ECOLOGICAL ROLES OF FUNGAL ENDOPHYTES

by

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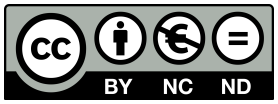

DISSERTATION ABSTRACT

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Title: Ecological Roles of Fungal Endophytes

Endophytic fungi live within tissues of plant hosts without causing symptoms of disease. These fungi are broadly split into the taxonomically and ecologically cohesive Clavicipitaceous endophytes, which infect grasses, and the taxonomically diverse non-Clavicipitaceous endophytes, which are found in nearly all plants and have diverse ecological strategies. My dissertation has two sections: Section A investigates the intersection of Clavicipitaceous endophyte ecology with other ecological theory, including invasion ecology (Chapter II) and community ecology and climate change (Chapter III); Section B investigates the ecology of one group of non-Clavicipitaceous endophytes, the Xylariaceae, using a culture-based study in Ecuador (Chapter IV) and a next-generation sequencing based endophyte survey in Taiwan (Chapter V). Section B is centered on testing the Foraging Ascomycete (FA) hypothesis—the idea that some decomposer fungi may adapt an endophytic lifestyle to escape limitations in primary substrate in both time and space.

In Chapter II, I utilized a host-specific *Epichloë* endophyte present ubiquitously in the European native range of the Pacific Northwest (PNW) invasive grass *Brachypodium sylvaticum* to test theories of invasion. In Chapter III, I examined the grass *Agrostis capillaris* in the context of a climate manipulation experiment in prairies in the PNW to

elucidate patterns of interaction between multiple symbionts (*Epichloë* endophytes, dark septate root endophytes, and arbuscular mycorrhizal fungi) within single hosts across climatic variation.

In Chapter IV, I began to test the FA hypothesis by examining spatial relationships of *Xylaria* endophytic fungi in the forest canopy with *Xylaria* decomposer fungi on the forest floor in a remote Ecuadorian cloud forest. In Chapter V, I build on the results from the previous study, using a novel technique to examine spatial ecology of the Xylariaceae, pairing traditional mycological collection with the preparation of a next-generation sequencing metabarcode library of endophytes over a much greater area.

This dissertation includes previously published and unpublished coauthored material.

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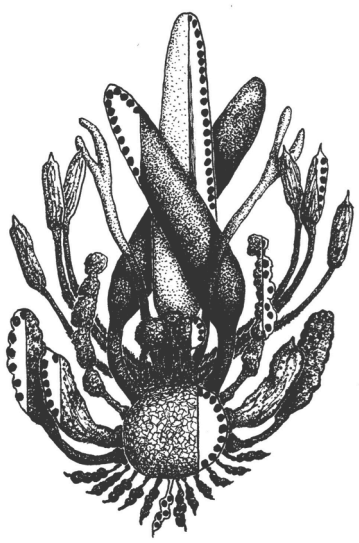

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CHAPTER I

INTRODUCTION

Since the concept of symbiosis—the 'living together of dissimilar organisms'—was first put forward by De Bary (1879), research into the ways that organisms can live together has revealed an incredible diversity of symbiotic lifestyles (Lewis 1985). Plants, in particular, seem to be reliant on symbioses; nearly all terrestrial plants host mycorrhizae and/or endophytes (Petrini 1986, Rodriguez et al. 2009, Porras-Alfaro and Bayman 2011). The effects of these fungal partners on their hosts' ecology, fitness, and evolutionary history cannot be understated (Brundrett 2006). It is probable that the association between plants and fungi dates back to the colonization of land, > 400 million years ago. Early fossils of terrestrial plants indicate associations with endophytic (Krings et al. 2007) and mycorrhizal fungi (Redecker et al. 2000), which may have had a role in setting the course of evolution for life on land. Chapter III, Box 1 outlines the use of terminology in this dissertation; though it is intended to clarify usage for that specific chapter, the usage is consistent throughout.

Fungal endophytes are defined functionally; they are fungi found within living, healthy plant tissues that make their living by not damaging their host (Clay 1990, Rudgers et al. 2009). Since their discovery, they have been found to be both ubiquitous and incredibly diverse in plants of all ecosystems (Arnold and Lutzoni 2007, Porras-Alfaro and Bayman 2011). Endophytes are commonly split into two broad groupings, *Clavicipitaceous endophytes*, which infect the foliar tissues of many cool-season grasses

(Poaceae), and *non-Clavicipitaceous endophytes*, which are incredibly diverse taxonomically and ecologically (Rodriguez et al. 2009).

Clavicipitaceous endophytes are fungi in the family Clavicipitaceae, and most often the genus *Epichloë* (anamorphic synonym: *Neotyphodium*), that are systemic foliar endophytes of grasses, colonizing the aerial tissues of their hosts. Although many *Epichloë* species may be seedborne, and thus tightly linked to their host's fitness (Schardl 1996), horizontal (contagious) transmission is possible via both sexual (Brem and Leuchtmann 1999) and asexual (Tadych et al. 2007) means. They are often considered strong mutualists because they produce fungal alkaloids, which can reduce herbivory on the host plant (Schardl 1996, Bush et al. 1997, Scott 2001). Fungi in this genus have also been linked experimentally with drought tolerance and increased competitive abilities (Malinowski et al. 2005). A growing body of work, however, demonstrates that they can be pathogenic in certain circumstances (Brem and Leuchtmann 2002). The metabolic cost to the plant of hosting an *Epichloë* endophyte must be balanced by the fitness increase that the endophyte provides.

Non-Clavicipitaceous endophytes are incredibly diverse, both taxonomically and ecologically. They are mostly in the fungal phylum Ascomycota—though Basidiomycota and Zygomycota endophytes have been observed as well (Arnold and Lutzoni 2007)—and represent many diverse and often poorly understood ecological roles. They have been found to be nearly ubiquitous in plants of all ecosystems and show staggering diversity, particularly in the tropics (Arnold and Lutzoni 2007, Porras-Alfaro and Bayman 2011).

While there has been an increase in the study of fungal endophytes recently (Rodriguez et al. 2009, Porras-Alfaro and Bayman 2011, Wani et al. 2015), a large

portion of this research has focused on descriptions of diversity (Ahlholm et al. 2002, Arnold and Lutzoni 2007, Arnold et al. 2007, Murali et al. 2007, Davis and Shaw 2008, Loro et al. 2012, Zimmerman and Vitousek 2012, Giauque and Hawkes 2013, Scholtysik et al. 2013, Vincent et al. 2015), the interactions between endophytic species and plant of agricultural interest (Douanla-Meli et al. 2013, Impullitti and Malvick 2013, Thom et al. 2013), and the potential for exploitation of the secondary metabolites of endophytes for drug development (Strobel and Daisy 2003, Bernardi-Wenzel et al. 2010, Carvalho et al. 2012, Kaul et al. 2012, Chen et al. 2013, Heinig et al. 2013, Hammerschmidt et al. 2015). Much attention has been given to the advantages that plants may gain from hosting endophytic fungi (Brem and Leuchtmann 2001, Gange et al. 2012, Saikkonen et al. 2012, Estrada et al. 2015), but relatively little attention has been given to the advantages that fungi may gain from adopting an endophytic lifestyle (but see: Carroll 1988, Saikkonen et al. 2004, Porras-Alfaro and Bayman 2011). While some endophytes have been observed to be latent pathogens or saprotrophs (Chapela and Boddy 1988, Osono 2006, Promputtha et al. 2007, 2010), the ecological roles of most fungal endophytes remain unclear (Lodge 1997, Porras-Alfaro and Bayman 2011).

My dissertation research can be broadly broken down into two sections, each containing two chapters each. The first section (Section A) deals with clavicipitaceous endophytes of grasses, and explores the intersection of host/symbiont interactions with other ecological theory, such as theories of invasion (Chapter II) and community ecology and climate change (Chapter III). The second section (Section B) deals with non-clavicipitaceous endophytes, and focuses on testing one ecological explanation for foliar

endophytism from a myco-centric viewpoint. The two chapters in this section both use fungi in the family Xylariaceae to test the Foraging Ascomycete (FA) hypothesis.

SECTION A: GRASS/*EPICHLOË*

This section includes two research-based chapters, both concerning aspects of the ecological roles of Clavicipitaceous endophytes in grasses in Oregon. Despite the well-developed and large body of research on this taxonomically restricted and ecologically cohesive group of endophytes (e.g. (Clay and Schardl 2002), there are intersectional issues where more research is needed. These two chapters attempt to address the intersection of endophyte ecology with invasion ecology (Chapter II), and with community ecology and climate change (Chapter III).

Chapter II.—For my first data chapter, I investigated the role that a host-specific Clavicipitaceous endophytes plays in the invasion ecology of an aggressive invasive grass here in Oregon. This work was co-authored with W. Blaser, F. Campos-Cerda, A. F. Heneghan, G. C. Carroll, and B. A. Roy.

The Enemy Release Hypothesis (ERH) postulates that one major factor facilitating invasion is the relative lack of specialized enemies in the invaded range, allowing for faster growth and spread (Keane and Crawley 2002). An alternative, the Evolution of Increased Competitive Ability Hypothesis (EICA), assumes that the success of invasive species is evolutionary in nature, driven by the change in selective pressures

of the new environment. Under the EICA framework, enemy release provides selective pressure to reallocate resources from defense to growth and reproduction (Blossey and Notzold 1995), though there are many complicating factors (Colautti et al. 2004). One complication is the distinct difference in defensive strategies used to protect against generalist versus specialist enemies (van der Meijden 1996, Müller-Schärer et al. 2004). Specialist enemies are theorized to be more important to plant invasions (Keane and Crawley 2002) because of the disproportionate effect they have on controlling populations in their native ranges, and the relative metabolic costliness of specific defenses. Thus, evolved increased competitive abilities may be due to reallocation of resources from specialist defenses to generalist defenses (Joshi and Vrieling 2005).

I used *Brachypodium sylvaticum* (Huds.) P. Beauv. to test the EICA hypothesis, with the ERH (phenotypic plasticity in the face of specialist enemy loss) as an explicit alternative hypothesis. This was possible because *Brachypodium sylvaticum* in its native Eurasian range appears to be almost ubiquitously infected with the host-specific fungal endophyte, *Epichloë sylvatica* Leuchtm & Schardl (Eckblad and Torkelsen 1989, Raynal 1994, Väre and Itämies 1995, Bucheli and Leuchtmann 1996, Enomoto et al. 1998, Zabalgogeazcoa et al. 2000, Roy et al. 2011, Leuchtmann, pers. com.), which may act as a pathogen rather than a mutualist (Brem and Leuchtmann 2002), despite common assumptions about *Epichloë* endophytes of grasses (Schardl 1996).

With my co-authors help, I documented the near total absence of *E. sylvatica* infection in the invaded range, and then we compared germination and growth rates of seedlings from the native and invaded ranges to explicitly test the EICA hypothesis. We utilized a greenhouse experiment using seeds collected during the same season in both

ranges, clearing the seeds of *Epichloë* infection and then selectively re-inoculating half of each group.

We found that, in its native range, *Epichloë sylvatica* seems to control *B. sylvaticum* in some ways (reducing growth rates and competitive abilities), but those detriments seem to be off-set by increased germination rates and potential protection from seed herbivores and pathogens. In the invaded range, the grass is released from control on growth and competitive abilities imposed by the fungus, likely because it is not necessary to harbor such a costly endophyte to maintain high seed viabilities in the invaded range. Whether that is through the additional release of control by a seed-damaging organism, or through novel genetic recombination that allows for high germination rates in the absence of the fungus is still to be determined.

Chapter III.—For my second data chapter, I also utilized the grass/Clavicipitaceous endophyte association, in addition to mycorrhizal associations and dark septate root endophyte (DSE) associations. All symbiotic fungi exist on a functional continuum from mutualist to pathogen (Carroll 1988, Porras-Alfaro and Bayman 2011). In theory, the position upon this continuum will depend upon the environmental context, in addition to the particular host/symbiont pairing (Carroll 1988, Johnson et al. 1997, Saikkonen et al. 1998, 2006, Faeth and Sullivan 2003). I was interested in the relationship between multiple symbionts, their host, and the climatic conditions that the assemblage was embedded within. This work was co-authored with B. A. Roy, L. Pfeifer-Meister, B. R. Johnson, and S. D. Bridgham.

To this end, I focused on three groups of symbionts. Fungi in the genus *Epichloë*; arbuscular mycorrhizal fungi (AMF), which colonize the roots of the vast majority of terrestrial plants (~80% of plant families; Schüβler et al. 2001) and provide access to inorganic soil nutrients in exchange for photosynthate (Harley and Smith 1985); and dark septate root endophytes (DSE), which are a poorly studied, phylogenetically diverse group of root-inhabiting fungal endophytes (Jumpponen 2001, Porras-Alfaro and Bayman 2011). Though previously assumed to be pathogens (Jumpponen and Trappe 1998), there is mounting evidence that DSE may function as pseudo-mycorrhizae in some contexts (Upson et al. 2009, Alberton et al. 2010). All three of these groups of symbionts may exist across the full spectrum of the mutualist/pathogen continuum.

To examine these multi-symbiont interactions, we quantified percent root length colonized (PRLC) by both AMF and DSE, and tested for the presence of systemic foliar *Epichloë* endophytes within a single host species (*Agrostis capillaris* L.) across a broad climatic gradient within the context of a manipulative climate change experiment (Pfeifer-Meister et al. 2013). We examined how these fungal symbionts interacted to affect host fitness across a broad range of environmental conditions.

We found a correlation between DSE and AMF PRLC across climatic conditions; we also found a fitness cost to increasing DSE colonization, which was negated by presence of *Epichloë* endophytes. These results suggest that selective pressure on the host is likely to favor host/symbiont relationships that structure the community of symbionts in the most beneficial way possible for the host, not necessarily favoring the individual symbiont that is most beneficial to the host in isolation. These results highlight the need for a more integrative, systems approach to the study of host/symbiont consortia.

SECTION B: THE FORAGING ASCOMYCETE

The second major section of my dissertation research switches from the grass/Clavicipitaceous endophyte system to foliar endophytes of tropical trees. The ecology of Clavicipitaceous endophytes is well studied (for a review, see: Clay and Schardl 2002), but the ecology of tropical foliar endophytes, which may represent a significant portion of the undescribed fungal diversity in the world (Arnold et al. 2000), remains largely mysterious.

My mentor, Dr. George C. Carroll, had proposed an ecological strategy that might explain some cases of foliar endophytism several years ago, known as the Foraging Ascomycete (FA) hypothesis (Carroll 1999). This hypothesis proposes that some decomposer fungi may utilize endophytism as a way to bridge spatial and temporal gaps in preferred substrate, and disperse with falling, senescent leaves.

Historically, microorganisms were thought to be functionally unlimited in their ability to disperse over the planet (Baas-Becking 1934, Fenchel and Finlay 2004). Despite this, many recent studies of microbes have uncovered evidence for dispersal limitation, or the inability of a strain or species to access and successfully establish itself in otherwise suitable habitat (Roy 2001, Green and Bohannan 2006, Telford et al. 2006, Grubisha et al. 2007, Peay et al. 2010, Galante et al. 2011). Such dispersal limitation may function to constrain the geographic ranges of some species, or the range of gene flow within or between local populations of a given species; indeed, such constraints on gene flow between populations are theorized as a major driver of speciation over evolutionary time scales (Clobert et al. 2012). There is evidence that at least some decomposer fungi

are dispersal limited, even at local scales (Norros et al. 2012). Dispersal limitation may reduce fitness of an organism relative to competitors (Hurtt and Pacala 1995), suggesting that fungi may be under selection to increase dispersal at both local and regional scales.

Dispersal involves successful transport and successful establishment of propagules (Clobert et al. 2012, Hanson et al. 2012, Peay et al. 2012). In many cases, there is the potential for an endophytic life-style to improve upon both of these processes: senescent leaves fall farther than the vast majority of spores are predicted to travel unassisted (Roper et al. 2010, Galante et al. 2011), carrying with them mycelium, avoiding the uncertainty inherent in the germination phase of growth from spores. In evergreen forests, leaves generally fall asynchronously, which provides low propagule density over relatively long periods of time (in tropical cloud forests, leaves live 12 mo to >5 years; (Reich et al. 1991, Bruijnzeel and Veneklaas 1998), in contrast to spore dispersal from a fruiting body, which provides high propagule density over relatively short periods of time (<1 year; Rogers 1979, Whalley 1996). Leaves may enhance colonization rates, by creating a sheltered microclimate favorable to inoculation. Additionally, living leaves may provide refugia for endophytic fungi, where fungi can wait out difficult conditions at low metabolic cost, benefiting from the protection afforded by the leaf tissue (Stone 1987, Schulz and Boyle 2005). The idea of endophytism as a secondary life-history strategy for decomposer fungi to span (i.e., disperse across) scarcity of primary substrates and challenging environmental conditions in both time and space is known as the Foraging Ascomycete (FA) hypothesis (Carroll 1999).

Chapter IV.—My third data chapter was designed as an initial test of George's FA hypothesis. Thinking about the framework of the FA ecological strategy, we hypothesized that fungi that utilize such a switching of life-styles must be linked—between the two life phases—by dispersal between the endophyte and decomposer, and vice versa. If there is a dispersal linkage between life phases, there is also likely a linkage in space between occurrences of the two phases. This is the major hypothesis that we set out to test. This work was completed and written in close collaboration with Daniel Thomas, as well as A. Ludden, G. C. Carroll, and B. A. Roy.

My co-authors and I attempted to critically examine the FA hypothesis in a cloud forest ecosystem, using the genus *Xylaria* Hill ex Schrank (Xylariaceae, Ascomycota) as an example of typical endophytic fungi that may utilize a FA strategy. Members of this genus are important saprotrophs, found primarily on decomposing dead wood—and, rarely, on leaves and fruits—on the forest floor (Whalley 1996, Lodge 1997, Rogers 2000). *Xylaria* are visible during sexual sporulation, forming relatively large, macroscopic stromata, or "fruiting" structures (Bayman et al. 1998, Davis and Shaw 2008). *Xylaria* are common in virtually every study that has ever been done on endophytes, especially in tropical ecosystems (see Davis et al. (2003) for an extensive list). We focus here on a common endophyte genus to avoid the problem of being swamped in the overwhelming diversity of fungal endophytes in the tropics (Arnold et al. 2000, Arnold and Lutzoni 2007). These two life stages in *Xylaria,* leaf endophyte and wood decomposer, have been observed within single, tightly defined clades (Okane et al. 2008). Additionally, *Xylaria* grow readily in culture, making them ideally suited for study in laboratory conditions (Whalley 1996, Bayman et al. 1998).

We used a spatially explicit sampling scheme: we looked for spatial clustering not attributable to environmental gradients or biotic interactions, but indicative of dispersal linkage between life phases. Additionally, if *Xylaria* endophytes display a FA lifestyle, we would expect endophytic host generalism in the tropics, as host selectivity would interfere with dispersal in systems where most available hosts are present in low densities (May 1991). The FA hypothesis also leads to the hypothesis that endophytes will be released from environmental constraints relative to their corresponding decomposers. Using ITS rDNA barcode sequence comparisons (Gardes and Bruns 1993, Schoch et al. 2012), we matched decomposer *Xylaria* with endophytes in leaves from the canopy, and compared habitat characteristics of both. Lastly, we expect the FA strategy to be a specialized survival/dispersal mechanism utilized by a subset of fungi within the genus *Xylaria*. Given the diversity of the genus, we expect variation in species' niches to modulate the selective advantage of endophytism.

We found evidence of spatial linkage between endophyte and decomposer life phases in some taxa, as well as no evidence for host preference in the isolated *Xylaria.* We found some evidence that *Xylaria* fruiting bodies are sensitive to distance to a small stream running through our plot, but that their corresponding endophytes are not, indicating that endophytes may be released from environmental constraints relative to decomposers. Lastly, we found endophytism in a subset of collected species, and no endophytes that did not have an associated decomposer phase. Additionally, we directly observed the ability of endophytic *Xylaria* to colonize available woody substrates and initiate stromata formation, explicitly linking the two life phases. These results are

consistent with the predictions of the Foraging Ascomycete Hypothesis, and a successful first step into the investigation of this intriguing and ecologically important hypothesis.

Chapter V.—My fourth and final data chapter builds intuitively on Chapter IV, expanding the scope both spatially and taxonomically of the investigation of the FA hypothesis. This work was co-authored with D. Thomas, Y.-M. Ju, H. Soukup, G. C. Carroll, and B. A. Roy. For this experiment, we altered our endophyte detection technique from culture-based to culture-independent NGS metabarcode library preparation, and expanded our taxonomic focus from the genus *Xylaria* to the entire fungal family Xylariaceae. This work allowed for close collaboration with Dr. Yu-Ming Ju, the world expert on the genus *Xylaria*, and one of the top Xylariaceae taxonomists in the world. Dan and I went to Taiwan to work with Dr. Ju, and with his help were able to conduct this field experiment at the Fushan Forest Dynamics Plot, part of the Smithsonian Tropical Research Institute's Center for Tropical Forest Studies (CTFS) worldwide network of forest monitoring plots (Losos and Leigh 2004).

The entire family are well known to be common foliar endophytes in the tropics (Bayman et al. 1998, Arnold et al. 2000, Davis and Shaw 2008) without any apparent host preference (Murali et al. 2007, Davis and Shaw 2008, Okane et al. 2012, Ikeda et al. 2014), and there is evidence that the genus *Xylaria* may utilize a FA strategy (Chapter IV). The Xylariaceae are an ancient lineage of predominantly wood decay fungi, which likely diversified heavily coincident with the radiation of angiosperms, ca. 100 mya (Rogers 1979, 2000, Ju and Rogers 1996). The fruiting structures of most xylariaceous fungi are macroscopic perithecial stromata occurring on wood—or occasionally other

plant-derived substrates—and are readily identified to family in the field (Ju and Rogers 1999). Endophytic and saprotrophic examples of the same taxa within Xylariaceae have been observed by both molecular and culture-based techniques (Okane et al. 2008, Thomas et al. 2016).

