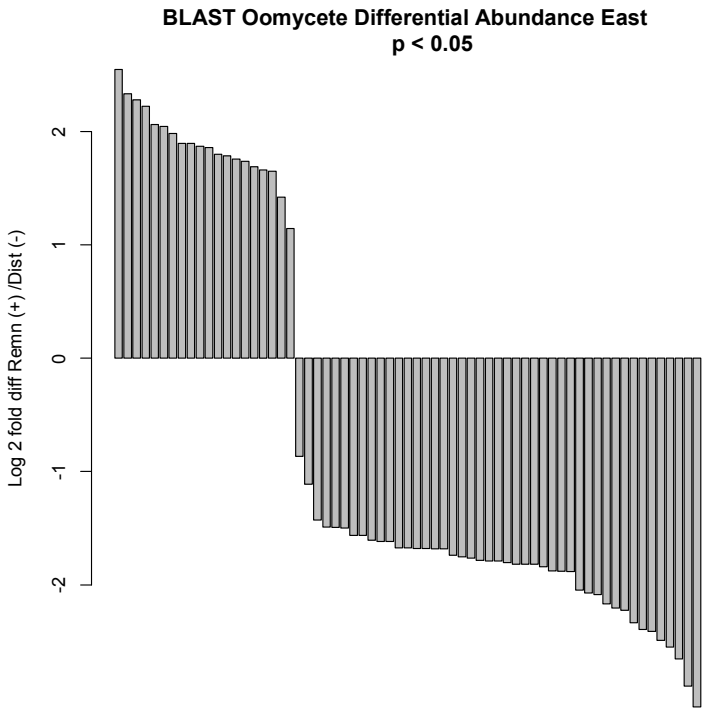
a. ii.


Phylogenetic Oomycete Differential Abundance East
p < 0.05
Log 2 fold diff Remn (+) /Dist (-)
2
1
0
-1
-2

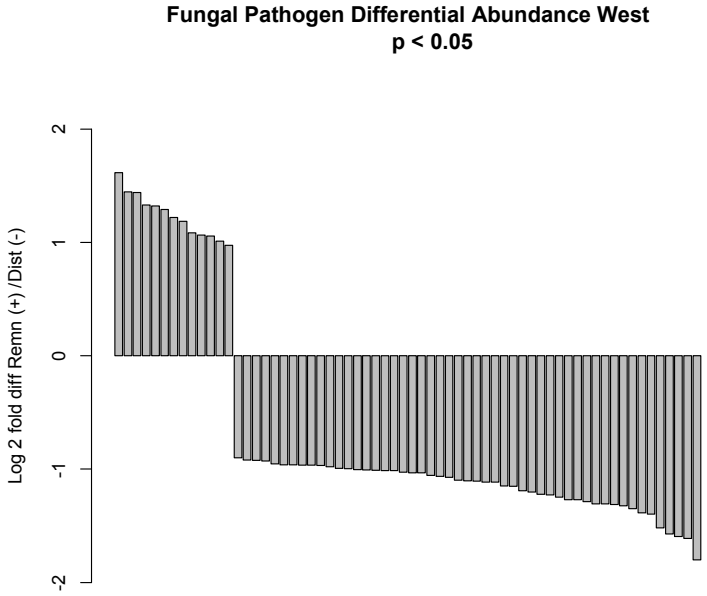
b. i.


BLAST Oomycete Differential Abundance West
p < 0.05
Log 2 fold diff Remn (+) /Dist (-)
2
1
0
-1
-2

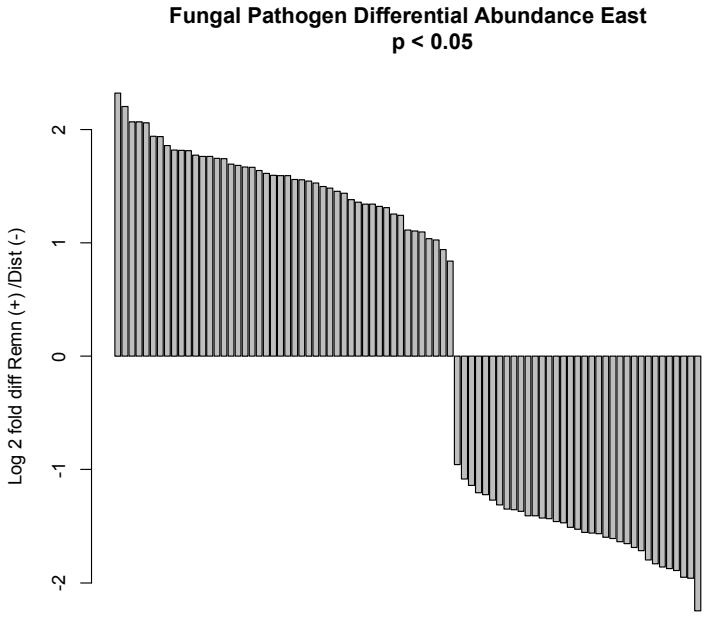
b. ii.