To test the predictions of the FA hypothesis with the Xylariaceae, we again utilized a spatially explicit sampling scheme explicitly designed to test turnover effects (Rodrigues et al. 2013) in an evergreen subtropical forest in northeastern Taiwan. We coupled traditional specimen-based collection with Illumina next-generation sequencing (NGS) metabarcode microbial survey techniques (Taberlet et al. 2012, Blaalid et al. 2013, Meadow et al. 2013, Schmidt et al. 2013). This sensitive, culture-independent detection technique allowed us to accurately determine presence of fungi in the endophytic community based on sequences generated from specimens collected. Furthermore, the spatial relationships between fungal fruiting bodies and occurrence in the endophytic community could be examined across the site. Lastly, trends in diversity and turnover were examined with respect to environmental gradients across the site.

We found evidence for diverse spatial ecologies in the Xylariaceae, including evidence in support of a FA strategy in some taxa, particularly in the subfamily Xylarioideae. We found that xylariaceous endophyte communities are less sensitive to distance and environment than the community of stromata, and that xylariaceous endophytes are generally not host-specific, though some influence of host remains. We also believe that the pairing of traditional mycological collection with NGS metabarcode libraries is a novel approach with much potential for elucidating the spatial patterns of these intriguing organisms. Though they produce macroscopic fruiting structures, for

most of their lives they are microorganisms, growing and dispersing hidden beneath the threshold of observable size.

CONCLUSIONS

In my final chapter (Chapter VI), I summarize results from the four foregoing data chapters (II-V), and the conclusions drawn therefrom. I also discuss briefly findings beyond the scope of the experiments presented, and avenues of future investigation.

CHAPTER II

MIXED FITNESS EFFECTS OF GRASS ENDOPHYTES MODULATE IMPACT OF ENEMY RELEASE AND RAPID EVOLUTION IN AN INVASIVE GRASS.

From Vandegrift, R., Blaser, W., Campos-Cerda, F., Heneghan, A. F., Carroll, G. C., & Roy, B. A. 2015. Mixed fitness effects of grass endophytes modulate impact of enemy release and rapid evolution in an invasive grass. *Biological Invasions*, *17*(4): 1239–1251.

Contributions

R. Vandegrift designed and carried out the greenhouse experiment and much of the analysis and writing. W. Blaser did the initial growth and germination assay. F. Campos-Cerda did the second germination assay. A. Heneghan did the PCR screen and immunoblots. G. C. Carroll did the culture and some conceptual work. Finally, B. A. Roy did design, analysis, and the remainder of the writing. All authors screened grasses.

Introduction

The Enemy Release Hypothesis (ERH) postulates that one major factor facilitating invasion is the relative lack of specialized enemies in the invaded range, allowing for faster growth and spread (Keane and Crawley 2002). An alternative, the Evolution of Increased Competitive Ability Hypothesis (EICA), assumes that the success of invasive species is evolutionary in nature, driven by the change in selective pressures

of the new environment. Under the EICA framework, enemy release provides selective pressure to reallocate resources from defense to growth and reproduction (Blossey and Notzold 1995), though there are many complicating factors (Colautti et al. 2004). One complication is the distinct difference in defensive strategies used to protect against generalist versus specialist enemies (van der Meijden 1996; Müller-Schärer et al. 2004). Specialist enemies are theorized to be more important to plant invasions (Keane and Crawley 2002) because of the disproportionate effect they have on controlling populations in their native ranges, and the relative metabolic costliness of specific defenses. Thus, evolved increased competitive abilities may be due to reallocation of resources from specialist defenses to generalist defenses (Joshi and Vrieling 2005).

There has been much debate and intensive research surrounding the EICA hypothesis (reviewed in: Atwood and Meyerson 2011; Felker-Quinn et al. 2013), much of it inconclusive or contradictory (e.g., Willis et al. 2000; van Kleunen and Schmid 2003; Bossdorf et al. 2004; but see also Vilà et al. 2003; Meyer et al. 2005). Founder effects, novel hybridization opportunities, and strong abiotic environmental selective pressures can drive rapid evolutionary change in invasion. This complicates EICA research, and many studies have not tested competitive abilities and defense in the same organisms (see Bossdorf et al. 2005; Atwood and Meyerson 2011 and citations therein). It is impossible to make inferences about energetic tradeoff without a measure of both competitive abilities (usually growth) and defense. Here, we test the EICA hypothesis using *Brachypodium sylvaticum* (Huds.) P. Beauv., an aggressive invasive species in the northwest of the USA (Roy 2010), with the ERH (phenotypic plasticity in the face of specialist enemy loss) as an explicit alternative hypothesis. Previous work indicates that

pathogens and herbivores of *B. sylvaticum* show some, but not all, of the characteristics predicted by the ERH (Roy et al. 2011). Insecticide and fungicide sprays were used to remove herbivorous insects and pathogenic fungi from the plants in multiple populations in both the native and invaded ranges. In accordance with the ERH, population growth rates were higher in the native range in the sprayed plots, where enemies were fewer than in the control plots. There was no statistically significant effect of enemy removal in the invaded range. Contrary to the ERH, all the common enemies were generalists and there was more herbivory in the invaded range relative to the native range (Roy et al. 2011; Halbritter et al. 2012).

Increased herbivory in the invaded range and reduced seed germination in the native range suggested that there might be differences in endophyte infection, since endophytes would not have been killed by the non-systemic fungicides used in previous studies (Roy et al. 2011; Halbritter et al. 2012). Endophytes are fungi that live between the cell walls of plants and cause no visible disease symptoms on the surface of the plant; they are common in grasses (Clay 1990; Rudgers et al. 2009). While it is not obvious that a plant is infected when endophytes are present, they may nonetheless have a range of consequences for their hosts, from true mutualism that increases insect or drought resistance, through commensalism, to antagonist pathogenicity that decreases survival and reproduction (Carroll 1988; Faeth and Sullivan 2003; Saikkonen et al. 2006). The same species of endophyte can either be a mutualist or pathogen depending upon its lifecycle stage, genotype, or environmental conditions. All symbioses exist on a continuum from pathogen to mutualist: if the benefit to the host (e.g., from reduced herbivory) is greater than the cost (e.g., reduced growth and seed-set) the fungus is a

mutualist. The environment within which a host is embedded will impact the position of a symbiont along this continuum because it will alter the balance between costs and benefits.

Brachypodium sylvaticum in its native Eurasian range appears to be almost ubiquitously infected with a host-specific fungal endophyte, *Epichloë sylvatica* Leuchtm & Schardl (Eckblad and Torkelsen 1989; Raynal 1994; Väre and Itämies 1995; Bucheli and Leuchtmann 1996; Enomoto et al. 1998; Zabalgogeazcoa et al. 2000; Roy et al. 2011; Leuchtmann, pers. com.), which may act as a pathogen rather than a mutualist, despite common assumptions about *Epichloë* endophytes of grasses (Schardl 1996). Small-scale studies done with infected and uninfected plants in Switzerland, by Brem and Leuchtmann (2002), indicate that while plants infected with an asexual strain of *E. sylvatica* have less herbivory, they also have decreased growth rates and competitive abilities. Recent research in our lab (Roy et al. 2011; Halbritter et al. 2012) suggests that *Epichloë* infection may decrease germination rates in *B. sylvaticum* by seed infection, as the fungus is spread vertically from mother plant to daughter (Brem and Leuchtmann 1999). Thus, *E. sylvatica* appears to be a specialist enemy of *B. sylvaticum.*

Here, we document the near total absence of *E. sylvatica* infection in the invaded range. If *E. sylvatica* is generally pathogenic, the near lack of fungal endophyte infection in *B. sylvaticum* within the invaded range may constitute strong support for some form of the ERH or the EICA hypothesis.

In addition to documenting the virtual absence of the endophyte in the invaded range, we compare germination and growth rates of seedlings, a common proxy for fitness (Poorter and Garnier 1999; Matzek 2012), from the native and invaded ranges to

explicitly test the EICA hypothesis. We utilized a greenhouse experiment using seeds collected during the same season in both ranges, clearing the seeds of *Epichloë* infection and then selectively re-inoculating half of each group. This bifactorial design permits us to effectively compare the effect of *E. sylvatica* and plant origin independent of each other, allowing us to distinguish between ERH, EICA, and potential founder effects (Fig. 1). If release of *B. sylvaticum* from control by *E. sylvatica* is sufficient to explain observed increases in fitness in the invasive range (Holmes et al. 2010; Roy et al. 2011; Halbritter et al. 2012), we expect that removing it from native range plants should increase their performance to be on par with those from the invasive range. Additionally, we would expect invasive range plants to be affected similarly to those from the native range. In short, under the hypothesis that *E. sylvatica* is directly impacting fitness in *B. sylvaticum*, such that it is a controlling specialist enemy in the native range, we expect the fungus to impact native and invasive range plants similarly (Fig. 1A).

Alternatively, if release from the specialist enemy *E. sylvatica* has provided selective pressure for *B. sylvaticum* to have evolved some reallocation of resources from defense to growth and reproduction, we expect to be able to observe the reduction in defensive capabilities by invasive range plants in the form of strongly reduced fitness when infected as compared to infected native range plants. In other words, we expect the invasive range plants to be disproportionately negatively affected by infection with *E. sylvatica*. Additionally, if such evolution has occurred, we would also predict that even in the absence of the controlling enemy, invasive range plants will out-perform native range plants (Fig. 1B).

Factors independent of enemy release may be driving the evolution of invasive

range populations of *B. sylvaticum,* such as founder effects and drift, or selection unrelated to enemy release. If the invasive range plants show increased fitness relative to

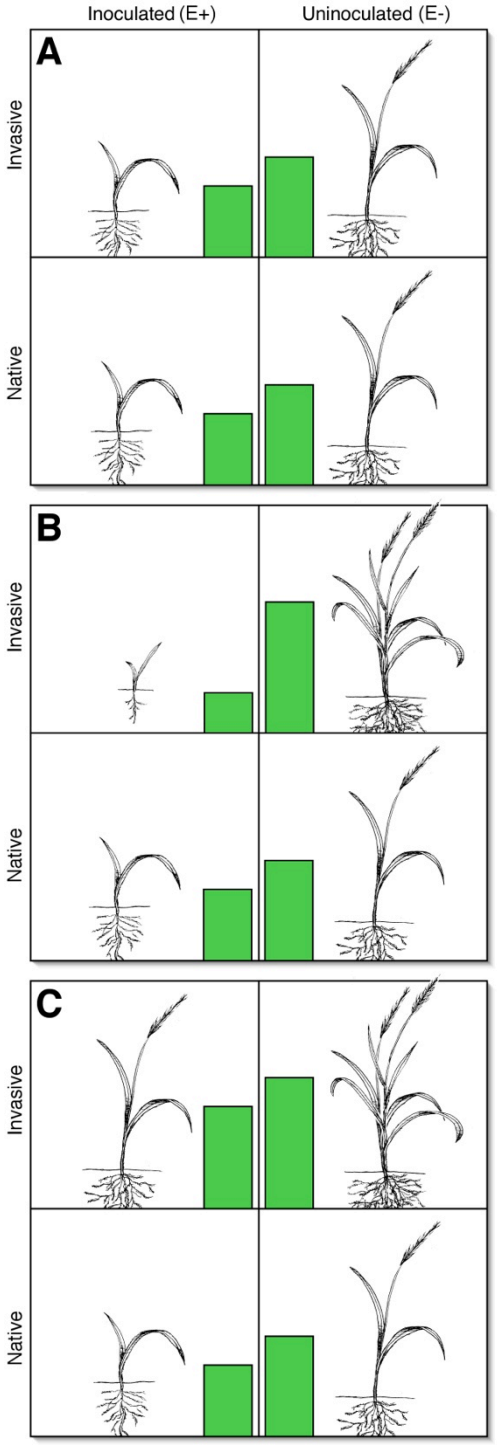


Fig. 1 Comparison of predictions of the ERH (A), the EICA hypothesis (B), and founder effects or other evolutionary forces unrelated to enemy release (C). Relative sizes of the cartoon grasses and the green bars indicate relative differences in fitness for the hosts. Presence (E+) or absence (E-) of *E. sylvatica* is shown on the X-axis, and range of origin on the Y-axis of each panel.

ERH (A): If the observed increase in fitness in *B. sylvaticum* is due only to escape from *E. sylvatica*, re-introduction of the fungus to invasive range plants should recapitulate native range fitness levels, while removal of the fungus from native range grasses should release them from control, increasing fitness to levels to those observed in invasive range plants.

EICA (B): If populations of *B. sylvaticum* have evolved in response to enemy release in the invasive range, we expect invasive range plants to experience a disproportionate fitness loss when infected by *E. sylvatica* compared to their native range conspecifics, as well as having increased fitness in the absence of the fungus.

Founder effects (C): If founder effects, or evolution not related to enemy release, is responsible for the increase in fitness observed in the invasive range, we expect invasive range plants to be more fit than native range plants regardless of infection with *E. sylvatica*, though infection will likely still negatively affect the host, likely in a manner proportionate to the effect on native range plants.

their native range counterparts in both infected and uninfected states, such other evolutionary forces may be the best explanation for the observed increases in fitness in the invasive range (Holmes et al. 2010; Roy et al. 2011; Halbritter et al. 2012), though a great deal more work will be needed to determine the extent to which founder effects, genetic drift, or selective pressures not related to enemy release are responsible for such evolution (Fig. 1C).

This experiment also allowed us to test explicitly for effects of infection by *E. sylvatica* on germination rates. All germination rates observed to date of uninfected seeds of European origin are from naturally infected seeds that were treated to kill the endophyte (Roy et al. 2011; Halbritter et al. 2012). It is necessary to compare germination rates of seeds from the same population produced with and without the endophyte to accurately determine the effect of *Epichloë* on germination rate, because infection of seeds at any time may negatively impact germination, including prior to heat treatment.

Materials and Methods

Focal Species

We are working with the grass *Brachypodium sylvaticum* (Huds.) P. Beauv., an aggressive invasive species in the USA (Roy 2010), introduced in the early 1900's by the US Department of Agriculture (USDA) for agronomic research. Records from the Office of Foreign Plant Introduction dating back to 1912 indicate that *B. sylvaticum* was being imported from India, Sweden, Russia, and probably other localities (Rosenthal et al. 2008). The grass was first collected in the wild in Oregon in 1939 (Chambers 1966), and

has become increasingly common during the last 15 years (Rosenthal et al. 2008). This grass is of particular concern because it is shade-tolerant (Holmes et al. 2010) and forms vast, virtually monospecific carpets in the forest, which crowd out other vegetation (Kaye and Blakeley-Smith 2006) and, similar to other grasses, may reduce conifer seed germination (Powell et al. 2006). It is found commonly and in high densities in the central Willamette valley, particularly from Eugene to Corvallis, and appears to be in the midst of rapid range expansion (Rosenthal et al. 2008; Roy 2010).

Study Sites

We sourced seed and tested for endophyte infection in Switzerland (center of the native range) and Oregon (USA, epicenter of the invaded range) at 21 field sites (Supplementary Table S1; Fig. 2), a subset of which were used for germination, growth

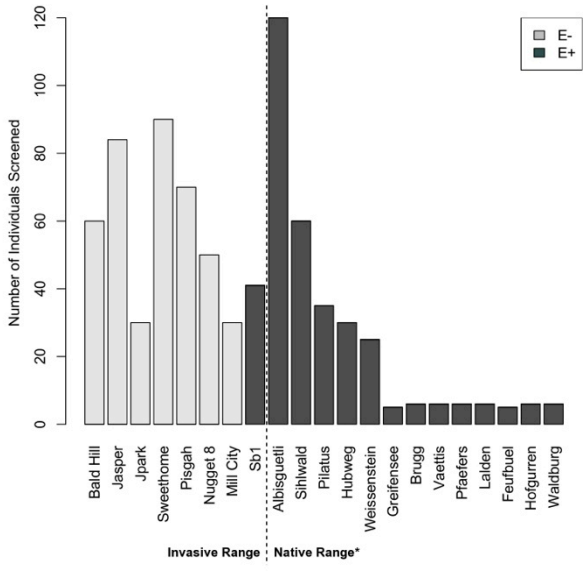


Fig. 2 Wild endophyte screen results. We only found evidence of *E. sylvatica* in one wild population in the invaded range, the Fisherman site near Mill City, Oregon. All individuals from all populations tested from the European native range were infected, however. *Native range data from Bucheli & Leuchtmann (1996).

rate, and mortality assays. Climate in the two areas are similar: Zürich (Switzerland) has an annual precipitation of 1086 mm and Eugene (Oregon, USA) has 1254 mm. Mean temperatures for Zürich and Eugene are 8.5 ˚C and 11.9 ˚C, respectively (climate information from www.meteoschweiz.admin.ch and the Western Regional Climate Center www.wrcc.dri.edu). There are, however, seasonal differences between the two sites: summer is much drier in Oregon (mean precipitation for July and August in Zürich is 124.5 mm, but it is only 20 mm for Eugene).

Infection Rates

To determine whether the fungus was present in the invaded range we used the Agrinostics Field Tiller immunoblot kit (Agrinostics Ltd. Co., Watkinsville, GA, USA). Because the kit was developed for a different species, we verified its efficacy by isolating *E. sylvatica* from *B. sylvaticum* seeds collected in Switzerland where infection rates are at or near 100% (Fig. 2; Bucheli & Leuchtmann 1996; Leuchtmann & Schardl 1998). For additional positive controls we used leaf tissue from plants grown from Swiss seeds and *Dactylis glomerata* showing choke symptoms caused by *Epichloë typhina* (Pers.:Fr.) Tul. Finally, we verified a subset of immunoblot results with an *E. sylvatica*-specific PCR screen; these indicated that the immunoblot results were valid (see Supplemental Methods for details).

Genetic data and historical records suggest that *Brachypodium sylvaticum* was likely initially introduced from two Bureau of Plant Introduction experimental plots, one near Eugene, Oregon, and one near Corvallis, Oregon (Rosenthal et al. 2008). We therefore screened three populations near Eugene (Mount Pisgah, Jasper, and Jasper State

Park) and two near Corvallis (Bald Hill and Sweet Home; see Table S1). The vegetation and other site characteristics have been described elsewhere (Roy et al. 2011).

We tested for *Epichloë sylvatica* in the invaded range at three times: peak growing season (20 June 2010), seven weeks later (4 August 2010), and at the end of the summer (26 Aug 2010). All tillers were collected at ground level within 24 hours of analysis, wrapped in a paper towel and placed on ice. Thirty tillers per population were randomly sampled by taking the nearest tiller to a meter mark along two parallel transects each 15 m long and approximately 10 m apart.

Initial Germination and Growth Rate Assays

For the initial assay, seed material of *B. sylvaticum* was collected in late August 2007 from three wild populations in the native range and six in the invaded range (Table S1). Seeds were stored at 4°C until needed. Prior to germination, seeds were deglumed and winnowed to remove aborted seeds. To reduce fungal attack, seeds were surface sterilized in 5% bleach solution for 30 sec and then rinsed twice with tap water. On May 22nd 2008 seeds were placed between four sheets of filter paper in a sterile Petri dish and dampened with a solution of gibberelic acid (50 mg GA_3/500 ml tap water). Petri dishes were kept at room temperature and checked daily to ensure correct moisture level. As they germinated the seedlings from each of the US populations were transplanted into 200 cm^3 Containers (D-40 cells, Steuwe and Sons, Corvallis, Oregon) filled with Rexius, Patio Potting Soil™ (one seedling per tube). The Swiss seeds had extremely low germination rates, and after day 11 we transferred the remaining seeds to trays filled with potting soil and transplanted them into containers upon germination.

We measured the aboveground height of seedlings nine days after transplanting (with a few exceptions of 8-11 days). Seedling growth rates, a proxy for fitness (Poorter and Garnier 1999; Matzek 2012), were calculated by dividing the height at the time of measurement by the number of days since emergence. To test differences between ranges we used a mixed-model analysis of variance (ANOVA) with restricted maximum likelihood (REML) estimation of variance components. Range was designated as a fixed effect and population as a random effect.

EICA Greenhouse Experiment

A large number of seeds were collected from two native (Flaach:654, Rafz:395) and two invaded (Pisgah:293, Jasper:291; see Table S1) range populations of *B. sylvaticum* at the end of summer 2011. These were deglumed by hand, and then treated to remove the endophyte, following Nott & Latch (1993). The seeds were surface sterilized by immersion in 95% EtOH for one minute, full-strength bleach (6.15% NaHClO) for 3 minutes, 30 seconds in 95% EtOH, then triple rinsed in autoclaved deionized water. The seeds were allowed to dry on sterile filter paper, then were placed in sterile petri dishes and placed in 100% humidity at 37˚C for three weeks in a sealed incubator. The seeds were then germinated on sterile water agar, with any seeds showing fungal infection being discarded.

Half of the germinants from each population were inoculated with *E. sylvatica*, for a total of 65 plants per population. Inoculation of *B. sylvaticum* with the endophyte was accomplished following Leuchtmann & Clay (1988). Working under a dissecting microscope, a 27-gauge sterile hypodermic needle was used to make a small incision just

above the apical meristem of the seedlings at the two- or three-leaf stage of development (typically five days post germination). The needle was then used to collect a small sample of cultured fungal hyphae (isolated from Swiss seeds as described above), which was then inserted into the incision. Control plants were injected with a small drop of sterile deionized water (Leuchtmann and Clay 1988). Plants were grown for five days on agar before transplantation into soil (Black Gold, Sun Gro Horticulture, Agawam, MA, USA) in 10 cm pots.

The plants were randomly distributed in racks in the greenhouse, and were re-randomized every week. Height (length from longest leaf tip to soil surface) was measured every other day, from initial transplant into soil (19 December 2011) to harvest (29 Feb 2012). Daily growth rates were calculated as the difference in height between two subsequent measurements, divided by two. The data were analyzed using a repeated measures, mixed model analysis of variance (ANOVA), including site, nested in range, and inoculation as fixed effects, population included as a random effect, and daily growth rate as the response variable. Number of tillers at harvest and oven-dried biomass were analyzed by mixed model analysis of variance (ANOVA) as well, though without repeated measures. Tukey's HSD was used to compare means. Mortality was analyzed using the log-rank Mantel-Haenszel test (Harrington and Fleming 1982).

A subset of both treatment groups for all populations (10 plants per treatment per population) were repotted into gallon pots and allowed to set seed, which was collected for second-generation germination assays. A subset of 100 seeds from each parent plant were deglumed by hand, surface sterilized as above, plated onto water agar to germinate, and observed for germination for 30 days. Ten seeds from each plant were also tested

using the Agrinostics Seed Immunoblot kit (Agrinostics Ltd. Co., Watkinsville, GA, USA) to confirm infection status of the parent. Because seed infection by *Epichloë* was not entirely all-or-nothing, germination rates were analyzed by linear regression to examine trends in germination in response to rate of seed infection. Additionally, a mixed model ANOVA and Tukey's test were used to examine treatment and range differences. Student's T-tests were used to examine pairwise differences.

All analysis was performed in R (version 2.15.1), using the packages vegan (Oksanen et al. 2012) and survival (Therneau 2012).

Results

Endophyte Screening

Endophyte infection in Oregon, epicenter of the invasion, appears to be limited to a single population of the eight we sampled. Using the immunoblot test we found *Epichloë sylvatica* in 41 of the 455 wild collected tillers from the invaded range (Fig. 2; Supplementary Table S1). The only infected plants from the invaded range were collected from Fisherman, near the northern limit of the invaded range. There was no effect of time sampled; within a population, all samples were either infected, or not infected. Our positive controls were consistently positive (see Methods). Immunoblot results were validated by screening a subset of samples (24 negative, 3 positive) with an *Epichloë*-specific PCR assay, which gave identical results to the immunoblot.

Germination Rates

In our initial assay, germination was significantly higher in the invaded range ($F = 13.10_{1,7.33}$, $P = 0.0079$), and all populations in the invaded range had higher germination than the native range (Fig. 3A).

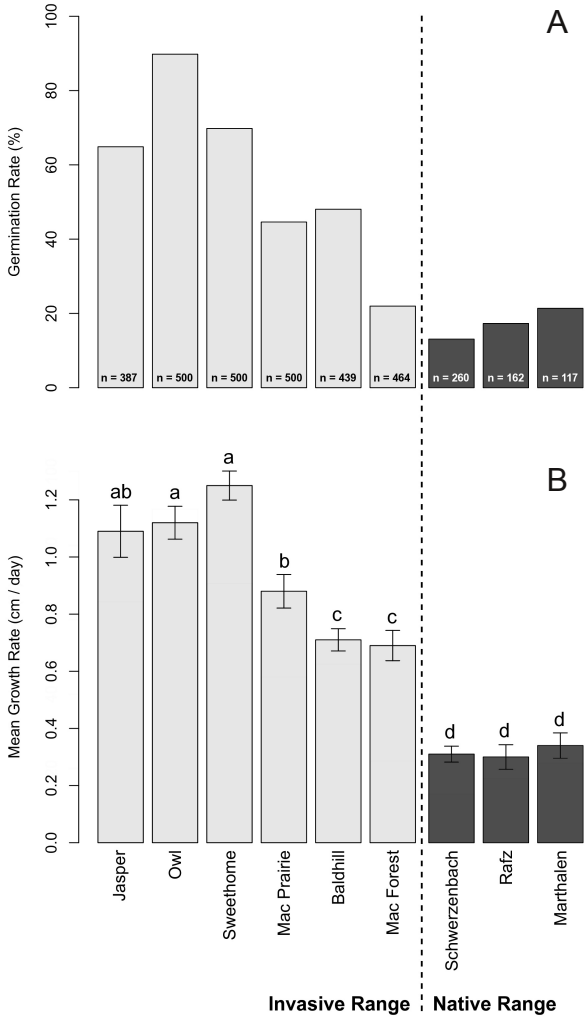


Fig. 3 Results from our initial germination rate (A) and growth rate assays (B), May-June 2008. There is a significant difference in germination rates for the two ranges ($t = 3.97_{5.588}$, $P = 0.0085$), which we initially attributed to the fact that all native range populations are ubiquitously infected with *Epichloë sylvatica*, and all invasive range populations tested lack the endophyte. There is also a significant difference in growth rates for the two ranges ($t = 16.48_{159.539}$, $P < 0.0001$), in addition to differences by individual populations (letters represent differences at $P < 0.05$ by pairwise t-test).

In our second germination assay with seeds originating from the common greenhouse study, endophyte infection and seed origin were decoupled (Fig. 4). We still found significantly higher germination in invasive populations ($t = 5.08_{20.788}$, $P <$

0.0001), but there was also a significant interaction between range and infection status ($F = 8.23_{1, 22}$, $P = 0.0087$), such that there is no difference in germination rates for infected seeds by range ($t = 1.59_{11.001}$, $P = 0.1397$), while differences in germination rates of uninfected seed drove the entire trend ($t = 9.31_{8.736}$, $P < 0.0001$). In invasive populations there was a marginally significant trend towards reduced germination with endophyte infection ($F = 3.79_{1, 10}$, $P = 0.0801$, r^2=0.20), but in native range seeds germination rates significantly increased with increasing rates of endophyte infection ($F = 5.11_{1,12}$, P = 0.0432, r^2=0.24). These results are in line with Brem & Leuchtmann (2002): they cite unpublished germination data showing higher germination rates in endophyte infected seeds in the native range.

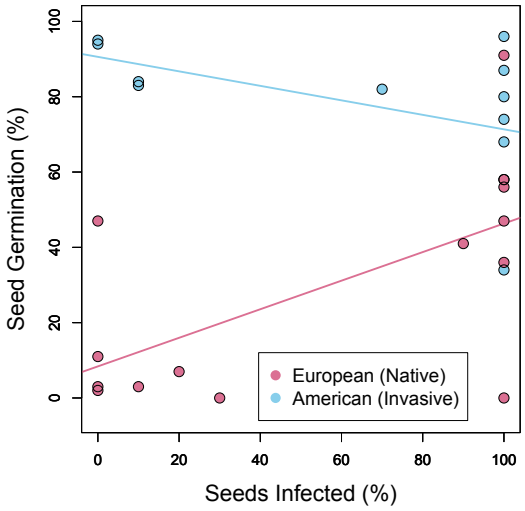


Fig. 4 Relationship between seed infection by *Epichloë sylvatica* and germination. Each point represents a single maternal genotype originating in the native range (red) or invaded range (blue), with seed produced being open-pollenated in a common greenhouse. Two native and two invasive range populations were included, and there were no statistically significant differences between populations within a given range (invasive: $t = 1.28_{9.966}$, $P = 0.2311$; native: $t = -0.48_{11.251}$, P = 0.6402). For linear regressions, the American range has $r^2 = 0.20$ with P = 0.080; the European range has r^2 = 0.24 with $P = 0.043$. Percentages were transformed using the standard arcsine square root transformation.

Growth Rates

In our initial seedling growth rate assay, we found that growth rates were significantly higher for plants from the invaded range ($F_{1,7.22} = 20.92$, $P = 0.0024$; Fig. 3B).

Our second growth rate assay was designed to de-couple endophyte infection from range of origin. We found that growth rates were not significantly reduced in the inoculated treatment for those seedlings from the native range (Fig. 5A; $F_{1,19} = 0.007$, $P = 0.933$), but were significantly reduced for seedlings from the invaded range (Fig. 5B;

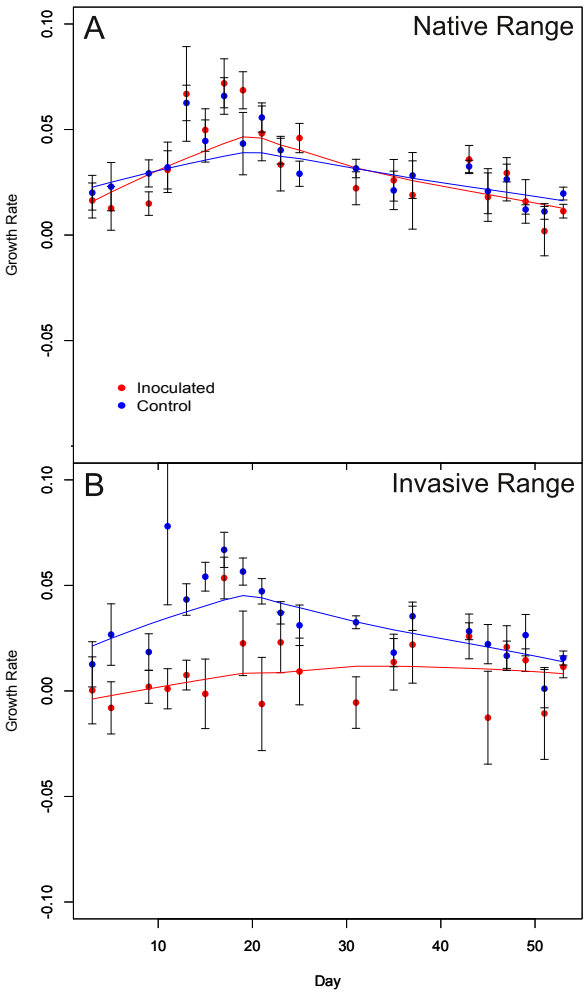


Fig. 5 Daily growth rate (ratio of change/day) of *B. sylvaticum* seedlings in the greenhouse. In seedlings originating in the plant's native range (Europe; A), there is no significant difference ($F_{1,19} = 0.007$, $P = 0.933$) between seedlings inoculated with *Epichloë sylvatica* (red) and those receiving the control treatment (blue). In seedlings originating in the invaded range (United States; B), however, there is significant effect of inoculation ($F_{1,19} = 26.04$, $P < 0.0001$).

$F_{1,19}$ = 26.04, $P < 0.0001$). Interestingly, there was no significant effect of range origin in this greenhouse experiment once the negatively affected invasive range inoculated plants were removed from the analysis ($F_{1,19} = 0.066$, $P = 0.797$). There was no significant effect of inoculation on final tiller number of surviving plants for either range (native: t = 1.894, $P = 0.2326$; invasive: t = 1.895, $P = 0.2321$), nor was there a significant effect of inoculation on the biomass of surviving plants for either range (native: t = 1.834, $P = 0.2586$; invasive: t = 0.103, $P = 0.9996$).

Seedling Mortality

Our second greenhouse experiment tracked mortality through time, in addition to growth rates. We observed a significant treatment by range interaction here, with inoculation not significantly changing mortality for *Brachypodium* originating from the native range (Fig. 6A; $\chi^2 = 1.1$, $P = 0.299$), but significantly increasing mortality for those seedlings originating from the invaded range (Fig. 6B; $\chi^2 = 34$, $P < 0.0001$).

Discussion

Pathogen or Mutualist?

Harboring this endophyte has fitness costs for *Brachypodium sylvaticum*, but whether or not an endophyte is a pathogen or a mutualist depends on the specific context of host, symbiont, and environment (Carroll 1988; Scholthof 2007). Theoretically, if herbivores are present that significantly decrease fitness, then infected plants will have an advantage, provided the herbivores are deterred by the fungal alkaloids produced (Richardson et al. 2000; Brem and Leuchtmann 2001). Our results indicate that the

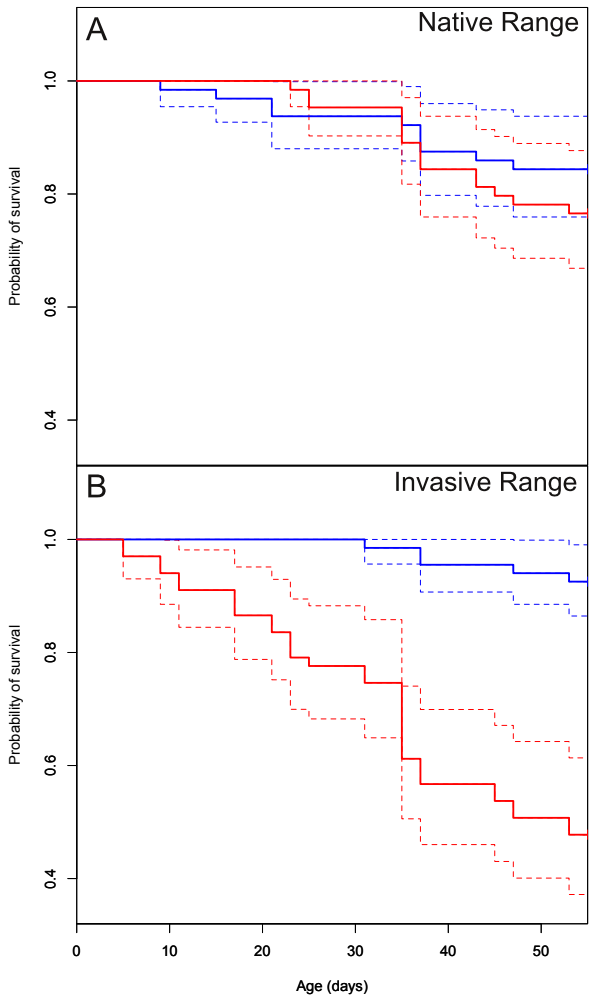


Fig. 6 Survival function estimates for *B. sylvaticum* seedlings. In seedlings originating in the plant's native range (Europe; A), there is no significant difference ($\chi^2 = 1.1$, $P = 0.299$) between seedlings inoculated with *Epichloë sylvatica* (red) and those receiving the control treatment (blue). In seedlings originating in the invaded range (United States; B), however, there is a significant effect of inoculation, leading to significantly reduced survivorship ($\chi^2 = 34$, $P < 0.0001$) within the first two weeks, and continuing to drop through time.

endophyte *Epichloë sylvatica* has low incidence in the invaded range (9% overall; Fig. 2), while literature indicates that it is nearly ubiquitous in the native range (Fig. 2). In addition to the Swiss infections reported in Fig. 2 (Bucheli and Leuchtmann 1996; Roy et al. 2011; Leuchtmann, pers. com.), there are also reports of infection in Scandinavia, Finland, France, Spain, and Japan (Eckblad and Torkelsen 1989; Raynal 1994; Enomoto et al. 1998; Zabalgogeazcoa et al. 2000; Väre and Itämies 1995). Additionally, Adrian Leuchtmann reports having seen 100% infection levels in populations of *B. sylvaticum*

from Holland, Sweden, England, and Italy. He does, however, note that in one population from Sardinia, only two out of three plants were infected (A. Leuchtmann, pers. com.).

Data from the native range indicates that the endophyte increases resistance to insect herbivory (Brem and Leuchtmann 2001), but also decreases competitiveness (Brem and Leuchtmann 2002). We found that in the invaded range it can be detrimental to growth rates (Fig. 5) and seed germination (Fig. 4), but increases seed germination in the native range (Fig. 4). While the loss of anti-herbivore properties conferred by the endophyte could have made it more susceptible to being eaten in the invaded range, data show that vegetative insect herbivory has little effect on fitness in either range (Brem and Leuchtmann 2001; Halbritter et al. 2012). However, the observed elevation in herbivory in the invaded range may be evidence that loss of the endophyte does mean a loss of protection (Halbritter et al. 2012).

Brachypodium sylvaticum may be controlled by the host-specific endophytic fungus *Epichloë sylvatica* in its native range, given the effects of the fungus on its host's competitive abilities (Brem and Leuchtmann 2002), and the differences in performance between ranges (Halbritter et al. 2012). This may contribute to the grass's success as an invader in the Pacific Northwest, where *Epichloë* is largely absent. Given preliminary germination data in both ranges (Fig. 3A), we expected to see control by *Epichloë sylvatica* acting through reduced germination; however, when infection status and range are deconfounded, it appears that lower germination in the native range is not caused by *E. sylvatica* (Fig. 4). In the absence of the endophyte, the European seeds germinate at significantly lower rates than the American seeds, indicating that differences in germination may be determined by genetic factors. The increase in germination with

Epichloë infection in the native range, and the trend towards decrease in germination rates with infection in the invaded range (Fig. 4), lends support to our hypothesis that *Brachypodium sylvaticum* in the USA has lost defense and/or tolerance mechanisms (the ability to survive and reproduce despite being infected; see Roy and Kirchner 2000) through evolution. Demonstrating that the loss of tolerance is in direct exchange for increased fitness will be interesting future work.

We show a near total absence of *E. sylvatica* infection in the invaded range, as well as a clear loss of tolerance of such infection by invasive-range *B. sylvaticum* from multiple populations throughout the invaded range (including the only population found to host the endophyte within that range). It is likely that ubiquitous infection of *B. sylvaticum* in Europe is maintained by a strong selection that is largely absent in the Pacific Northwest. This selective pressure may be acting through the seeds: while protection from folivores proved not to be important in previous studies (Halbritter et al. 2012), *Epichloë* may provide protection from seed-damaging insects or pathogens in the native range. There is, indeed, higher incidence of seed-associated insects and pathogens in the native range than the invasive (Halbritter et al. 2012). Further studies will be necessary to clarify the role of *Epichloë* endophytes in protection of seed.

EICA vs. ERH

With regards to enemy release, EICA can be construed as a sub-case of ERH (Joshi and Vrieling 2005), though the mechanisms are distinct. Enemy Release Hypothesis can be explanatory in the absence of evolution where populations of an organism are directly controlled by co-evolved enemies (Keane and Crawley 2002; Liu

and Stiling 2006), for example as in *Ambrosia artemisiifolia*, which seems to not have lost any defensive capabilities despite herbivore release upon invasion in France (Genton et al. 2005). Evolution of Increased Competitive Abilities, however, is important when the release from those enemies provides selective pressures to re-allocate resources from defense to competitive traits, such as increased growth and reproduction (Blossey and Notzold 1995), or production of allelopathic chemicals (Uesugi and Kessler 2013). These two hypotheses lead to different predictions in our study system (Fig. 1).

Our experimental design allowed us to assess evolutionary change in the invaded range, such that we can effectively distinguish between the ERH and the EICA hypothesis. We found significantly increased mortality of inoculated *B. sylvaticum* originating from the invaded range as compared to their native range equivalents (Fig. 6). We also found reduced seedling growth rates in inoculated invasive-range plants (Fig. 5). Both of these results show a loss of tolerance for the host-specific fungal enemy in the invasive range populations tested, consistent with evolutionary loss of defensive mechanisms against this specific enemy, as predicted by the EICA hypothesis. The difference in germination rates seems to point to genetic mechanisms for increased germination in the invaded range, as well as loss of other factors controlling germination in the native range, such as seed-damaging pathogens and herbivores. These facts, taken together, are strong support for the Evolution of Increased Competitive Abilities hypothesis, which predicts such a loss of defensive mechanisms to specific enemies in exchange for increased fitness in the invasive range.

Enemy Release and Invasion History

The story of any invasive species is unique, and while there may be unifying trends, each species has a particular history of introduction and a particular biology that influences its success. *Brachypodium sylvaticum* is no different; the success of this grass as an invader in the Pacific Northwest is no doubt influenced by the way in which it was introduced. During introduction, seed stock from all over the native range was planted in USDA test plots near Corvallis and Eugene (Rosenthal et al. 2008), promoting novel genetic combinations. Rapid range expansion may also have contributed to evolutionary changes (Rosenthal et al. 2008), independent of selective effects of enemy release. Such evolutionary drivers are theorized to be more important generally (Felker-Quinn et al. 2013), but it is necessary to keep in mind the individual nature of species invasions (Mitchell et al. 2006). This confluence of genotypes and brisk range expansion may have led to the rapid spread and fixation of resistance genes in the population, likely before subsequent dispersal, which is theorized to have been facilitated by logging in the region of the abandoned USDA test plots (Rosenthal et al. 2008). This argument supposes that there is selection for endophyte infection in the native range that is absent in the invaded range. If this is so, then it is unlikely to be leaf attacking insect herbivores, as these do not reduce fitness in *B. sylvaticum*, and insect herbivory is conspicuously elevated in the invaded range (Roy et al. 2011). Similarly, it is unlikely to be a large herbivore, as *Brachypodium sylvaticum* is unpalatable to most macroherbivores due to high silica content: rabbit, deer, and other macroherbivore browsing makes up an extremely small portion of total plant herbivory for this grass (Brem and Leuchtmann 2001). This appears to be true in both ranges (Roy et al. 2011). It is more likely to be a seed eating insect or

seed pathogen, or an enemy affecting young seedlings, as these have stronger effects on fitness (Roy et al. 2011; Halbritter et al. 2012), and are in line with our germination results (Fig 3A, Fig. 4).

Alternatively, the endophyte may have been lost during introduction: seeds could have been treated, either accidentally or purposefully, in ways that would have killed seed endophytes. During slow shipment or uncooled storage in the early 20th century, seeds were likely subjected to conditions of heat (37°C) and high humidity (~100%) that would have led to loss of infection. Storage duration has also been shown to result in endophyte loss, with endophyte viability decreasing before seed viability (Gundel et al. 2009).

Endophytes and Invasion

Vertically transmitted endophytes are commonly assumed to be mutualists, and are expected to have a positive effect on invasiveness (Richardson et al. 2000). For example, Rudgers et al. (2004) said "Specifically, vertically transmitted fungal endophytes may confer predictable advantages to invading grasses when they accompany their host to new environments (pp. 47)". However, there is no reason to believe that all endophyte infections lead to more fit plants. In our first assay, we found greatly reduced growth rates of plants whose seeds originated in the native range (Fig. 3B), where infection is 100%, and in our second assay, we found that endophyte infection significantly reduced growth rates of plants originating in the invaded range (Fig. 5). Given the trade-off between the costs of hosting a given endophyte and the benefits that such a symbiont can provide in a given context, it is no surprise that endophyte effects in

invasion ecology are context-dependent. Our results are consistent with the published results of Brem & Leuchtmann (2002), who found that when they removed the endophyte from seeds, the resultant uninfected plants were faster growing, larger, and more competitive than infected plants.

Enemy release, in this case, is more complicated than loss of a single controlling organism upon invasion. In its native range, *Epichloë sylvatica* seems to control *B. sylvaticum* in some ways (reducing growth rates and competitive abilities), but those detriments seem to be off-set by increased germination rates and potential protection from seed herbivores and pathogens. In the invaded range, the grass is released from control on growth and competitive abilities imposed by the fungus, likely because it is not necessary to harbor such a costly endophyte to maintain high seed viabilities in the invaded range. Whether that is through the additional release of control by a seed-damaging organism, or through novel genetic recombination that allows for high germination rates in the absence of the fungus is still to be determined.