BLAST Oomycete Differential Abundance East
p < 0.05
Log 2 fold diff Remn (+) /Dist (-)
2
1
0
-1
-2

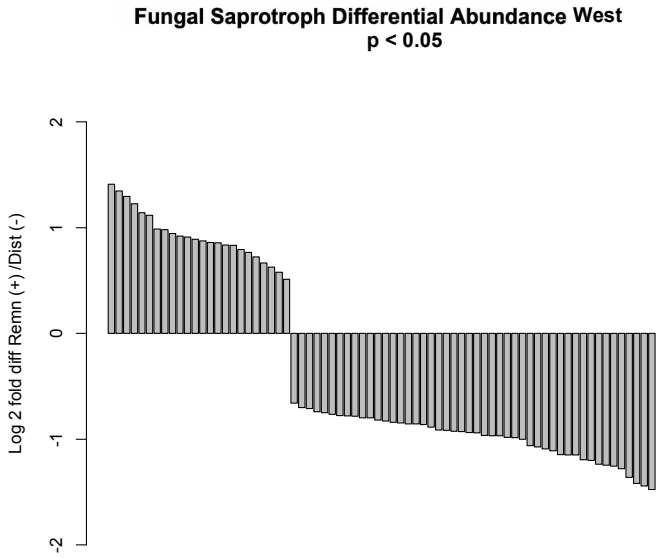
c.i.


Fungal Pathogen Differential Abundance West
p < 0.05
Log 2 fold diff Remn (+) /Dist (-)
2
1
0
-1
-2

c. ii.


Fungal Pathogen Differential Abundance East
p < 0.05
Log 2 fold diff Remn (+) /Dist (-)
2
1
0
-1
-2

d. i.


Fungal Saprotroph Differential Abundance West
p < 0.05
Log 2 fold diff Remn (+) /Dist (-)
2
1
0
-1
-2

d. ii.