Bridge to Chapter III

Chapter II likely represents the neatest, cleanest set of results that I have had the pleasure of encountering in my doctoral research. Given the body of knowledge that exists around Clavicipitaceous endophytes, the results of this study—while satisfying—were not altogether surprising. I wanted to sink my teeth deeper into the problem of understanding plant/symbiont relationships, and for that, I knew I would need to delve into more complex, complicated realms. The crispness of the *Brachypodium* experiment left me feeling uncomfortable: after all, ecology—or the romanticized notion of ecology

in my mind, at least—happens in the field, deep in the dirt and muck of the real world, not the pseudo-sterile conditions of a lab and a greenhouse, locked under cold, heartless glass. I wanted to get my hands dirty, both metaphorically, and literally; I wanted to know how these *Epichloë* symbionts functioned in the real world—'do they play well with others?', I asked myself. The heart of ecology is in the interaction between organisms and the biotic and abiotic environment, so in Chapter III, I set out to add both of those factors to the study of the *Epichloë* symbiosis, and examined their relationships with other symbionts in the same host across a great range of environmental conditions. I was not disappointed where dirt, both literal and metaphorical, was concerned.

CHAPTER III

THE HERBACEOUS LANDLORD: INTEGRATING THE EFFECTS OF SYMBIONT CONSORTIA WITHIN A SINGLE HOST

From Vandegrift, R., Roy, B. A., Pfeifer-Meister, L., Johnson, B. R., & Bridgham, S. D. (2015). The herbaceous landlord: integrating the effects of symbiont consortia within a single host. *PeerJ*, *3*, e1379.

Contributions

R. Vandegrift conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper. B. A. Roy and S. D. Bridgham conceived and designed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper. L. Pfeifer-Meister conceived and designed the experiments, performed the experiments, reviewed drafts of the paper. B. R. Johnson conceived and designed the experiments, reviewed drafts of the paper.

Introduction

There has been a surge in interest in the microbiome of terrestrial plants (Porras-Alfaro and Bayman 2011, Turner et al. 2013), largely driven by the increasing recognition that the microbial associates of plants play major roles in plant health (Carroll 1988, Chaparro et al. 2012, Berendsen et al. 2012, Berlec 2012). Furthermore, microbial associates of

plants may be integral to plants' responses to disease and climate change (Köberl et al. 2011, Woodward et al. 2012). Here we ask: how do the associations of microbes change under different climatic conditions within the same host plant species, and does this matter to host fitness?

Particularly important components of the plant microbiome are fungal symbionts, especially mycorrhizal fungi (Munkvold et al. 2004, Glassman et al. 2015) and fungal endophytes (Arnold and Lutzoni 2007, Porras-Alfaro and Bayman 2011) (Box 1). Fungal endophytes are defined functionally, rather than phylogenetically—they are fungi found within living, healthy plant tissues (Clay 1990, Rudgers et al. 2009). Endophytes make their living by not harming their host enough to induce a defensive reaction; many of these fungi are assumed to be mutualists, but both fungal endophytes and mycorrhizal fungi exist on a functional continuum from mutualist to pathogen (Carroll 1988, Porras-Alfaro and Bayman 2011). The position upon this continuum will depend upon the environmental context, in addition to the particular host/symbiont pairing (Carroll 1988, Johnson et al. 1997, Saikkonen et al. 1998, 2006, Faeth and Sullivan 2003).

While there is a growing body of research examining the interactions among multiple symbionts within a single host (Müller 2003, Lingfei et al. 2005, Novas et al. 2005, 2011, Omacini et al. 2006, Mack and Rudgers 2008, Scervino et al. 2009, Kandalepas et al. 2010, Urcelay et al. 2010, 2010, Liu et al. 2011), most studies of fungal symbionts of plants have examined individual relationships in isolation (Kuldau and Bacon 2008, Porras-Alfaro and Bayman 2011, White and Bacon 2012), despite recognized need for an integrative, systems biology perspective (Porras-Alfaro and Bayman 2011, Schlaeppi and Bulgarelli 2014). In this study we examined the interaction of three symbionts within a

Box 1: Definitions of terms

***Symbiosis*:** We use the word *symbiosis* in the literal sense, meaning "to live together", for the relationship between a host and an associated fungus. The *symbiont* is the fungal partner, deriving nutrition from the host; the words *symbiont* and *symbiosis* are not intended to convey any sense of whether or not the association is beneficial or harmful to the host, only that the association exists.

***Mutualist*:** A *mutualist* is a symbiont that provides a net fitness benefit to its host. Mutualism implies that both partners benefit—we take the nutritional mode of the fungal partner (i.e., carbon derived from the host) to be the symbiont's benefit. For example, some *Epichloë* endophytes of grasses are mutualists, because they produce fungal alkaloids that can lead to a dramatic reduction in herbivory of the host (Brem and Leuchtmann 2001, Kuldau and Bacon 2008, Gange et al. 2011).

***Pathogen*:** We define a *pathogen* as a symbiont that causes a net fitness decrease in its host. There are many obvious and direct plant pathogens, such as ergot (*Claviceps purpurea* (Fr.) Tul.), which reduces host fitness by forming sclerotia on the developing seeds of its host (Langdon 1954). There are, however, many much less direct modes of pathogenicity: some *Epichloë* endophytes, for example, have been shown to reduce growth rates and seedling survival (Brem and Leuchtmann 2002, Vandegrift et al. 2015)—if these fitness costs of hosting the fungus are not offset by fitness benefits provided by the fungus, the net effect is pathogenic.

single grass host, as well as the shift in host/symbiont interactions within the context of a manipulative climate change experiment.

We focus on three groups of symbionts (Box 2). Fungi in the genus *Epichloë* are endophytes that systemically infect the aboveground tissues of many grasses (Fig. 1A), and are often assumed to be strong mutualists (Schardl 1996, Bush et al. 1997, Scott 2001), though they may also be pathogenic (Faeth and Fagan 2002, Brem and Leuchtmann 2002, Vandegrift et al. 2015). Arbuscular mycorrhizal fungi (AMF; Fig. 1B) colonize the roots of the vast majority of terrestrial plants (~80% of plant families) (Schüβler et al. 2001) and provide access to inorganic soil nutrients in exchange for photosynthate (Harley and Smith 1985). Dark septate endophytes (DSE; Fig. 1C) are a poorly studied, phylogenetically diverse group of root-inhabiting fungal endophytes

(Jumpponen 2001, Porras-Alfaro and Bayman 2011). Though previously assumed to be pathogens (Jumpponen and Trappe 1998), there is mounting evidence that DSE may function as pseudo-mycorrhizae in some contexts (Upson et al. 2009, Alberton et al. 2010). All three of these groups of symbionts may exist across the full spectrum of the mutualist/pathogen continuum.

Box 2: Overview of symbionts examined

Epichloë (Fig. 1A; anamorphic synonym: *Neotyphodium*) are a genus of predominantly endophytic fungi in the family Clavicipitaceae. Although many *Epichloë* species may be seedborne, and thus tightly linked to their host's fitness (Schardl 1996), horizontal (contagious) transmission is possible via both sexual (Brem and Leuchtmann 1999) and asexual (Tadych et al. 2007) means. These are systemic foliar endophytes of cool-season grasses (Poaceae), colonizing the aboveground tissues of their hosts (Schardl 1996); since these fungi do not colonize root tissues, we presume that interactions with root symbionts are primarily via signaling or competition for host photosynthate. They are generally considered strong mutualists because they produce fungal alkaloids, which can reduce herbivory on the host plant (Schardl 1996, Bush et al. 1997, Scott 2001). Some fungi in this genus have also been linked experimentally with drought tolerance and increased competitive abilities (Malinowski et al. 2005). A growing body of work, however, demonstrates that they can be pathogenic in certain circumstances (Faeth et al. 1999, Faeth 2000, Faeth and Fagan 2002, Brem and Leuchtmann 2002, Vandegrift et al. 2015). The metabolic cost to the plant of hosting an *Epichloë* endophyte must be balanced by the fitness increase that the endophyte provides.

Arbuscular mycorrhizal fungi (AMF; Fig. 1B) are well known fungal symbionts of plants that provide access to inorganic soil nutrients, most notably phosphorus, in exchange for host photosynthate (Harley and Smith 1985). AMF have also been linked to uptake of other soil nutrients (Li et al. 2006, Smith and Read 2008), protection from root pathogens (Newsham et al. 1995, Smith and Read 2008), and drought tolerance (Ruiz-Lozano et al. 1995).

The definitive demonstration that a carbon "marketplace" exists between host plants and AMF (wherein plants can allocate carbon to mycorrhizal partners that provide more phosphorous) did not come until relatively recently (Kiers et al. 2011). The existence of such a marketplace provides a mechanism for the discouragement of cheaters, and demonstrates that plants can control where carbon is allocated over fairly fine spatial scales within their root systems (Selosse and Rousset 2011, Kiers et al. 2011, Grman et al. 2012).

This is not to say that AMF cannot be pathogenic in certain contexts. For example, if there is an abundance of available phosphorous, non-mycorrhizal plants perform better than those colonized by AMF (Johnson 1993, Klironomos 2003,

Johnson et al. 2004, Landis and Fraser 2008). Environmental conditions determine the benefit of the symbiosis for the host.

Dark septate endophytes (DSE; Fig. 1C) are a poorly studied group of fungal endophytes found in plant roots; they are, however, starting to receive more attention (Collins et al. 2008, Urcelay et al. 2010, Porras-Alfaro and Bayman 2011). These common, widely distributed root endophytes are distinguished by their brown cell walls, which are darkly pigmented by fungal melanins. Dark septate root endophytes colonize hosts from across the plant kingdom, and include fungi from multiple phyla, though Ascomycota predominate (Jumpponen 2001). They are known to co-exist with mycorrhizal fungi within plant roots (Girlanda et al. 2002, Li and Guan 2007). Previously assumed to often be root pathogens (Jumpponen and Trappe 1998), DSE have recently been linked to increased plant nutrient uptake, particularly of nitrogen (Upson et al. 2009, Alberton et al. 2010), and growth (Jumpponen et al. 1998, Newsham 1999, Arnold et al. 2000). As with AMF, these fungi exist upon a continuum—the benefits to the host must outweigh the metabolic costs incurred for these fungi to be truly mutualistic (Mandyam and Jumpponen 2015).

There is evidence of competition between *Epichloë* endophytes and AMF in multiple grass species (*Brachypodium sylvatica*, *Lolium perenne*, *Lolium multiflorum*, and *Schedonorus phoenix*) (Müller 2003, Omacini et al. 2006, Mack and Rudgers 2008, Liu et al. 2011). There is also some evidence of a more cooperative relationship in some cases (Novas et al. 2005, 2011). It is reasonable to expect that these two types of fungi may interact within all their hosts. Though there is little research on the subject to date, there are some reports suggesting AMF/DSE competition (Kandalepas et al. 2010, Urcelay et al. 2010), as well as potential facilitation (Lingfei et al. 2005, Scervino et al. 2009).

To examine these multi-symbiont interactions, we quantified percent root length colonized (PRLC) by both AMF and DSE, and tested for the presence of systemic foliar *Epichloë* endophytes within a single host species (*Agrostis capillaris* L.) across a broad climatic gradient within the context of a manipulative climate change experiment

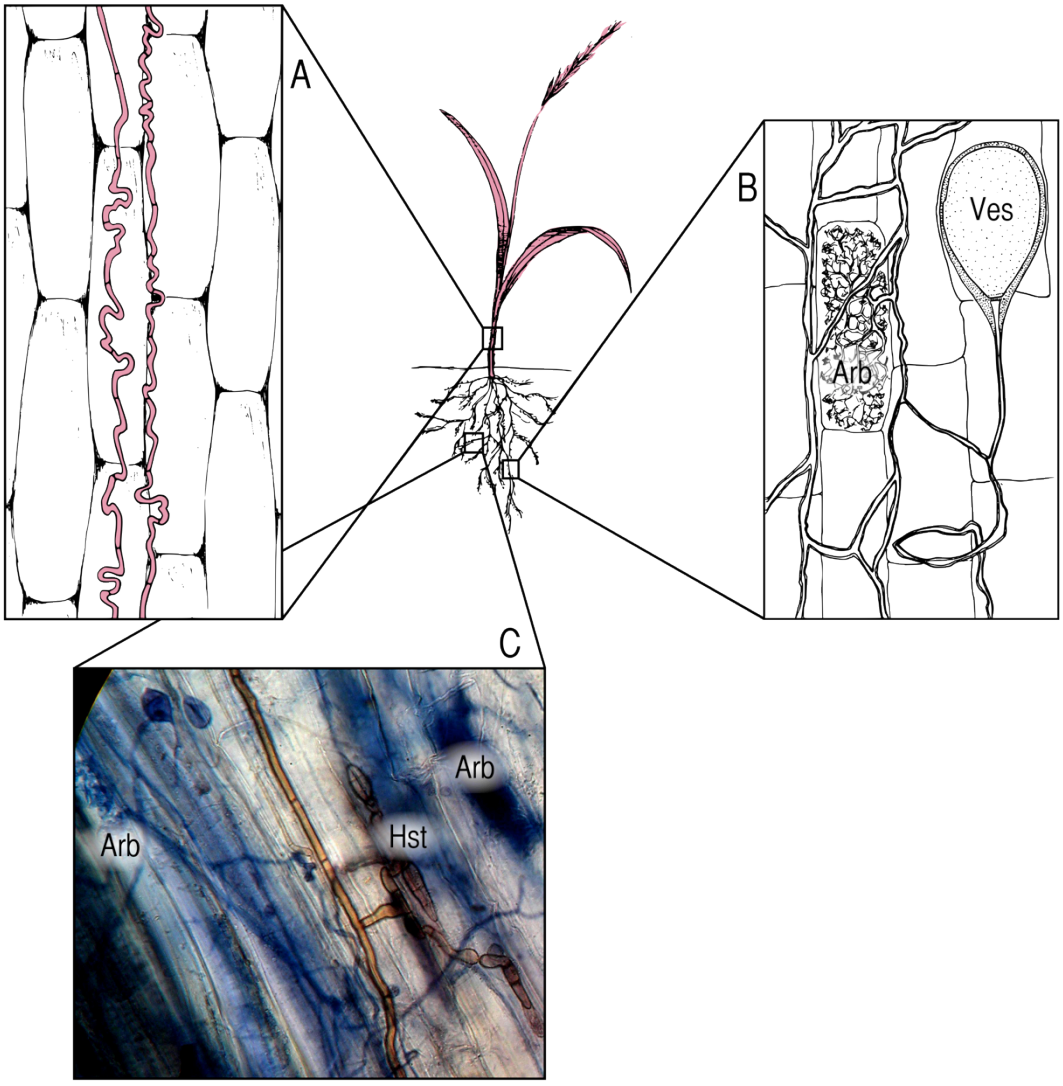


Figure 1: Symbionts and locations within the host plant. (A) *Epichloë* endophytes, pictured in red, systemically infect the aboveground tissues of host grasses, growing between cells; (B) AMF colonize the roots of their hosts, forming characteristic nutrient exchange structures called arbuscules (Arb.) and storage vesicles (Ves.); (C) DSE colonize roots as well, and are often found in association with AMF. The photomicrograph (400x) shows brown DSE colonizing the same segment of an *Agrostis capillaris* root as AMF, with DSE haustoria (Hst.) in close proximity to mycorrhizal arbuscules (Arb.).

(detailed in: Pfeifer-Meister et al. 2013). We examined how these fungal symbionts interacted to affect host fitness across a broad range of environmental conditions.

Considering that plant symbionts largely exist on a mutualist/pathogen continuum, we hypothesized that we would find evidence that the symbionts in our system were in competition for host resources—photosynthate, space within roots, etc.—and that the outcome of that competition could be driven by the benefit to the host. In other words, we hypothesized that the host plants would be able to allocate carbon to the symbionts in such a way as to maximize fitness benefit within a particular environmental context. Specifically, we expected changes along soil nutrient and soil moisture gradients to alter the balance between symbionts, favoring AMF over DSE and *Epichloë* in drier and more nutrient-poor soils. We also expected that symbionts could alter host response to environmental conditions; in particular, we expected that correlations between AMF/DSE PRLC and fitness would change predictably along environmental gradients.

Methods

Site descriptions

This study was conducted within the framework of a large manipulative climate change experiment in PNW grasslands, described fully in Pfeifer-Meister et al. (2013). We utilized two (of three) experimental sites: one at The Nature Conservancy's Willow Creek Preserve at the southern end of the Willamette Valley in Eugene, Oregon (44°1'34"N/ 123°10'56"W), and one at The Nature Conservancy's Tenalquot Prairie Preserve, managed by the Center for Natural Lands Management, in western Washington (46°55'6"N/ 122°42'47"W). Willow Creek has mean annual precipitation of 1201 mm,

while Tenalquot Prairie has 1229 mm; mean annual temperatures at the two sites are are 11.4 ºC and 9.8 ºC, respectively (Pfeifer-Meister et al. 2013). The soil at Tenalquot Prairie is a gravelly sandy loam Andisol (sandy-skeletal, amorphic-over-isotic, mesic Typic Melanoxerand), whereas the Willow Creek soil is a silty-clay loam Mollisol (very-fine, smetitic, mesic Vertic Haploxeroll).

Prairies and oak savannas historically dominated much of the interior valleys along the Pacific coast from central California to southern British Columbia. The two study sites occupy the Willamette Valley and Puget Lowland Level III ecoregions, respectively (U.S. EPA 2011). These ecosystems were maintained by drought-season fire, often of anthropogenic origin, which prevented succession to woodland or forest (Boyd 1986, Walsh et al. 2010, Walsh et al. 2010). Before Euro-American colonization, 50% the Willamette Valley floor and lower foothills was prairie or savanna (Christy and Alverson 2011). Presently, however, only 2% of this remains (Baker et al. 2002), and such grasslands are among the most endangered ecosystems in the United States due to fire suppression, land-use change, habitat fragmentation, and invasions by exotic plants and animals (Noss et al. 1995).

Climate manipulations and plot measures

All plots were treated with spring and autumn applications of the herbicide glyphosate, followed by mowing and thatch removal. In December 2009 all plots were seeded with the same mixture of 32 native upland prairie graminoids and forbs.

Each site had twenty 3 m diameter circular plots (7.1 m^2) fully crossing heat (+3.0˚C) and precipitation (+20%) treatments. Temperature was increased in the

experimental plots with six overhead 2000-W infrared heat lamps (Kalglo Electronics, Inc., Bethlehem, PA) angled at 45° to the surface (Kimball et al. 2008). Precipitation intensity was increased by 20% by hand-watering from an on-site rainwater collection system using a gauged hose within two weeks of the most recent rainfall. This led to most of the increased precipitation being applied during the wet season, and very little being applied in the summer, mirroring GCM predictions for the region (Meehl et al. 2007, Mote and Salathé 2010). All ambient temperature plots had wooden imitation heaters suspended overhead, to control for any effect of shading by the infrared heaters. Precipitation treatments were initiated in the spring of 2010, and heating treatments were initiated by autumn of 2010.

Soil temperature was measured continuously at the center of each plot at 10 cm depth by thermistors (model 107, Campbell Scientific, Logan, UT, USA); volumetric water content (0-30 cm) was measured continuously at the center of each plot by time-domain reflectometry (model CS616-L, Campbell Scientific, Logan, UT, USA). Soil nitrogen and phosphorous availabilities (5-10 cm depth) were measured using anion/cation exchange resin probes (PRS™ Western Ag Innovations Inc., Saskatoon, Canada) from April–July 2011. Nitrogen from ammonium and nitrate ions were combined into a single measure of inorganic nitrogen, though nitrate predominated at both sites.

Focal species and sample collection/preparation

Agrostis capillaris L. (colonial bentgrass) is a perennial bunchgrass native to Eurasia with a stoloniferous habit and an observed preference for dry soils (Hubbard 1984).

Despite this observed preference, reports of its drought tolerance are conflicting (Hubbard 1984, Dixon 1986, Ruemmele et al. 2000). Since our central questions resolved around the interactions between *Epichloë*, AMF, and DSE within a single host, we chose a grass species that hosts all three symbionts. We focused on an introduced species so that harvesting for our study did not affect the community ecology experiments that were concurrently underway at these sites. *Agrostis capillaris* plants within the treatment plots were most likely germinants from the seed bank following the herbicide treatments, or potentially germinants from seeds dispersed in from the surrounding fields; there is also a small potential that some stolons survived the herbicide treatment.

In June–July of 2011 we collected four first-year *A. capillaris* plants from each plot, selecting one plant from the center each of the four quadrants of the plot, at both the Tenalquot Prairie and Willow Creek sites (4 plants × 20 plots × 2 sites = 160 total plants). At the time of flowering, the plants were collected whole, dug up with the root systems intact. Shoot and root tissues were separated, and the shoot tissues were tested for *Epichloë* infection using the Agrinostics Field Tiller immunoblot kit (Agrinostics Ltd. Co., Watkinsville, GA, USA), and then all aboveground biomass (AGB) was dried at 60˚C for three or more days. Aboveground biomass is well established as a reliable measure of plant fitness (Shipley and Dion 1992) and is frequently used in studies where counts of reproductive output are not feasible. In particular, it is highly correlated with reproduction in *A. capillaris* in our own research (Goklany 2012).

Root tissues were cleaned and stained for quantification of percent root length colonized (PRLC) by focal symbionts. Arbuscular mycorrhizal fungi are often assessed by PRLC, providing a measure of the host/symbiont interface linked to plant fitness and

phosphorus transfer (Treseder 2013). The PRLC methods traditionally applied to AMF have only recently been applied to other root colonizing fungi, such as DSE (Weishampel and Bedford 2006, Mandyam and Jumpponen 2008, Upson et al. 2009, Dolinar and Gaberščik 2010, Zhang et al. 2013). We used a modified version of Vierheilig's ink and vinegar staining technique (Vierheilig et al. 1998), soaking roots overnight at room temperature in 10% (w/v) KOH to clear them, rinsing several times in deionized water, then staining overnight in a 5% (v/v) ink-vinegar solution using white household vinegar (5% (w/v) acetic acid) and Shaeffer's Black drawing ink. Roots were then rinsed several times in deionized water acidified with a few drops of vinegar (Vierheilig et al. 1998). Eleven one-centimeter segments were selected at random from each root system and mounted to glass slides in polyvinyl lacto-glycerol. Slides were examined at 200x magnification and colonization percentages were obtained using McGonigle's magnified intersections method (McGonigle et al. 1990). Arbuscules, vesicles, and hyphae were quantified. In *Agrostis capillaris*, we found that colonization, where present, was generally very dense, with overlapping arbuscules, vesicles, and hyphae. As such, all analyses are presented with an aggregate measure of total AMF colonization.

Statistical analyses

Soil volumetric water content was converted to soil matric potential using site-specific values of soil texture and organic matter (Saxton and Rawls 2006), allowing for direct comparisons between sites. The average plot values of data for a twenty-day window before harvest were used in all analyses. We considered other windows, as well as temporally local maxima and minima, and found that the twenty-day window explained

the most variance in the data (though 5- to 30-day windows had similar explanatory power).

Analysis of variance (ANOVA), analysis of covariance (ANCOVA), and regression analyses were used to examine effects of heating and precipitation on the fungal partners and the AGB of the host plants. We used individual plants as the replicate unit. Proportional data was transformed with the logit transformation to meet ANOVA's requirements of normality. All ANOVA, ANCOVA, and regression analyses were performed in R version 2.15.1 (R Core Team 2012). Site, treated as a random effect, was not significant in any model that took into account the differential N:P and soil moisture between sites, so it was excluded from analyses in favor of these variables. More extensive site characterization supports this approach to analysis (Wilson 2012, Pfeifer-Meister et al. 2013).

Structural equation modeling (SEM), a classic multivariate technique related to multiple regression and path analysis (McCune & Grace 2002), was used to examine hypothesized relationships among multiple symbionts within a single host, environmental conditions, and host response in the form of AGB. Given our relatively small sample size (n = 155), we attempted to meet the guideline of a 5:1 ratio of samples to free parameters (Bentler & Chou 1987), and limited the number of selected variables within the confines of our hypothesis (Tanaka 1987). We used bivariate scatter-plots, Pearson's correlations, and linear regression to evaluate whether these relationships met the normality and linearity assumptions for SEM (Grace 2006). No variables were found to possess strong co-linearity, but the soil nitrogen and phosphorus data were found individually to have almost zero explanatory power, and were thus omitted from the models. However,

nitrogen-to-phosphorous ratios were kept in the models, and have been suggested by others to be a more powerful predictor of AMF responses than net availability of either nutrient alone (Johnson 2010).

Our *a priori* hypotheses defined the models we tested (Fig. 2). We expected AMF and DSE to be correlated, and we expected each environmental variable (soil temperature and matric potential, as well as nitrogen-to-phosphorous ratio) to be able to affect percent root colonized by either symbiont, as well as AGB of the host. Additionally, we expected soil temperature to have a strong effect on soil matric potential, and for matric potential and temperature to have an effect on N:P ratio. We specified separate models for *Epichloë*-infected (E+) and *Epichloë*-free (E−) host plants, comparing changes in direction, magnitude, and significance of relationships to examine the effect of *Epichloë* infection on relationships between other symbionts, the host, and the environment. Proportional data were logit transformed to satisfy distributional and linearity assumptions. Plant was again used as the unit of replication.

The relationships amongst all variables were modeled as path coefficients, which represent the magnitude and direction of the effect of each predictor variable on a response variable with all other variables held constant. SEM analysis was conducted in IBM's SPSS Amos (v20.0) software (SPSS Inc., Chicago IL, USA), using a maximum likelihood approach to model evaluation and parameter estimation. Model fit was evaluated using the χ^2 goodness-of-fit statistic and associated *p*-values, Bentler Comparative Fit Index (CFI), and Root Mean Square Error of Approximation (RMSEA). CFI values range between 0 and 1, with higher values indicating better model fit (Bentler

& Chou 1987), and tend to underestimate model fit when sample sizes are small (Bishop & Schemske 1998).

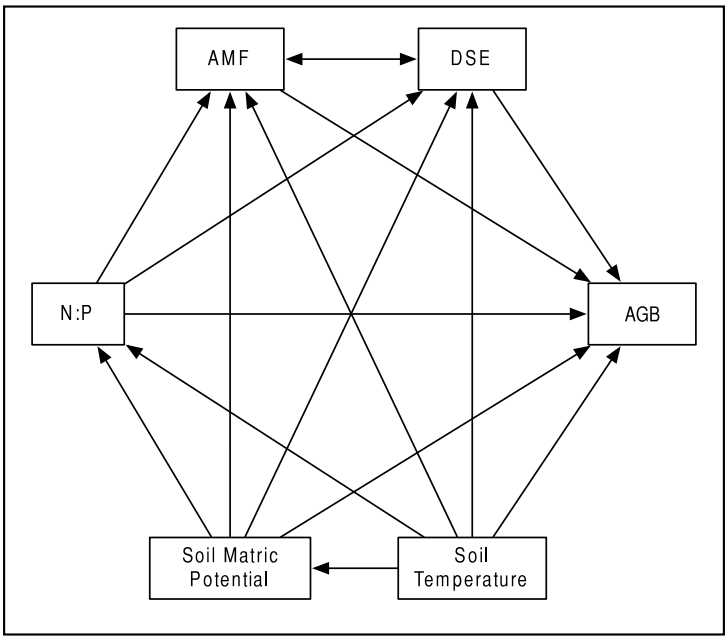


Figure 2: Schematic of our Structural Equation Model, which illustrates our *a priori* hypotheses. Arrows represent predicted direct effects of one variable on another; double headed arrows represent correlations. Variables included in the model were AMF percent root length colonized (AMF), DSE percent root length colonized (DSE), aboveground biomass of the plants (AGB), soil matric potential, soil temperature, and soil nitrogen-to-phosphorus ratios (N:P).

Results

For ease of comparison throughout this paper, figure-elements representing groups/samples hosting *Epichloë* endophytes are shown in red (E+), while those groups/samples not hosting *Epichloë* endophytes are shown in blue (E−).

Infection with *Epichloë* was 36% (n = 155), and was uncorrelated with any environmental variable (see Supplemental Figures S1-S11 in Appendix B). We found no

evidence of competition between symbionts. *Epichloë* infection did not affect root length colonized by either AMF (Fig. 3A; $F_{1, 153} = 0.956$, $P = 0.330$) or DSE (Fig. 3B; $F_{1, 153} = 0.083$, $P = 0.774$). Percent root length colonized by AMF and DSE were correlated positively (Fig. 4, Fig. S1; Adjusted $R^2 = 0.107$, $F_{1, 153} = 19.51$, $P < 0.001$), indicating facilitation rather than competition.

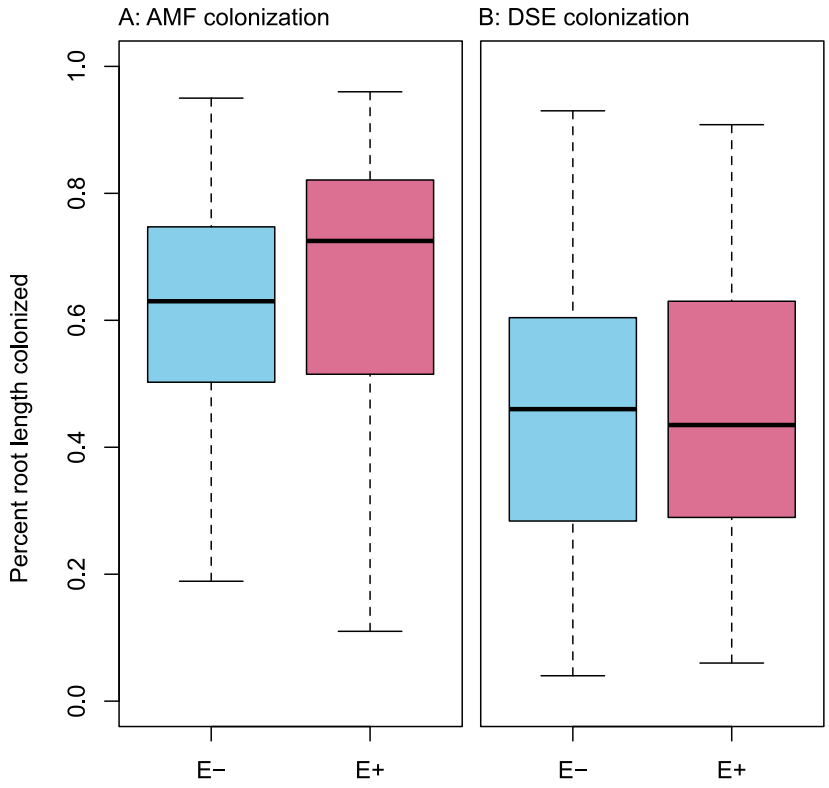


Figure 3: Percent root length colonized by AMF (A) and DSE (B) for plants without *Epichloë* endophytes (E−, blue), and those hosting *Epichloë* endophytes (E+, red).

Structural equation model fit was good; both the E+ (n = 56) and E− (n = 99) structural equation models had non-significant χ^2 values ($P > 0.10$) and Bentler CFIs > 0.90. Magnitude of standardized path coefficients differed between the E+ and E− models, but these were relatively minor. The only substantial difference between the models was a negative correlation between DSE root length colonized and plant biomass,

but only in the absence of *Epichloë* infection (Fig. 4 & S2; E+ Adjusted $R^2 = 0.029$, $F_{1, 54} = 2.644$, $P = 0.110$; E− Adjusted $R^2 = 0.053$, $F_{1, 97} = 6.437$, $P = 0.013$). DSE colonization decreased when more water was available to plants (Fig. 4 & S3; Adjusted $R^2 = 0.107$, $F_{1, 153} = 19.5$, $P < 0.001$). There was a direct negative effect of warmer soil temperatures on

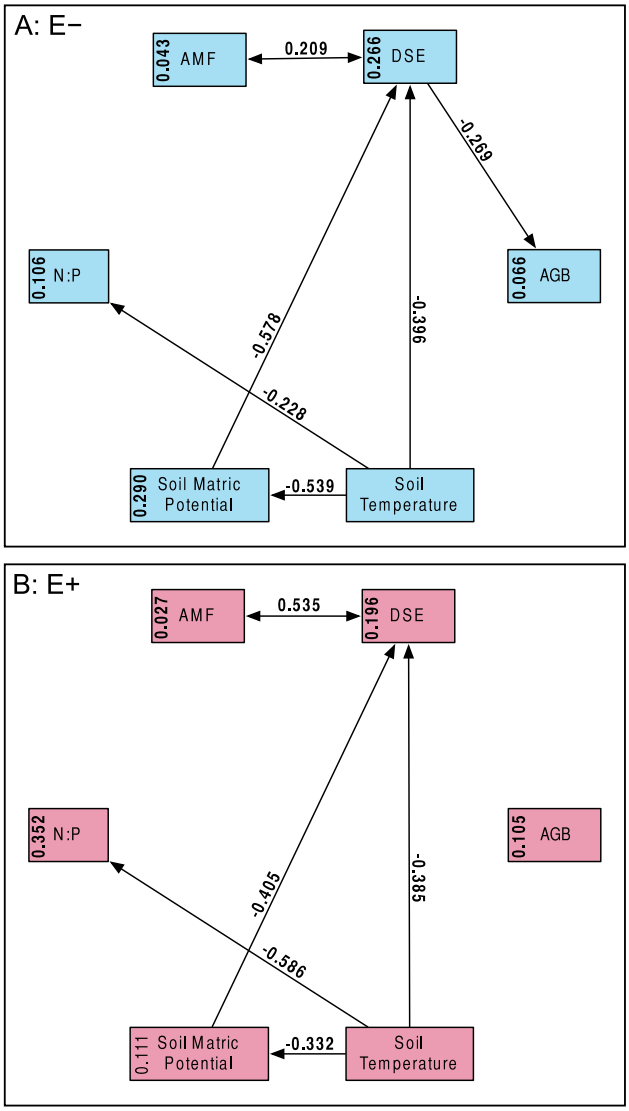


Figure 4: Overall SEMs, with different models for those plants without *Epichloë* endophytes (A: E−, blue), and those with *Epichloë* endophytes (B: E+, red). Model fit was good for both models (A: $\chi^2 = 2.50$, $P = 0.114$; CFI = 0.981; RMSEA = 0.124; n= 99 | B: $\chi^2 = 0.63$, $P = 0.427$; CFI = 1.000; RMSEA < 0.001; n = 56). The numbers above the arrows are the standardized path coefficients. Non-significant ($P > 0.05$) path coefficients are not shown. Numbers in the boxes are total explained variance (R^2) of each variable.

DSE colonization as well, which regression analysis does not recover (Fig. 4). Neither AMF colonization nor proportion of plants hosting *Epichloë* varied significantly with measured edaphic conditions (soil moisture, soil temperature, soil N:P ratios; Fig. 4 & Figs. S4–S9).

Discussion

Our initial hypotheses centered on competition between symbionts within a shared host: we expected to find evidence that consortia of symbionts changed with environmental conditions in such a way as to minimize changes to host fitness (and maximize fitness in a given environmental context). In other words, we expected there to be interactions among environmental variables (soil temperature, moisture, and N:P ratios) and the fitness costs/benefits of colonization by different symbionts. In addition, we expected to find evidence of competition between symbionts. Lastly, we expected the outcomes of that competition to be stabilized by the fitness benefits to the host.

What we found instead was no evidence of competition between symbionts: neither root symbiont seems to be affected by presence of *Epichloë* endophytes in the aboveground tissues of the host (Fig. 3). If anything, AMF and DSE appeared to have a facilitative rather than a competitive interaction (Fig. 4 & Fig. S1). We also did not find any effect of AMF colonization or *Epichloë* presence on plant fitness as measured by AGB (Fig. 4 & Figs. S10–S11). Aboveground biomass is known to be highly correlated with reproduction in *A. capillaris* (Goklany 2012), and is often used as a surrogate for overall fitness (Shipley and Dion 1992). We did find a negative effect of DSE colonization on AGB, but only in the absence of *Epichloë* endophytes (Fig. 4 & Fig. S2),

suggesting that the presence of *Epichloë* counteracts the otherwise negative effects of dark septate root endophytes.

*DSE/*Epichloë *interaction*

To our knowledge, this is the first study to examine interactions between DSE and *Epichloë* endophytes. We found a significant effect of DSE root length colonized on plant biomass, but only when the host plants did not also host foliar *Epichloë* endophytes.

Dark septate root endophytes have been studied very little, though there has been broader interest recently (Porras-Alfaro and Bayman 2011, Mandyam and Jumpponen 2015). These fungi show a wide range of effects on their host plants, from mutualism to pathogenicity (Jumpponen 2001, Mandyam and Jumpponen 2005, Grünig et al. 2008, Alberton et al. 2010, Newsham 2011, Mandyam et al. 2012, 2013, Mayerhofer et al. 2013). The variability of response of the host plant is likely linked to the variability of the DSE species being studied, the genetic combinations of particular host/symbiont pairs, and the environmental context within which the experiment takes place (Mandyam et al. 2012, Mandyam and Jumpponen 2015). That environmental context includes the entire consortium of interacting fungal symbionts within a given host (Munkvold et al. 2004, Grünig et al. 2008, Mandyam et al. 2012, 2013), as our findings demonstrate.

Inoculation studies support the function of DSE as 'pseudo-mycorrhizal' in that they have been shown to translocate N or P into their hosts (Jumpponen et al. 1998, Newsham 2011), but N uptake seems to be the more common role for DSE in this context (Upson et al. 2009, Alberton et al. 2010, Newsham 2011). *Epichloë* endophytes are well known for producing fungal alkaloids which discourage herbivory (Schardl 1996, Bush et

al. 1997), including those species known to associate with *Agrostis capillaris* (Funk et al. 1993, Porter 1995, Schardl & Phillips 1997, Leuchtmann et al. 2000). These alkaloids are costly to produce, particularly in terms of nitrogen (Belesky et al. 1988, Faeth and Fagan 2002)—although, it has been suggested that carbon may also limit alkaloid biosynthesis (Rasmussen et al. 2008). Thus, herbivory reduction by *Epichloë* endophytes may be dependent upon soil nutrient levels (Lehtonen et al. 2004). We theorize that the interaction we saw between DSE root length colonized and *Epichloë* infection may be the intersection of these two things: in the absence of an *Epichloë* infection, the fitness increase from N gained by hosting more DSE is not offset by the metabolic (i.e., carbon) cost of hosting the DSE, but when also hosting *Epichloë* endophytes, the increased N uptake can be allocated to plant defense by way of fungal alkaloids, thus offsetting the costs of hosting the DSE. Such interactions may also be affected by priority effects, and may be differential in the case of seedborne *Epichloë* transmission versus horizontal transmission. Much more work will be required to investigate this theory.

AMF/DSE interaction

We found a positive correlation between AMF root length colonized and DSE root length colonized, as well as generally high colonization values for both fungi (Figs. 3–4, Fig. S1). This correlation was not influenced by *Epichloë* endophyte infection, site, or climate treatment.

The few studies to date examining the interactions between these two common root symbionts have found conflicting results. Competition between AMF and DSE is reported from wetland plants in Louisiana by Kandalepas and colleagues (2010), who

found that plants that had greater AMF colonization generally had lower DSE colonization, and vice versa. These results are similar to those of Urcelay and colleagues (2011), who report that high alpine species of the Altiplano in Bolivia display evidence of a tradeoff between AMF and DSE root colonization. However, a study in Chinese grasslands found that DSE colonization was generally positively correlated with AMF hyphal—but not arbuscular or vesicular—colonization (Lingfei et al. 2005).

However, these studies examined variation in AMF/DSE colonization *between* host species, not *within* a single host species. Within the bounds of variation for a particular host species, the relationship between the two symbionts might be quite different; for example, Scervino and colleagues (2009) found that exudates from a particular DSE could stimulate lengthening and branching of AMF hyphae *in vitro*, which indicates a facilitatory effect, consistent with our results; interestingly, similar effects have been observed with exudates from *Epichloë* endophytes (Novas et al. 2011). Future research should focus on these host/symbiont pair-specific interactions within single plant host species.

Context-dependence

Given the broad importance of AMF, DSE, and *Epichloë* symbioses to ecological (Porras-Alfaro and Bayman 2011, Mohan et al. 2014, Mandyam and Jumpponen 2015) and economic systems (Hoveland 1993, Dodd 2000), we feel it is important to emphasize that the system of interaction we have observed here represents a single set of symbioses. As discussed above, the identities and genetic backgrounds of the particular host/symbiont partners are of great importance to the outcome of the association

(Ahlholm et al. 2002, Klironomos 2003, Mandyam and Jumpponen 2015); additionally, the environmental context within which a particular host/symbiont pair interact is of great importance to the outcome of the association (Ahlholm et al. 2002, Landis et al. 2004, Roy et al. 2004, Mandyam and Jumpponen 2015).

In an attempt to examine the generalizability of these results, we initiated a small, similar study, also within the context of the larger manipulative climate change experiment (data available in Vandegrift et al. 2015). We used the annual grass *Bromus hordeaceus* L. for this experiment, and collected data in a similar manner, but only at the southern-most site, which has much greater soil nutrient availability and total precipitation, but also much more extreme seasonal climate variation (see Pfeifer-Meister et al. 2013). These samples from only a single site covered a much narrower climatic envelope than the *Agrostis* dataset, and were much more limited in sample size, particularly the E+ samples (n = 19). With these caveats in mind, we found very different results: in the *Bromus* dataset, *Epichloë* infection changed the response of AMF, DSE, and host AGB to environmental variables; there was no correlation between AMF and DSE; and while *Epichloë* infection still modulated the effect of DSE colonization, the effect of DSE colonization on E− plants was positive, not negative (Fig. S12).

These differences highlight that the spectrum of host responses to symbiont consortia and environmental conditions is very much dependent upon the identities of the host and symbionts, as well as the particular set of environmental conditions within which the host/symbiont groupings are set. The importance of context-dependence, and species-specific idiosyncratic responses to abiotic factors has long been noted (Brown & Ewel 1987, Wardle et al. 2004, Roy et al. 2004, Agrawal et al. 2007).

This study relies on microscopic observation of DSE and AMF, and immunoblot identification of *Epichloë* infections, which limits our ability to determine specific species-by-species interactions between the symbionts. As discussed in the introduction, DSE are very phylogenetically diverse (Porras-Alfaro and Bayman 2011), and though they are often treated as a single functional group, they may play very different roles in different contexts simply because they are different organisms (Mandyam and Jumpponen 2015). Similarly, different species of AMF have been shown to have different functional roles (Munkvold et al. 2004), which may interact differently with DSE and *Epichloë* symbionts. Future work should focus on connecting the functional roles of these various symbionts with particular taxonomic groups, and attempt to link fungal microbiome data with careful microscopic observation across climatic gradients.

Integration of effects of symbiont consortia

Given the preponderance of emerging data about the complexity of AMF, DSE, and *Epichloë* endophyte ecology, a conceptual framework that synthesizes these advances is clearly necessary. Such a conceptual framework must take into account evidence for all partners, including: host specificity (Leuchtmann 1993, Vandenkoornhuyse et al. 2003, Martínez-García and Pugnaire 2011) and host generalism (Bever et al. 2001, Grünig et al. 2008, Smith and Read 2008); the functional diversity of fungal partners, even within single functional groups like AMF (Helgason et al. 2002, Öpik et al. 2009); colonization of the same host individual by multiple species of fungi (Palmer et al. 2010, Mandyam and Jumpponen 2015); changes in symbiont communities with changes in the abiotic environment (Martínez-García and Pugnaire 2011), including seasonal changes (Bever et

al. 2001); and the co-evolutionary history between terrestrial plants and their mycobiota (Carroll 1988).