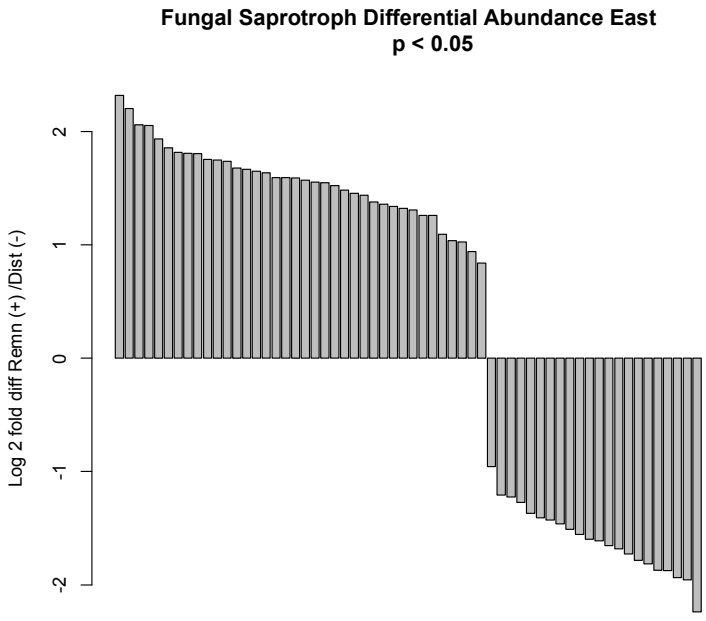
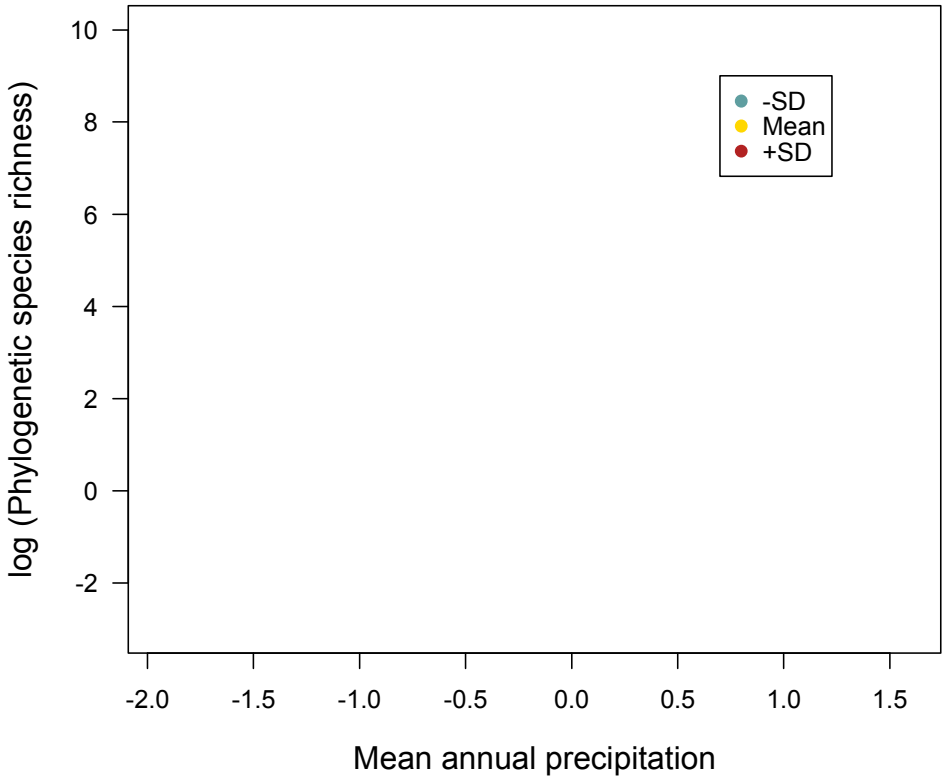

Fungal Saprotroph Differential Abundance East
p < 0.05
Log 2 fold diff Remn (+) /Dist (-)
2
1
0
-1
-2

Figure S5. Precipitation by temperature interaction for saprotrophic fungi in remnant grasslands.

The interaction between precipitation and temperature for saprotrophic fungi in remnant grasslands ($p = 0.02$), based on PSR (phylogenetic species richness) GLM (Table S**2a**). With higher temperature (+ SD), precipitation predicts an increase in PSR; with lower temperature (- SD), precipitation predicts a decrease in PSR. Points represent the raw data; the trendlines are the predicted probability from the GLM.



Appendix F: Supplementary Information for Evidence for the Evolution of Mycorrhizal Response in Post-Agricultural Grasslands

Table S1. Mycorrhizal inocula used in the study.

Eleven cultures were used for native prairie AMF (all from native KS tallgrass prairies); ten cultures were used for non-native (INVAM) AMF (all not from native KS tallgrass prairies).

Native AMF		**Non-native AMF**	
Name	Likely species	Name	Species
Sanders Mound B	*Ambispora sp.*	NC119-24	*Gigaspora gigantea*
Sanders Mound F	*Rhizophagus sp.*	IN212	*Racocetra fulgida*
Trap 18C	*Rhizophagus sp.*	BR208A	*Cetraspora pellucida*
Scut 305-3 Insta B	*Cetraspora pellucida*	FL327C	*Archaeospora trappei*
Trap 18G	*Cetraspora pellucida*	IA122	*Paraglomus occultum*
Sanders Mound 0	undetermined	IN218	*Ambispora leptoticha*
508-2	*Gigaspora gigantea*	MT106	*Ambispora gerdemannii*
514-1	*Gigaspora gigantea*	BR851	*Rhizophagus clarus*
507-2	*Funniliformis mosseae*	FL328	*Septoglomus constrictum*
513-2	*Glomus mortonii*	AZ242	*Funneliformus mosseae*
Rock GZ 187 E. inf	*Claroidoglomus claroideum*		

Table S2. Greenhouse experimental design.