Facilitation between fungal species within a host may play a role in symbiont community determination: it has been demonstrated that both DSE and *Epichloë* derived exudates can affect the growth of AMF (Scervino et al. 2009, Novas et al. 2011), and our study supports facilitation between AMF and DSE, as well as synergistic effect of DSE colonization and *Epichloë* infection on host fitness. Indeed, facilitatory interactions need not be restricted to within single hosts: given the demonstrated movement of photosynthate between host species through mycorrhizal networks (Martins and Read 1996, Martins and Cruz 1998, Pringle 2009), connectivity between hosts by different species of fungi may be just as important to supporting struggling populations of fungi as it is to struggling plants.

Given this community framework, it is reasonable to expect that selective pressure on the host will favor host/symbiont relationships that structure the community of symbionts in the most beneficial way possible for the plant, not necessarily the individual symbiont that is most beneficial to plant fitness in isolation. The fitness effect of the consortium of symbionts is the integration of all fitness costs and benefits of all partners. The particular community assemblage of symbiotic fungi associated with a particular host will then be predicated upon the physiology of the host, the available inoculum, the interactions of the symbionts, and the abiotic environment's effects on both the host and the fungal partners (Schlaeppi and Bulgarelli 2014).

Bridge to Chapter IV

And so we come to the end of Section A of my dissertation. In Chapter III, I had set myself the challenge of pushing the boundaries of the extensive grass/*Epichloë* knowledge base, and I succeeded, though the results where perhaps a bit more ambiguous than I had desired. The theoretical underpinnings of this study were that the consortium of symbionts interact in the ways that communities of organisms always interact, but that the balance of competition and facilitation might be nudged by the host, which is the environment to these organisms, to the host's own benefit. This was interesting, however centered solidly on the host. A common theme in the field is the centering of theory on the hosts, rather than on the endophytic fungi themselves. This was beginning to bother me. For my next chapter, I wanted to take not a plant-centric view of symbiosis, but a myco-centric view; I wanted to know what the fungal symbiont got out of the deal, not what it did for its landlord. I decided to stop asking 'how much do they pay the slumlord in rent?', but instead 'why are they living in a tenement in the first place?'. To this end, I started exploring ecological theories that might explain why fungi want to be endophytes at all. The fortuitous congruence of having George C. Carroll (the archetect of the Foraging Ascomycete hypothesis) as a mentor, at the same time that my advisor Bitty A. Roy was doing work in Ecuador, was too much to ignore. For my next chapter, I traveled to Ecuador with Bitty to put George's old theory to the test, thus embarking on Section B of my dissertation.

CHAPTER IV

SPATIAL ECOLOGY OF THE FUNGAL GENUS *XYLARIA* IN A TROPICAL CLOUD FOREST

From Thomas, D.*, Vandegrift, R.*, Ludden, A., Carroll, G. C., & Roy, B. A. 2016. Spatial ecology of the fungal genus *Xylaria* in a tropical cloud forest. Biotropica. DOI: 10.1111/btp.12273

*D. Thomas and R. Vandegrift contributed equally to this work.

CONTRIBUTIONS

D. THOMAS AND R. VANDEGRIFT CONTRIBUTED EQUALLY TO THIS WORK; both did field work and lab work, conceptual work, statistical work, and co-wrote the paper. A. Ludden did much of the molecular work. G. C. Carroll contributed to conceptual design and theoretical grounding. B. A. Roy contributed to design work, and contributed reagents/materials/analysis tools. All authors reviewed drafts of the paper.

INTRODUCTION

MUCH OF THE REASON FOR RECENT INTEREST IN THE PLANT MICROBIOME is economic, as awareness grows that the plant microbiome is vital to plant health (Carroll 1988, Berendsen *et al.* 2012, Berlec 2012, Chaparro *et al.* 2012), and may be important in mitigating effects of disease and climate change on human food plants (Köberl *et al.*

2011, Woodward *et al.* 2012). Fungal endophytes, an important component of the plant microbiome, are receiving particular attention (Porras-Alfaro & Bayman 2011, Jones 2013). Fungal endophytes are defined functionally, as those fungi found within living, healthy plant tissues; they make their living by not harming their host enough to induce a defensive reaction (Clay 1990, Rudgers *et al.* 2009). Since their discovery, they have been found to be both ubiquitous and incredibly diverse in plants of all ecosystems (Arnold & Lutzoni 2007, Porras-Alfaro & Bayman 2011).

While numerous benefits to fitness for host-plant partners in the endophytic symbiosis have been observed, and many more proposed (Rodriguez *et al.* 2009), benefits for the fungal partners remain something more of a mystery. To date, the majority of endophyte research has been on temperate-zone clavicipitaceous endophytes of grasses, which often affect herbivory and host physiology, and are thus both ecologically and economically important (Clay & Schardl 2002, Schardl *et al.* 2004, Saikkonen *et al.* 2006). These fungi infect their hosts systematically and are passed on directly to their host-plant's offspring (Clay 1988). The fitness of these fungi increases with increased health and survival of their plant host. On the other hand, many non-clavicipitaceous fungal endophytes are very closely related to known plant pathogens (Carroll 1988, Freeman & Rodriguez 1993), and are well armed with energetically expensive arrays of enzymes for digestion of plant-tissues (Carroll & Petrini 1983, Schulz *et al.* 1999). Some endophytes have been observed to be latent pathogens or saprotrophs, waiting for host-plant weakness or death to be the first to colonize and digest host tissues (Chapela & Boddy 1988, Osono 2006, Promputtha *et al.* 2007, Promputtha *et al.* 2010), an obvious fitness benefit for the fungi involved.

However, many fungal endophytes neither vertically transmit to host-plant offspring, nor act as latent pathogens or saprotrophs of host tissues (Lodge 1997). The benefit of endophytism, if any, for these fungi remains unknown. Endophytism appears on the surface to be detrimental to fitness because these fungi undergo an extended period with reduced metabolic rate (Stone *et al.* 2004), and reduced or non-existent rates of sexual reproduction.

How then could the endophyte life-history strategy, which is observed in hundreds of species of fungi, and every major lineage of non-lichenized Pezizomycotina, possibly be adaptive? There are many potential benefits of endophytism to the fungal partner: the period of quiescence, or reduced metabolic rate (Stone *et al.* 2004), may allow for persistence in the environment. The host plant potentially provides a stable carbon source, and the host may provide protection from environmental pressures such as desiccation (Chaves *et al.* 2002) and harmful UV radiation (Krauss *et al.* 1997). Endophytism may also play a role in dispersal, as we examine here

Much discussion has taken place in recent years over questions of microbial dispersal (Green *et al.* 2004, Green & Bohannan 2006, Martiny *et al.* 2006, Hanson *et al.* 2012). Dispersal is defined as any transport of propagules, individuals, or gametes that creates gene flow within or between populations (Ronce 2007, Clobert *et al.* 2012). Historically, microorganisms were thought to be functionally unlimited in their ability to disperse over the planet (Becking 1934, Fenchel & Finlay 2004). Despite this, many recent studies of microbes have uncovered evidence for dispersal limitation, or the inability of a strain or species to access and successfully establish itself in otherwise suitable habitat (Roy 2001, Telford *et al.* 2006, Green & Bohannan 2006, Grubisha et al.

2007, Peay *et al.* 2010, Galante *et al.* 2011). Such dispersal limitation may function to constrain the geographic ranges of some species, or the range of gene flow within or between local populations of a given species; indeed, such constraints on gene flow between populations are theorized as a major driver of speciation over evolutionary time scales (Clobert *et al.* 2012). There is evidence that at least some decomposer fungi are dispersal limited, even at local scales (Norros *et al.* 2012). Dispersal limitation may reduce fitness of an organism relative to competitors (Hurtt & Pacala 1995), suggesting that fungi may be under selective pressure to increase dispersal at both local and regional scales.

Dispersal involves successful transport and successful establishment of propagules (Hanson *et al.* 2012, Peay *et al.* 2012, Clobert *et al.* 2012). An endophytic life stage may enhance both of these processes: senescent leaves fall farther than the vast majority of spores are predicted to travel unassisted (Roper *et al.* 2010, Galante *et al.* 2011; Fig. S1; see Appendix C for all supplemental figures for this chapter), carrying with them mycelium, avoiding the uncertainty inherent in the germination phase of growth from spores. In evergreen forests, leaves generally fall asynchronously, which provides low propagule density over relatively long periods of time (in tropical cloud forests, leaves live 12 mo to >5 years; Bruijnzeel & Veneklaas 1998, Reich *et al.* 1991), in contrast to spore dispersal from a fruiting body, which provides high propagule density over relatively short periods of time (<1 year; Rogers 1979, Whalley 1996). Leaves may enhance colonization rates, by creating a sheltered microclimate favorable to inoculation. Additionally, living leaves may provide refugia for endophytic fungi, where fungi can wait out difficult conditions at low metabolic cost, benefiting from the protection

afforded by the leaf tissue (Stone 1987, Schulz & Boyle 2005). The idea of endophytism as a secondary life-history strategy for decomposer fungi to span (i.e., disperse across) scarcity of primary substrates and challenging environmental conditions in both time and space is known as the Foraging Ascomycete (FA) hypothesis (Carroll 1999).

Here, we attempt to critically examine the FA hypothesis in a cloud forest ecosystem, using the genus *Xylaria* Hill ex Schrank (Xylariaceae, Ascomycota) as an example of typical endophytic fungi that may utilize a FA strategy (Fig. 1). Members of this genus are important saprotrophs, found primarily on decomposing dead wood—and, rarely, on leaves and fruits—on the forest floor (Whalley 1996, Lodge 1997, Rogers 2000). *Xylaria* are visible during sexual sporulation, forming relatively large, macroscopic stromata, or "fruiting" structures (Bayman *et al.* 1998, Davis & Shaw 2008). *Xylaria* are common in virtually every study that has ever been done on endophytes, especially in tropical ecosystems (see Davis *et al.* (2003) for an extensive list). We focus here on a common endophyte genus to avoid the problem of being swamped in the overwhelming diversity of fungal endophytes in the tropics (Arnold *et al.* 2000, Arnold & Lutzoni 2007). These two life stages in *Xylaria,* leaf endophyte and wood decomposer, have been observed within single, tightly defined clades (Okane *et al.* 2008). Additionally, *Xylaria* grow readily in culture, making them ideally suited for study in laboratory conditions. (Whalley 1996, Bayman *et al.* 1998).

Following the FA hypothesis, we hypothesized that (1) distributions of wood-decomposing *Xylaria* should be spatially coupled to the distributions of those same *Xylaria* in the endophytic life stage. To test this hypothesis, we used a spatially explicit sampling scheme: we looked for spatial clustering not attributable to environmental

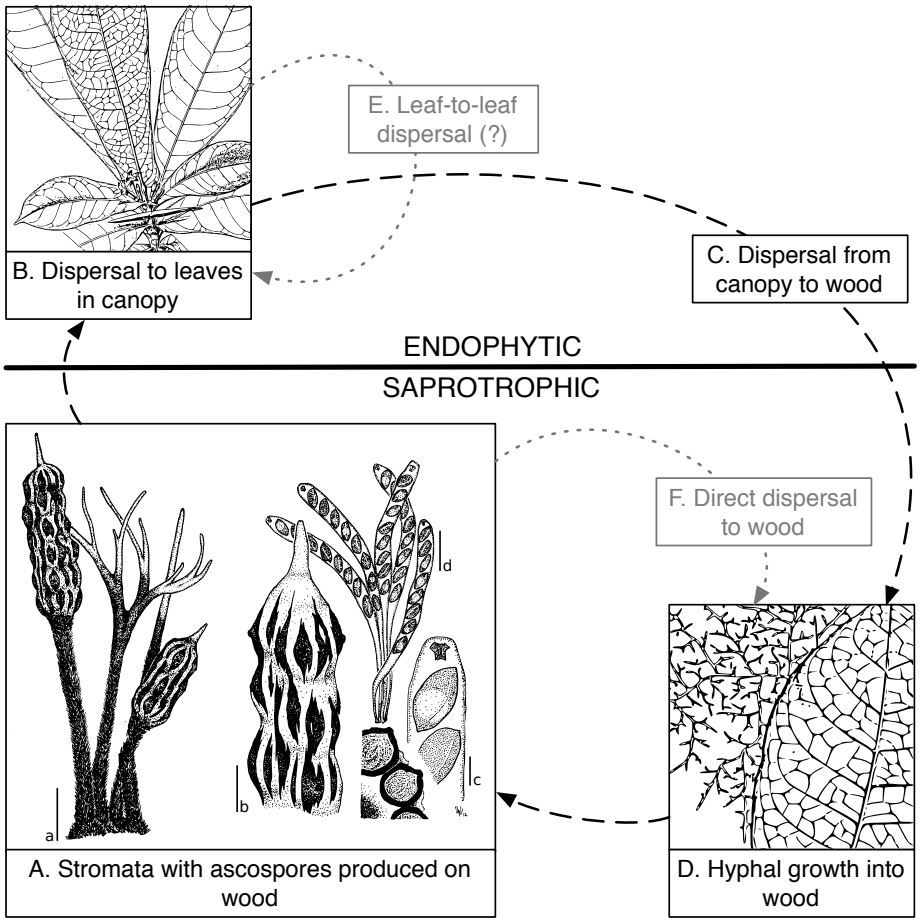


FIGURE 1. Schematic of proposed *Xylaria* life cycle, illustrating the Foraging Ascomycete hypothesis. Stromata (A) are produced on suitable substrate (generally wood); *Xylaria apiculata* Cooke, one of five *Xylaria* species present in both endophytic and decomposer life stages in this study, is illustrated as a typical example of the genus (scale bars: a = 2 mm; b = 1 mm (including stromatal section); c = 10 µm; d = 50 µm). The fungus disperses into the canopy (B) where it initiates endophyte infection; we presume ascospores to be the predominant mechanism of dispersal. When leaves are shed from the canopy (C), they take their endophytes with them; entire leaves may become dispersal vectors. The fungus grows from shed leaves into suitable substrate (D; see also Fig. S8), and the cycle continues. Not explicitly considered in this study are other potential courses of dispersal (in grey): there may be leaf-to-leaf dispersal in the canopy (E), which would maintain endophyte infection even in the absence of sexual reproduction on the forest floor. We find no evidence for this in the literature, however, and expect it to be rare or non-existent. Direct dispersal of ascospores to suitable substrate (F) is undoubtedly a common means of dispersal in this genus. While an interesting and important mechanism, we do not explicitly examine direct dispersal; this study focuses on elucidated the role of endophytism in the dispersal ecology of *Xylaria*. Panel B re-drawn from J. Seboth (1881).

gradients or biotic interactions, but indicative of dispersal linkage between life stages. This is in opposition to Beckman's hypothesis that microbes are unlimited in their dispersal abilities; if this is the case, *Xylaria* in both life stages should be distributed randomly and independent of each other, save for the selective impacts of the environment. Additionally, (2) if *Xylaria* endophytes display a FA lifestyle, we would expect endophytic host generalism in the tropics, as host selectivity would interfere with dispersal in systems where most available hosts are present in low densities (May 1991). The FA hypothesis also leads to the hypothesis (3) that endophytes will be released from environmental constraints relative to their corresponding decomposers. Using ITS rDNA barcode sequence comparisons (Gardes & Bruns 1993, Schoch *et al.* 2012), we matched decomposer *Xylaria* with endophytes in leaves from the canopy, and compared habitat characteristics of both. Lastly, we expect (4) the FA strategy to be a specialized survival/dispersal mechanism utilized by a subset of fungi within the genus *Xylaria*. Given the diversity of the genus, we expect variation in species' niches to modulate the selective advantage of endophytism.

METHODS

FIELD.—All field work described was performed at Reserva Los Cedros, a private, protected forest preserve in the western slope of the Andes, in northwestern Ecuador (00°18′31.0″ N, 78°46′44.6″ W), at 1200 m asl. The reserve lies within the Andean Chocó bioregion, one of the most biodiverse habitats on the planet (Gentry 1992). The reserve protects approximately 6800 hectares of forest, approximately 80 percent of which is primary, premontane tropical wet and cloud forest. The Reserve also shares a

border with the 305,000 hectare government-protected Cotocachi-Cayapas Ecological Reserve. Rainfall averages 2,903±186 mm per year (Policha 2014). Humidity is typically high (~100%), and daily temperatures at the site range from 15ºC to 25ºC (Policha 2014). Seasonal variation in climate is minimal. Our sampling occurred during the early part of the wettest season, in January 2012, when fungal fruiting was presumed to be highest.

We sampled within a previously established, "permanent" tree monitoring plot (Peck *et al.* 2008). Sampling occurred in primary forest at 1300 m, on the banks of a perennial stream and the surrounding area. The sampling area consisted of 120 individual points, spaced 10 m apart in the east-west direction and 5 m apart in the north-south direction. At each point, the two lowest leaves of the nearest tree or tree-like plant (Table S1) were collected for culturing of endophytes, as well as additional material for host-identification, if necessary. All xylarioid stromata within a 1.2 m radius of the point were collected from the forest floor and any aerial substrate within reach.

Previous environmental data for the plot were inaccessible, so stream mapping and individual point data were recollected later, in March 2014. Site characteristics in the plot are expected to change slowly (Policha, 2014). Slope by clinometer, canopy cover by densitometer, and aspect were measured for each point. Our sampling area was small (~0.5 ha) and is presumed to be homogeneous in soil quality and precipitation regime (Policha, 2014).

SAMPLE PROCESSING.—Leaves were washed gently in a basin of water (~30 s) to remove epiphyllous debris. Endophytes were recovered from two 2-mm diameter discs taken from each leaf using a Harris® micropunch sampling tool, for a total of 480 individual

leaf discs. Discs were surface sterilized by immersion in 70 percent ethanol for 1 min, 5 percent sodium hypochlorite for 2 min, then rinsed thoroughly in sterile water and placed on water agar (2% agar) petri dishes. Fungi were individually isolated onto MEA plates (2% malt extract, 0.1% yeast extract, acidified to pH 4) as they grew out from the discs of leaf tissue (methods modified from Okane *et al.* 2008). Water agar plates with leaf discs were examined daily for a period of 9 weeks, with new isolations made as needed.

All culture work was done in a portable sterile laminar flow hood constructed using a Dayton® Blower (model MG1104058171010), $^{1}/_{4}$ inch Plexiglass®, and a Hepa-sep® filter (model STD12-12-05PEADC50). Power was supplied by a micro-hydrological power plant installed at Reserva Los Cedros.

Cultures were grown on MEA until sufficient hyphae were present for DNA extraction. Under laminar flow, all aerial mycelium were harvested, and then pressed into a Whatman FTA® card with the aid of a standard claw hammer (Dentinger *et al.* 2010). Stromata were sampled by removing outer carbonaceous layers using a flame-sterilized scalpel, and preservation of inner tissues in Whatman FTA® cards.

ENDOPHYTE TRANSFER EXPERIMENT.—In April 2014, we also collected leaves from a randomly selected tree within the plot (*Nectandra lineatifolia* (Ruiz & Pav.) Mez) for an experiment to examine the transmission of endophytic Xylariaceae to woody substrates. Eight 2-cm sections were cut from each of twelve leaves, surface sterilized as described above, and placed on sterile (twice-autoclaved) white birch tongue depressors (Puritan, Guilford, Maine, USA) as a standardized angiospermous woody substrate. Four sections from the same leaf were placed on each tongue depressor. These were incubated at room

temperature in EtOH-sterilized Ziploc storage boxes (with an open container of sterilized water to maintain humidity) at the field station for 6 weeks, after which time the leaf segments were removed, the tongue depressors were air-dried in open, downward-facing, sterile plastic zipper bags, in which they were then transported back to the United States.

In our lab in Oregon, we started initial cultures from the first three tongue depressors in early August, 2014. We split each tongue depressor into three pieces lengthwise and extracted the middle piece; this was split into 12 equal pieces (~4 mm^2 each), each of which was plated onto water agar for fungal isolation, and incubated indefinitely. Subcultures were made on MEA as described above; cultures were identified to genus by a combination of morphology and DNA sequence.

DNA EXTRACTION.—Lab protocols followed Dentinger *et al.* (2010). Samples were excised from the Whatman FTA cards using a 2 mm punch tool and sterilized cutting mat. The punch tool was flame sterilized between uses, and its sterility was confirmed with extraction and PCR tests of DNA from sterile filter paper segments cut by the tool between each use.

Sigma Extract-N-Amp™ Plant PCR Kit reagents were used for extraction from Whatman© FTA cards. With each sample disc, 25 µL of Extraction reagent was added to each well and incubated for 10 minutes at 95˚C (using an Applied Biosystems© Vereti© model thermal cycler). After incubation, 25 µL of Dilution reagent was added to halt further extraction.

PCR AMPLIFICATION.—Template DNA was diluted. Generally, 1:19 dilutions worked best, though optimal dilution ranged from 1:1-1:99. DNA amplification was carried out using the fungal-specific ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC3-') primer sets (Gardes & Bruns 1993). DNA amplification was conducted in a standard 96-well plate with 10-uL reaction volumes (2 µL of template, 5 µL of Sigma Aldrich Jumpstart™ Taq Readymix™, 2.2 µL sterile water, 0.4 µL 25 mM $MgCl_2$, and 0.2 µL of each primer).

PCR amplification was done with an Applied Biosystems© Vereti© model thermal cycler with the following parameters: initial denaturation at 95˚C for 2 min, five cycles of denaturation at 95˚C for 30 s, annealing at 60˚C for 30 s, and extension at 72˚C for 1 min; followed by 25 cycles of denaturation of 95˚C for 30 s, annealing at 55˚C for 30 s, and extension at 72˚C for 1 min; a final extension at 72˚C for 10 min and a final step of indefinite duration at 4˚C.

DNA SEQUENCING AND ANALYSIS.—PCR products were visualized on a 1 percent agarose gel. Before sequencing, all successful PCR reactions were cleaned by adding 0.4 volumes of a master mix containing 10 percent FastAP© thermosensitive shrimp alkaline phosphatase (Thermo Scientific©) and 1 percent exonuclease I solution (New England Biolabs©) to the PCR product, and incubation for 15 min at 37˚C followed by 15 min at 85˚C. Samples were then frozen until shipping for sequencing at Functional Biosciences, Inc (Madison, WI, U.S.A.) on ABI 3730xl instruments using Big Dye V3.1.

Forward and reverse sequences were aligned and curated in Geneious v6.0.3 (Biomatters, Auckland, New Zealand). Sequences were grouped into 97 percent

similarity clusters using UClust as implemented in MacQIIME v1.7.0 with default settings. Specimens were identified morphologically with the help of Dr. Yu-Ming Ju (Academia Sinica, Taipei, Taiwan, ROC), and sequences were named via confirmed morphological identification wherever possible. In nearly all cases, 97 percent was an adequate cut-off to delineate previously defined morphological species. In one case two species occurred within a grouping (*Xylaria schweinitzii* Berk. & M.A. Curtis and *Xylaria ophiopoda* Sacc.). A maximum likelihood tree was constructed using the PhyML plugin in Geneious, and the two major branches of the tree corresponded perfectly to the two morphological species. Species groupings were adjusted to accommodate splitting that cluster. Some *Xylaria* species were unable to be identified morphologically due to immaturity or poor condition of specimens. When not in a cluster with identifiable specimens, these were assigned a species identifier, but no name. Finally, a species occurrence matrix was built for all species of *Xylaria*, both endophytes and decomposers.

STATISTICAL METHODS.—Data were analyzed using R Statistical Software, version 3.1.0 (R Core Team 2014), including the *sp* (Pebesma & Bivand 2005), *bipartite* (Dormann *et al.* 2008), and *vegan* packages (Oksanen *et al.* 2013). All scripts are publicly available online (Thomas *et al.* 2014).

Estimates of xylariaceous species richness within our plot were estimated using Chao2 and Jacknife1 estimators (Burnham & Overton 1978, Chao 1984, Colwell & Coddington 1994). Sampling effort was visualized with species accumulation curves constructed using the vegan package in R.

Spatial clustering of endophyte and decomposer *Xylaria* life stages of each species was analyzed using nearest neighbor analysis (Clark & Evans 1954) with randomization (Fortin & Dale 2005), using a customized script in R (Thomas *et al.* 2014). Four spatial relationships were examined: clustering of (1) stromata around stromata, (2) endophytes around endophytes, (3) endophytes around stromata, and (4) stromata around endophytes. For some taxa, not all stages were present; in these cases the subset of possible comparisons was performed.

Spatial clustering of fungal observations around a stream that dissected the plot were also analyzed using custom scripts in R. When all life stages were present, combined life stages (all fungi), stromata alone, and endophytes alone were examined.

The nearest-neighbor with randomization statistic we employ here is not often utilized in ecology (but see Clark and Evans (1954) and Dixon (1994) for related usages). In each case, a nearest neighbor test statistic was generated using the average of distances of up to five (Liu 2001) nearest neighbor observations from each point, for all observations of a species and life stage. A test-statistic distribution was generated for each species using 20,000 randomly generated sampling areas with the same number of both endophytes and decomposer fungi as the actual sampling area. In each rank of nearest neighbor, or "distance class", the observed mean nearest neighbor distance ($\bar{d}_o$) was compared to the randomly generated distribution of expected mean nearest neighbor distances ($\bar{d}_e$), and the proportion of $\bar{d}_e$ values lower than the observed were taken as the probability that a given species was spatially under-dispersed significantly more than as predicted by a completely spatially random null model (i.e., that the distance between points is less than that expected by chance; this is often called "clustering" or

"clumping"). P-values were considered significant at $P = 0.05$ or below; all nearest neighbor distances are reported in meters.

If the real distance to the nearest neighbor is less than the randomly generated distance to the nearest neighbor more than 95 percent of the time ($P < 0.05$), we take this to mean that the points are significantly clustered. In other words, it is more likely that observations of these species will occur in proximity to other observations of the same species than expected by chance. In the absence of environmental gradients controlling this spatial structuring within a life stage, we take this as evidence of spatial dependence: in the case of life stages clustering to themselves (e.g., endophytes around endophytes), this is likely a signal of "true" or "autogenic" autocorrelation (Fortin & Dale 2005), or the tendency of neutral processes to cause organisms to cluster in space and time. In the case of different life stages clustering together (e.g., endophytes around decomposers), we take this as evidence that dispersal is occurring between these different life stages. Tests for spatial correlation ("autocorrelation") of environmental variables were conducted using a Mantel correlogram of environmental dissimilarity of plots against a physical distance matrix. Testing for community turnover, or decay of similarity in *Xylaria* species composition among plots with distance, was done using a Mantel correlogram of *Xylaria* species composition distance matrix against a physical distance matrix of all plots sampled (Fortin & Dale 2005).

In addition to determining if clusters are non-random, the nearest-neighbor metric we employ here allows us to examine the direction of clustering *between* life stages—that is, we can compare the distance to nearest stromata from an endophyte, or vice versa. When determining whether there is clustering of the two life stages, two *P*-values are

obtained: one for stromata clustering around endophytes, and one for endophytes clustering around stromata.

We examined host-preference by endophytes using two methods: (1) we used chi-squared goodness-of-fit tests of **host preference** by our most common *Xylaria* endophyte (*X. adscendens*) and **endophyte preference** in the most common host tree (*Faramea* aff. *oblongifolia* Standl.); (2) we used bipartite network analysis to examine strength of interactions between host-plants and endophytes.

In the goodness-of-fit analysis of host-preference, the null hypothesis was that infection depended only on host commonness, and was generated from the respective ratios of species of all host trees from our plot that were found to host any xylariaceous endophyte. Reciprocally, the null hypothesis for endophyte preference was that the most common host tree would be infected by xylariaceous endophytes in roughly the same frequency that these endophytes were collected from all hosts in the plot. These hypothesized ratios were then compared to the observed ratios of host trees from which *Xylaria adscendens* (Fr.) Fr. was isolated and the frequencies of endophyte species observed solely in *Faramea* aff. *oblogifolia*, using a chi-squared goodness-of-fit test with Monte Carlo simulation (from the base R *stats* package).

Network analysis followed Ikeda *et al.* (2014). Using the *bipartite* package in R (Dormann 2008) species interaction matrices were constructed and a network-wide H_2' value (Blüthgen 2006) was calculated to characterize the level of preference ("specialization") among host-plants and endophytes. These results were then compared to a null model of network assembly (Vásquez *et al.* 2007), with 10000 randomization cycles.

Tests for grouping of species by habitat characteristics—slope, canopy, distance-to-water, and aspect (separated into component northern and eastern exposures)—were done using Permutational Multiple Analysis of Variance (PerMANOVA), with the *adonis* function in *vegan* package in R. These data were visualized with non-metric multidimensional scaling (NMDS). Differences among the above characteristics for all sites containing a *Xylaria* observation were summarized in an environmental distance matrix as input for the *metaMDS* function in the *vegan* package in R (Oksanen *et al.* 2013); points were then categorized by the species of *Xylaria* observed. The *metaMDS* considers multiple possible solutions using Procrustes analysis and employs Wisconsin double standardization to reduce Kruskal stress in ordination. We considered solutions with stresses below 0.15 to be informative. Linear models of differences in habitat, used for weighting relative importance of habitat variables, were also constructed using the *adonis* function.

RESULTS

Endophytes were isolated from 38 tree species in 19 different families, as well as a species of large fern and several large herbaceous plants when no woody hosts were present within the sampling plot (Table S1). From the 480 total leaf segments, 720 unique cultures were isolated; no leaf segment yielded zero fungi. Of the endophyte isolates, 104 (14.4%) were in the Xylariaceae (19 species in *Xylaria, Hypoxylon, Nemania,* and *Annulohypoxylon*). We collected stromata in two genera of Xylariaceae, *Xylaria* and *Kretzschmaria,* from 79 (65.8%) of the points within the plot. We found 36 species of *Xylaria*, 31 of which were found to only occur as fruiting bodies, and five of which were

found as both stromata and endophytes. All five species of *Xylaria* found as endophytes were also found as fruiting bodies; there were no endophytic *Xylaria* not also recovered as stromata (Table 1; Table S2). *Xylaria* leaf endophyte species were found to be a subset of wood decomposer species: all *Xylaria* endophyte species were also recovered as decomposer species. There were species-specific differences in the frequencies of occurrence of the leaf endophyte and decomposer (stromatal) life stages (Table 1): that is, frequency of one life stage does not predict frequency of the other; they are specific to particular species.

Chao2 and Jackknife1 species richness estimators predicted 52.33 (SE = 11.7) and 49.9 (SE = 4.2) *Xylaria* decomposer (stromatal) species, and 5.00 (SE = 0) and 8.0 (SE = 1.7) *Xylaria* endophyte species. This is in agreement with species accumulation curves of our sampling effort indicating that we sampled nearly completely for culturable endophyte species but that decomposer species remain to be discovered within the plot (Fig. 2).

Five species of *Xylaria* were found both in the leaves and as decomposers. Of these, two species demonstrated non-random clumping of differing life stages (i.e., endophyte-stage fungi were found to clump around decomposer-stage fungi, or *vice versa*): *X.* aff. *curta* ($\bar{d}_o$ (1) = 18.10, $\bar{d}_e$ (1) = 43.90 ± 17.48, P = 0.048) and *X. fissilis_1* ($\bar{d}_o$ (2) = 13.83, $\bar{d}_e$ (2) = 19.94 ± 3.84, P = 0.036) (Table 2; Fig. 3; Figs. S2-S3). For these five species, significant clumping within a life stage was only observed for endophytic *X. adscendens* ($\bar{d}_o$ (2) = 11.91, $\bar{d}_e$ (2) = 13.43 ± 0.89, P = 0.044; Table S3, Figs. S4-S5).

Of the five *Xylaria* species exhibiting both decomposer and endophytic life stages, three species in the decomposer life stage appear to be closely clustering around

TABLE 1. List of all *Xylaria* species recovered and the number of points in the study area (out of 120) from which each species was recovered in each life stage. Distinct ITS clusters in otherwise indistinguishable taxa are indicated by an underscore followed by a clade number on the specific epithet.

Taxa	Points with **Stromata**	Points with **Endophytes**
Xylaria adscendens (Fr.) Fr.	3	26
Xylaria anisopleura (Mont.) Fr.	3	
Xylaria apiculata_1 Cooke	9	1
Xylaria apiculata_2 Cooke	1	
Xylaria atrosphaerica (Cooke & Massee) Callan & J.D. Rogers	4	1
Xylaria aff. *comosa* (Mont.) Fr.	5	
Xylaria cristata Speg.	1	
Xylaria cuneata Lloyd	4	
Xylaria curta_1 Fr.	1	
Xylaria curta_2 Fr.	1	
Xylaria aff. *curta* Fr.	2	1
Xylaria enterogena Mont.	11	
Xylaria fissilis_1 Ces.	11	5
Xylaria fissilis_2 Ces.	2	
Xylaria globosa (Pers.) Mont.	5	
Xylaria meliacearum Læssøe	3	
Xylaria multiplex (Kunze) Fr.	3	
Xylaria ophiopoda Sacc.	5	
Xylaria schweinitzii Berk. & M.A. Curtis	16	
Xylaria scruposa_1 (Fr.) Fr.	12	
Xylaria scruposa_2 (Fr.) Fr.	4	
Xylaria subtorulosa Speg.	2	
Xylaria telfairii (Berk.) Sacc.	7	
Xylaria xanthinovelutina (Mont.) Fr.	2	
Xylaria sp. 01	1	
Xylaria sp. 02	1	
Xylaria sp. 03	1	
Xylaria sp. 05	1	
Xylaria sp. 06	1	
Xylaria sp. 07	1	
Xylaria sp. 08	1	
Xylaria sp. 10	1	
Xylaria sp. 11	1	
Xylaria sp. 12	2	
Xylaria sp. 13	2	
Xylaria sp. nov. 2	1	

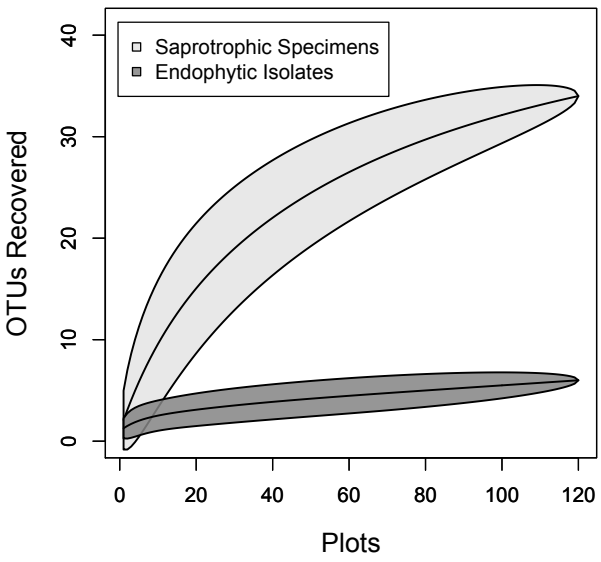


FIGURE 2. Species accumulation/sampling effort curve of both decomposer stromata collected on the forest floor and endophytes cultured from leaves; shaded areas are 95% confidence intervals.

the stream present in our sampling area (Fig. 4; Table S4): *X.* aff. *curta* ($\bar{d}_o$ (2) = 29.67, $\bar{d}_e$ (2) = 52.75 ± 12.81, P = 0.016)*, X. atrosphaerica* ($\bar{d}_o$ (2,3) = 21.11 and 33.17, $\bar{d}_e$ (2,3) = 34.23 ± 8.01 and 47.30 ± 9.91, P = 0.007 and 0.048)*,* and *X. apiculata* ($\bar{d}_o$ (1,2,3) = 6.85, 12.74, 18.08, $\bar{d}_e$ (1,2,3) = 13.49 ± 3.50, 21.62 ± 3.99, 28.17 ± 4.54, P = 0.006, 0.002, 0.001). None of the species in the endophytic life stage were clustered around water (Fig. S6; Table S4).

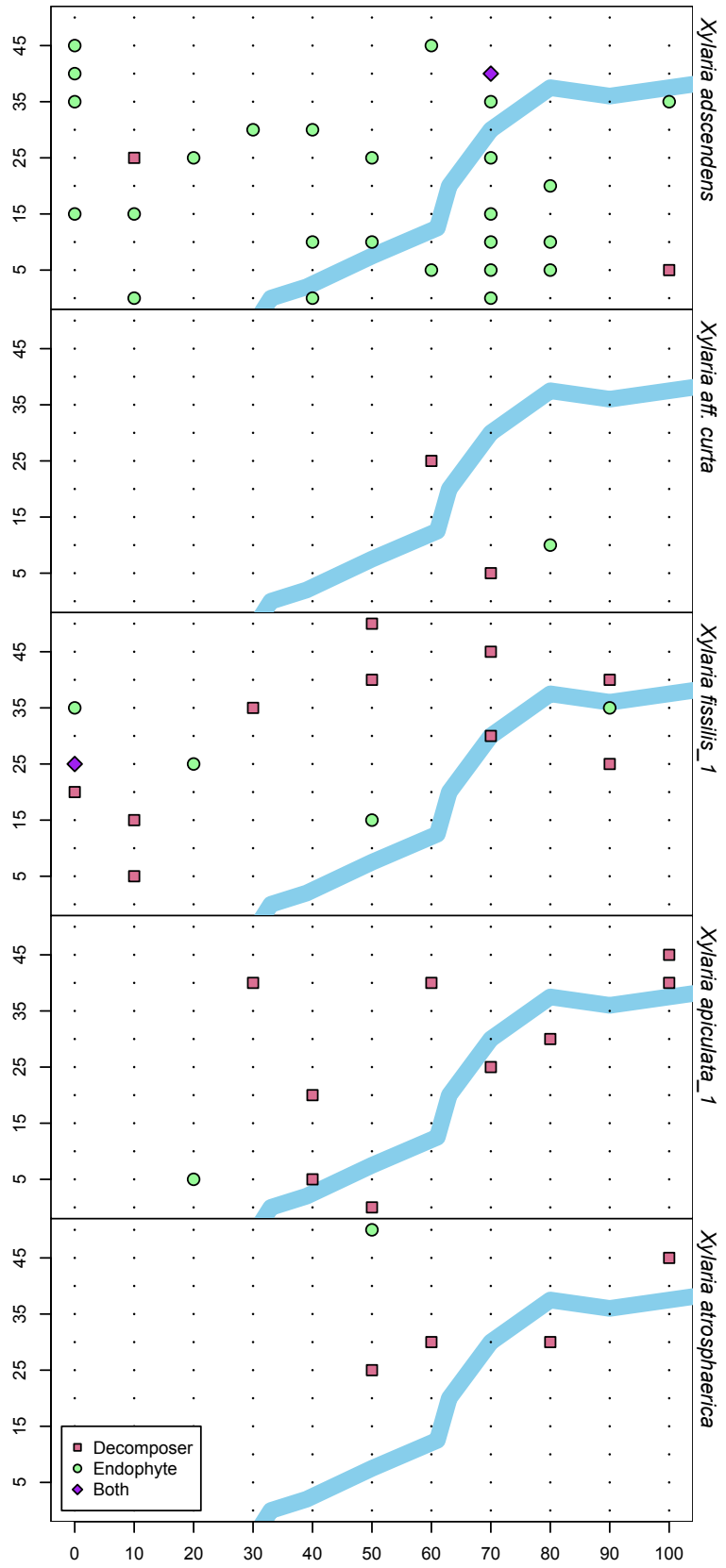
Among the 36 species of *Xylaria* detected as decomposers, significant clustering of stromata to stromata was observed in two species (*X. multiplex* and *X. ophiopoda*; Table S5). Significant clustering of stromata around streams was observed in eight species (*X.* aff. *curta, X. cuneata, X. apiculata_1, X. subtorulosa, X. multiplex, X.* sp. *13, X. enterogena,* and *X. atrosphaerica*; Table S5).

Spatial correlation of environmental variables was significant only at distances below 15 m, and variance explained was extremely low (Mantel's r = 0.06, R^2 = 0.004, P

TABLE 2. Nearest-Neighbor analysis of spatial clusters in five species of *Xylaria*. Values shown are the observed mean nearest neighbor distance ($\bar{d}_o$), the expected mean nearest neighbor distance ($\bar{d}_e$) from a Monte Carlo simulation null model assuming complete spatial randomness (CSR), the standard deviation around the expected mean nearest neighbor distance (s_e), and the *P* values, calculated as the proportion of simulations where $\bar{d}_e < \bar{d}_o$. Bold indicates $P < 0.05$; italics indicate $0.05 < P < 0.10$; dashes indicate insufficient sample size to conduct the analysis at a given neighbor class.

		Stromata around **Endophytes**				**Endophytes** around **Stromata**			
Taxa	Neighbor class	$\bar{d}_o$	$\bar{d}_e$	s_e	*P*	$\bar{d}_o$	$\bar{d}_e$	s_e	*P*
Xylaria aff. *curta*	1	11.2	31.5	18.2	0.152	18.1	43.9	17.5	**0.048**
	2	25.0	56.3	21.5	*0.068*	—	—	—	—
Xylaria apiculata_1	1	20.0	13.9	8.5	0.794	51.2	43.7	10.5	0.771
	2	25.0	22.3	9.3	0.705	—	—	—	—
	3	30.4	29.4	10.4	0.612	—	—	—	—
	4	36.4	36.0	11.6	0.609	—	—	—	—
	5	53.2	42.8	13.0	0.819	—	—	—	—
Xylaria fissilis_1	1	10.8	12.3	3.5	0.354	15.8	19.1	4.3	0.210
	2	13.8	19.9	3.8	**0.036**	28.4	31.5	5.3	0.285
	3	21.1	26.1	4.3	0.108	41.2	43.0	6.0	0.406
	4	26.1	31.7	4.8	0.102	58.0	55.5	8.3	0.634
	5	32.4	37.1	5.3	0.178	76.6	69.6	8.8	0.771
Xylaria adscendens	1	22.1	25.3	5.5	0.302	10.0	7.2	3.0	0.836
	2	46.2	43.4	6.2	0.715	11.9	12.4	2.7	0.494
	3	72.9	62.7	9.6	0.839	16.8	15.9	3.1	0.653
	4	—	—	—	—	19.7	19.0	3.3	0.627
	5	—	—	—	—	23.5	21.9	3.5	0.703
Xylaria atrosphaerica	1	22.4	21.8	12.9	0.639	33.4	43.9	13.4	0.230
	2	25.0	36.3	15.5	0.277	—	—	—	—
	3	36.1	50.6	17.8	0.236	—	—	—	—
	4	50.3	66.8	18.8	0.221	—	—	—	—

FIGURE 3 (next page). Maps of the five species of *Xylaria* displaying both endophyte and decomposer life stages. All collection points are marked; the stream is indicated with a blue line. Scale in meters.