Greenhouse experimental design to test mycorrhizal response between native and post-agricultural plant population types to native and non-native mycorrhizal inocula. Numbers represent number of replicates per species per site.

Site	***Apocynum cannabinum***	***Solidago Canadensis***	***Vernonia faciculata***	***Asclepias sp.* (Milkweed)**
Native Plant Populations				
Rockefeller	5	5	5	5

Dogleg	5	5	5	5
Kill Creek	5	5	5	5
Prairie Nature	0	5	0	0
Post-agricultural Plant Populations				
Welda	5	5	5	5
Plot 4010	5	5	5	0
Land Institute	5	5	5	5
Rock Chalk	5	5	5	0

Table S3. Mycorrhizal assessment results.

Mycorrhizal identification in a subset of sapmles using the gridline intersect method. The occurrence of AMF in any structure type is noted (out of 25 root intersects). Next, the occurrence of different structures is noted (out of the same 25 root intersects).

	AMF		structure type				
Sample	NO	YES	hyphae	coil	spore	arbuscule	other
0IW51	24	1	0	0	0	0	0
0IW55	25	0	0	0	0	0	0
0IW71	25	0	0	0	0	0	0
1IW33	1	24	24	4	10	10	0
1IW52	4	21	21	2	4	1	1
1IW15	2	23	23	1	7	2	0
2IW86	16	9	9	2	8	5	0
2IW35	8	17	17	2	4	6	0
2IW46	9	16	16	2	0	3	0
1IW13	24	1	1	0	0	0	0
0SL39	25	0	0	0	0	0	0
0SL14	24	1	1	0	1	0	0
0SL17	25	0	0	0	0	0	0
0DB74	25	0	0	0	0	0	0
0DB86	25	0	0	0	0	0	0
0DB49	25	0	0	0	0	0	0
0MW52	25	0	0	0	0	0	0
0MW86	24	1	0	1	0	0	0
0MW15	25	0	0	0	0	0	0
1DB45	25	0	0	0	0	0	0
1DB34	8	17	15	0	17	0	0
1DB33	0	25	25	1	24	8	0
2DB46	13	12	12	1	3	4	0

2DB11	21	4	4	0	0	0	0
1MW48	6	19	14	0	8	2	0
1MW85	24	1	1	0	0	1	0
1MW81	12	13	13	0	8	4	0
2MW23	21	4	4	0	0	2	0
2MW12	13	12	9	1	2	6	0
2MW81	12	13	13	1	0	10	0
1SL10	17	8	8	0	0	2	0
1SL29	18	7	7	0	6	0	0
1SL37	17	8	8	0	0	1	0
2SL48	10	15	15	4	0	3	0
2SL25	9	16	15	0	12	2	0
2SL49	13	12	11	0	0	9	0

Figure S1. Mycorrhizal colonization across study treatments.

GLM shows that mycorrhizal treatments showed significantly higher colonization than sterile treatments ($p < 0.001$).

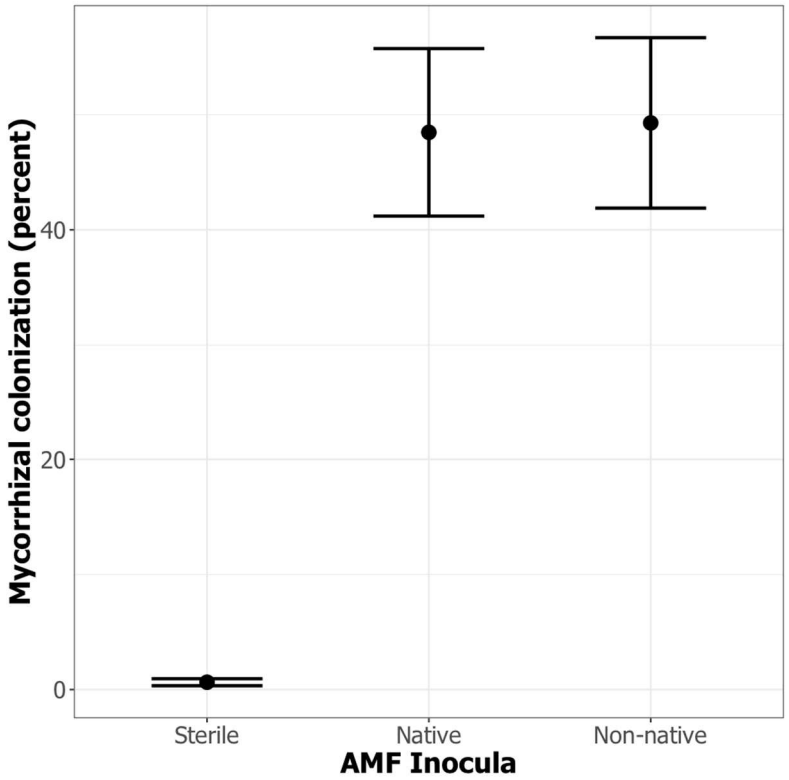


Figure S2. Root length differences across study species.

Linear model shows that there is a significant difference in root length between species ($p < 0.001$). Between species, Dogbane and Solidago have significantly greater root length than Ironweed (all $p < 0.0001$).

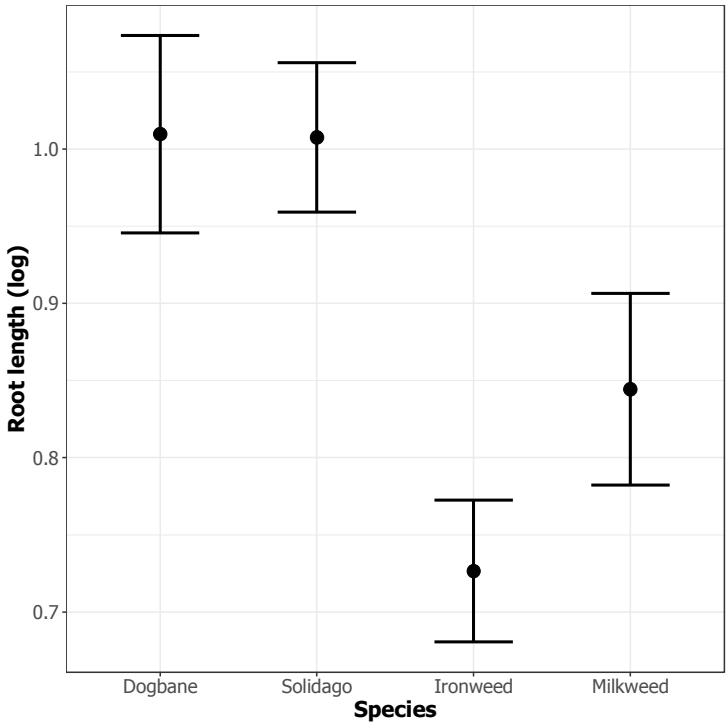
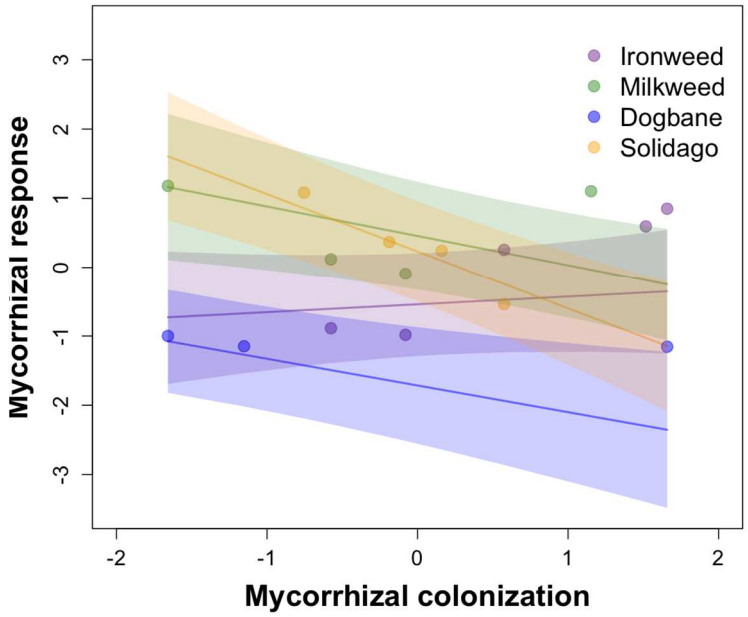


Figure S3. Species differences in relationship between mycorrhizal colonization and response.

The relationship between logit colonization proportion and mycorrhizal growth response depends on species, with Ironweed showing a more positive relationship ($p = 0.03$).



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