Xylaria adscendens
Xylaria aff. curta
Xylaria fissilis_1
Xylaria apiculata_1
Xylaria atrosphaerica
Decomposer
Endophyte
Both
0
10
20
30
40
50
60
70
80
90
100
5
15
25
35
45

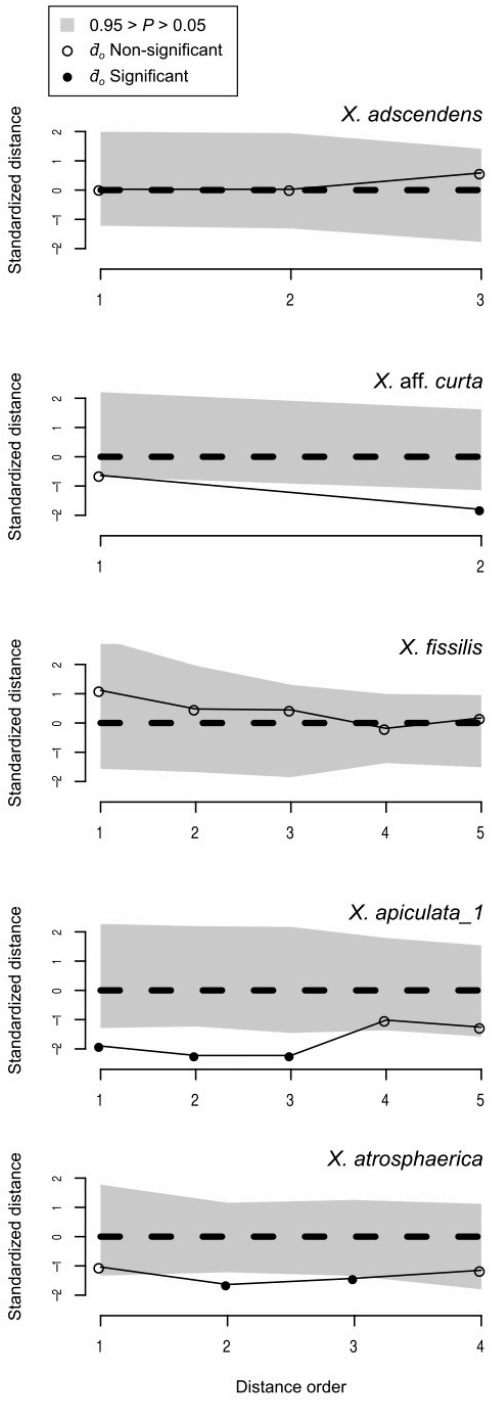


FIGURE 4. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for clustering of stromata around the stream (see also Table S4). For each species, the standardized mean distance to nearest neighboring point along the stream ($\bar{d}_o$) for all available distance classes is plotted. The dashed line represents the mean distance to points along the stream of the permutations on complete spatial randomness ($\bar{d}_e$), standardized to zero for all distance classes; the y-axis units represent deviation from the permutational mean (s_e). Open points are non-significant ($P > 0.05$); closed points are significant ($P < 0.05$); the grey area represents the region where $0.95 > P > 0.05$.

< 0.05). *Xylaria* species composition was not found to be significantly autocorrelated on the scale of this study (Mantel's r = 0.01, $R^2 < 0.001$, $P = 0.394$).

Habitat preferences were not found to be significantly different among the five *Xylaria* species when we examined combined life stages (PerMANOVA, $F_{4, 58} = 1.57$, $R^2 = 0.10$, $P = 0.112$). However, when examined separately, decomposer *Xylari*a may show species-specific habitat preferences (PerMANOVA, $F_{4, 24} = 1.84$, $R^2 = 0.23$, $P = 0.07$; Fig. S7); endophytic *Xylaria* do not ($F_{4, 29} = 0.45$, $R^2 = 0.06$, $P = 0.94$; Fig. S7). In decomposer fungi, differences among habitats were defined most strongly by proximity to water (PerMANOVA, $F_{1, 23} = 112.42$, $R^2 = 0.44$, $P = 0.001$), followed by slope ($F_{1, 23} = 31.36$, $R^2 = 0.12$, $P = 0.001$), canopy cover ($F_{1, 23} = 20.61$, $R^2 = 0.08$, $P = 0.001$), and aspect, in its components of northern and eastern exposure ($F_{1, 23} = 11.84$, $R^2 = 0.05$, $P = 0.001$ and $F_{1, 23} = 6.20$, $R^2 = 0.02$, $P = 0.006$, respectively).

We found no evidence for host preference by endophytes from the family Xylariaceae. Host trees for the most common endophyte, *Xylaria adscendens,* did not vary from general abundances of host trees within the total plot (χ^2, 10000 replicates, ($N = 10$) = 2.45, $P = 0.74$*).* Relative abundances of endophytes recovered from the most common host, *Faramea* aff. *oblongifolia,* did not show a significant difference in endophyte abundances within the entire plot*,* (χ^2, 10000 replicates, ($N = 26$) = 19.80, $P = 0.86$). Network specialization did not exceed levels expected by chance alone given abundances of endophytes and host-plants ($H_2' = 0.261$, mean randomized $H_2' = 0.290$, 10000 cycles, $P = 0.62$; Fig. 5).

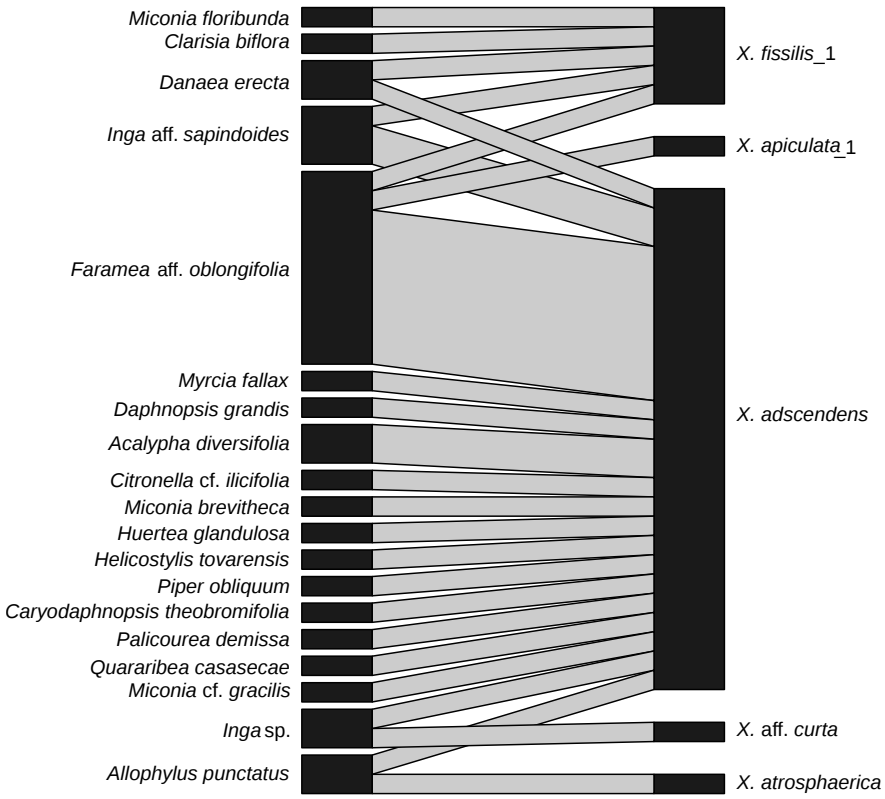


FIGURE 5. Bipartite network visualization of *Xylaria* endophytes (right) and plant-hosts (left). Widths of links are scaled to number of points at which endophytes were isolated from hosts.

ENDOPHYTE TRANSFER EXPERIMENT.—We isolated *Xylaria* from 8 of 12 segments from one of three sampled tongue depressors (22% of segments). By the sixth month, the *Xylaria* had established competitive dominance in these tongue depressor segments, and was observed to initiate fruiting in 7 of the 8 segments from which it was isolated (Fig. S8); all stromatal primordia displayed classic *Nodulisporium* anamorphs. Unfortunately, we have not been able to obtain usable ITS sequence for these isolates, presumably due to co-extraction of PCR inhibiting fungal cell wall polysaccharides.

DISCUSSION

The Foraging Ascomycete hypothesis challenges two classical assumptions about fungal dispersal: first, that fungi are unlimited in their dispersal abilities (Becking 1934, Fenchel & Finlay 2004), and second, that sexual spores of decomposers are the sole major source of dispersal for these fungi (Norros et al. 2012, Bayman et al. 1998, Malloch & Blackwell 1992). Following these assumptions, endophytism has been supposed by some to be an accidental "dead end" infection of living plants (Bayman *et al.* 1998). The FA hypothesis proposes that for some fungi, endophytism is not an accidental "dead end", but an important mechanism of fungal dispersal—an adaptation for bridging temporal or spatial scarcity of primary substrates. Under this model, a host-plant acts as a reservoir of mycelium, distributing fungi across the range of leaf-fall.

As such, the FA hypothesis yields several testable predictions: (1) A measurable spatial linkage between endophyte and decomposer life stages for fungi utilizing a FA strategy, wherein stromata serve as sources of endophytic infection (in addition to being sources of direct dispersal) but represent relatively short "bursts" in time, while areas of endophytic infection serve as slower, more "trickling" dispersal centers. (2) A prediction of endophytic host generalism in diverse tropical forests, as strong host preference would interfere with dispersal abilities in systems where the density of any one host species is usually quite low (May 1991). This prediction may not hold in systems where strong dominant hosts are available, as in many temperate forests. (3) The FA hypothesis leads to a prediction that endophytes will be less constrained by environmental conditions than their corresponding decomposers. And, (4) we predict the FA strategy to be a specialized survival/dispersal mechanism utilized by a subset of fungi. Variation in niche or preferred

habitat would modulate the selective advantage of endophytism. Thus, we predict some species in a group to be more adapted to endophytism than others.

We found significant clustering between life stages in two of the five species of *Xylaria* with both life stages, *Xylaria* aff. *curta* Fr. and *Xylaria fissilis* Ces. This suggests spatial linkage of life stages, consistent with prediction (1). It is worth noting that the genetic marker used to link endophytic and decomposer life stages, ITS, has certain limitations. This marker is composed largely of two highly variable introns, and as such is excellent for species identification where reference sequences are available, but is not appropriate for phylogenetic approaches to clustering (Schoch et al. 2012), and is not useful for determining relatedness of individuals within a taxon. As we expect that meiotically produced ascospores are the source of endophytic infection, markers that allow the determination of relatedness between isolates, in addition to the identity of isolates, may complement ITS in future studies. Additionally, the utilization of next-generation sequencing techniques in the elucidation of endophytic communities will allow much greater depth of sampling, regardless of locus selected. Such depth of sampling will be particularly useful in further examination of the environmental constraints and host specificity of fungi suspected of utilizing a FA life history strategy.

Demonstrating the possibility of transfer from endophytic to a decomposer life stage, we have observed endophytic strains of *Nemania serpens* (Xylariaceae)—close relative of *Xylaria* (Hsieh *et al.* 2010)—from conifer needles to colonize dead *Acer macrophyllum* wood in laboratory conditions (G. C. Carroll, unpub. data). Here we explicitly tested the ability of endophytic members of the Xylariaceae to successfully transfer from leaves at our Ecuadorian site to dead woody substrates in laboratory

conditions. This test conclusively demonstrates the link between endophytic and saprotrophic *Xylaria,* showing that endophytic isolates can colonize dead woody substrates from within leaves (Fig. S8). These observations are contrary to the predictions of Bayman *et al.* (1998), who hypothesized that *Xylaria* endophytes are one-way "dead ends"—purely a sink for dispersal.

Consistent with prediction (2), we did not detect host preference by xylariaceous endophytes. However, the power of our study to detect host preferences may be limited due to the large number of hosts with few samples. Our culture and sampling efforts, though quite extensive, were insufficient to populate multivariate community analyses of host-associated xylariaceous communities (see, for example, Veresoglou & Rillig 2014).

Culture-based studies may be particularly disadvantaged when dealing with questions of endophyte host specificity because of culture bias and other limitations of culture-based studies, such as sampling depth (species accumulation curves generally saturate at impractical levels of effort per leaf) (Arnold *et al.* 2000, Arnold & Herre 2003, Lau *et al.* 2013). Some culture-based studies have addressed culture bias through the use of specialized extracts of host-plants in growth medium (Arnold *et al.* 2000, Arnold & Herre 2003, Lau *et al.* 2013), or through direct PCR/cloning methods (Higgins *et al.* 2011), but these approaches are very labor intensive in experiments involving more than a few species or hosts. We chose to work with *Xylaria* species, in particular, because they typically culture readily both from spores and from leaves as endophytes (Bayman *et al.* 1998), reducing potential culture bias. In a direct comparison of direct PCR (using cloning) versus culturing, Higgins *et. al.* (2011), reporting at the order level, found that

Xylariales were somewhat more common in cultures (48%) versus clones (38.9%), but that they were common in both.

When examining questions of host specificity, endophytes are probably best analyzed as multivariate communities within hosts, or as networks of host/endophyte co-occurrences (Peršoh 2013, Higgins *et al.* 2014, Ikeda *et al.* 2014). In future efforts, culture-independent, high-throughput meta-barcode sequencing techniques combined with whole community analysis of endophytes will more adequately address host-endophyte affinities (see, for example, Peršoh 2013).

Nonetheless, our results are in agreement with many studies that indicate that most non-clavicipitaceous tropical foliar endophytes, and especially *Xylaria,* are host generalists (Bayman *et al.* 1998, Cannon & Simmons 2002, Suryanarayanan *et al.* 2002, Arnold & Lutzoni 2007, Higgins *et al.* 2011), and are supportive of the idea that plant-associated fungi in hyper-diverse regions of the tropics will tend towards host generalism (May 1991). Some have suggested that endophyte communities should be regionally unique, due to dispersal limitation (Higgins *et al.* 2014, Vaz *et al.* 2014), and that endophytes of individual plants are predicted as much by location as by host affinities. Higgins *et al.* (2011, 2014), for example, found that tropical forest grass endophyte communities are more similar to leaves of nearby woody plants than those of distant grasses.

We found that endophytes are released from environmental constraints as compared to corresponding decomposers, as expected from prediction (3). Decomposers exhibited sensitivity to environmental variables that was not observed in endophytes, particularly to proximity of water. This is not surprising, as moisture is important for

spore germination and decomposition by most free-living fungi (Moore 1986, Eveling *et al.* 1990, Gange *et al.* 2007). Indeed, it has been speculated that the evolutionary origins of the Xylariaceae are linked to adaptation for water conservation (Rogers 1979, 2000). Our findings, that *Xylaria* are found fruiting in closer proximity to water sources than expected by chance, seem to indicate a strong role of water use in the ecological and evolutionary constraints for the genus. Endophytic fungi, however, exist in the highly buffered environment of the internal tissues of their host-plants; it is predictable that environmental conditions would have a less direct effect on their distributions. We see this in our spatial clustering analysis, where endophytes are not constrained by proximity to the stream (Table S4; Fig. 3). The unconstrained endophytic life stage may be a way that these fungi can bridge spatial and temporal gaps in suitable habitat; this is the core of the FA hypothesis, and our results here are consistent with this.

Lastly, in agreement with prediction (4), in our study all endophytic species of *Xylaria* were also recovered as decomposers from rotting wood on the forest floor. The reverse was not true; many decomposers were found only as stromata and were not detected as endophytes. Our diversity estimators and sampling effort curves indicate that we recovered most of the culturable *Xylaria* species from the leaves, but that decomposer *Xylaria* were undersampled. Okane *et al.* (2008) suggest that there may be Xylariaceae that exist solely as endophytes, but did not undertake concurrent systematic stromata collection to verify this. It is clear from our study that there are species-specific differences in the frequencies at which *Xylaria* displaying both life stages were found in the endophytic and saprotrophic phases (Table 1), supporting the notion that there are

dispersal or habitat differences among species. Our results suggest that endophytism is a specialist strategy for some members of the genus *Xylaria*.

We observed probable dispersal linkage in the form of spatial clustering of fungi. We also observed release from moisture limitation by two decomposer fungi through endophytism, suggesting that the endophytic life stage may be serving as a method to span dry habitats or persist during times of low moisture. We also directly observed the ability of endophytic *Xylaria* to colonize available woody substrates and initiate stromata formation. Finally, we found no evidence for host preference in endophytic *Xylaria* species. The limitations of a single observational study must be acknowledged: it remains to be seen if similar trends will be observed in some endophytic fungi of temperate zones or outside of montane cloud forests in the tropics. Nevertheless, we find these results to be consistent with the predictions of the Foraging Ascomycete Hypothesis, and a successful first step into the investigation of this intriguing and ecologically important hypothesis.

BRIDGE TO CHAPTER V

Having discovered that I truly loved working with Xylariaceous fungi, and that it was actually possible to reasonably test spatial relationships, I wanted to continue the work. The study presented in Chapter IV started as a small pilot, and quickly expanded to a full-blown study. This meant that in the course of the study, I learned many things that I wanted to try to put into practice, and my understanding of the theory behind the FA ecological strategy evolved considerably. To capitalize on all that I had learned, and to maximize the possibility for further learning, I arranged to go to Taiwan and work with

Dr. Yu-Ming Ju, who is probably the leading taxonomist of the genus *Xylaria* in the world, and one of the top taxonomists for the entire family Xylariaceae. I wanted to apply new techniques to the study of endophytes, blending modern molecular tools with solid ecology and traditional culture/collection techniques, crossing the intellectual bridge between studies that are interesting technically and studies that are interesting theoretically. To that end, I planned a relatively large undertaking to capstone my dissertation: Chapter V would include (A) the monolithic technique of next-generation sequencing metabarcoding to survey the endophytes; (B) an innovative nested logarithmic squares sampling design, intended to capture changes in community turnover with distance; (C) sampling over a quarter of a square kilometer of primary rainforest; and (D) collection of not just *Xylaria*, but the entire family Xylariaceae, made possible by collaboration with Dr. Ju. This chapter was intended to synthesize all the technique and theory that I had learned in the execution of the previous chapters.

CHAPTER V

SPATIAL ECOLOGY IN THE XYLARIACEAE: COMBINING TRADITIONAL COLLECTION AND NEXT-GENERATION SEQUENCE BASED MICROBIAL SURVEY TECHNIQUES

CONTRIBUTIONS

R. Vandegrift wrote the paper, did field work, taxonomic identification, conceptual and experimental design work, statistics, and lab work. D. Thomas did field work, conceptual and experimental design work, statistics, and lab work. H. Soukup did lab work and taxonomic identification. Y.-M. Ju provided materials and lab space, and did taxonomic identification. B. A. Roy provided materials and lab space and did conceptual/experimental design work. G. C. Carroll did conceptual/experimental design work.

INTRODUCTION

There are fungal symbionts, called *endophytes*, living asymptomatically in nearly all plant species and all plant tissues sampled to date (Rodriguez et al. 2009). Fungal endophytes are defined functionally: they are those fungi that occur beneath the surface of living, healthy plant tissues and do not harm their hosts enough to induce a defensive reaction (Clay 1990, Rudgers et al. 2009). They have been found to be nearly ubiquitous in plants of all ecosystems and are incredibly diverse, particularly in the tropics (Arnold

and Lutzoni 2007, Porras-Alfaro and Bayman 2011). The best understood endophytic associations are those between fungi in the family Clavicipitaceae and the pooid grasses; in agricultural systems, these endophytes have been shown to often enhance plant resistance to herbivory in a classic example of defence mutualism (Clay and Schardl 2002, Saikkonen et al. 2004, 2006, Schardl et al. 2004), though the they can at times be pathogenic to their hosts (Carroll 1988, Faeth and Sullivan 2003, Vandegrift et al. 2015). The vast majority of endophytic associations, however, are non-clavicipitaceous—the nature and ecology of these associations are more poorly understood, in part because they represent a much more variable group of fungi, both taxonomically and ecologically (Rodriguez et al. 2009).

There has been a great surge in the study of fungal endophytes in recent years (Rodriguez et al. 2009, Porras-Alfaro and Bayman 2011, Wani et al. 2015). Much of this research has focused on describing patterns of endophytic diversity (Ahlholm et al. 2002, Arnold and Lutzoni 2007, Arnold et al. 2007, Murali et al. 2007, Davis and Shaw 2008, Loro et al. 2012, Zimmerman and Vitousek 2012, Giauque and Hawkes 2013, Scholtysik et al. 2013, Vincent et al. 2015) and the potential economic impacts of endophytes—particularly, the interaction between endophytic species and agronomically important plant hosts (Douanla-Meli et al. 2013, Impullitti and Malvick 2013, Thom et al. 2013) and the exploitation of the rich secondary chemistry of endophytic fungi for use in drug development (Strobel and Daisy 2003, Bernardi-Wenzel et al. 2010, Carvalho et al. 2012, Kaul et al. 2012, Chen et al. 2013, Heinig et al. 2013, Hammerschmidt et al. 2015). Much attention has been given to why it may be adventitious for plants to play host to endophytic fungi (Brem and Leuchtmann 2001, Gange et al. 2012, Saikkonen et al. 2012,

Estrada et al. 2015), but relatively little attention has been given to the advantages that fungi may gain from adopting an endophytic lifestyle (but see: Carroll 1988, Saikkonen et al. 2004, Porras-Alfaro and Bayman 2011). While some endophytes have been observed to be latent pathogens or saprotrophs (Chapela and Boddy 1988, Osono 2006, Promputtha et al. 2007, 2010), most observed endophytes do not have such clear ecological roles (Lodge 1997, Porras-Alfaro and Bayman 2011). The benefits of endophytism, if any, remain unclear for these taxa.

Here, we examine one potential ecological explanation for some cases of endophytism. Some decomposer fungi may utilize endophytism as a way to bridge spatial and temporal gaps in preferred substrate—this is known as the Foraging Ascomycete (FA) hypothesis (Carroll 1999, Thomas & Vandegrift et al. 2016).

Endophytism may be advantageous within the framework of the FA hypothesis for several reasons: In evergreen forests, leaves are often shed asynchronously as they are no longer photosynthetically productive, which leads to contrasting patterns of dispersal—fungal fruiting bodies produce relatively high densities of propagules over relatively short periods of time, while endophytic-phase fungi provide relatively low densities of propagules over relatively long periods of time. These leaves may fall farther from their source than most fungal spores are predicted to travel unassisted (Roper et al. 2010, Galante et al. 2011), though the influence of turbulent air should not be ignored. Dispersal within senescent leaves may also aid in colonization through the creation of a sheltered microclimate favorable to inoculation and the provision of initial labile nutrients. Lastly, living leaves may provide protection for endophytic fungi from difficult

environmental conditions, allowing the fungi to persist at low metabolic cost within the highly buffered environment of the leaf tissue (Stone 1987, Schulz and Boyle 2005).

Several testable predictions follow logically from the FA hypothesis: (1) In diverse tropical forests, a FA strategy would be more adventitious to fungi capable of infecting a wide range of hosts, because in such forests most available host species would be present in relatively low densities (May 1991). A prediction of host generalism may not be valid in temperate evergreen forests, however, which tend to be dominated by one or a few species (Sherwood-Pike et al. 1986). (2) Given that there is dispersal linkage between the two life-phases, there should be a measurable spatial linkage in the distributions of saprotroph- and endophyte-phase fungi of a given species using the FA strategy. (3) If the general endophytic community is composed of fungi utilizing a diversity of ecological strategies (Rodriguez et al. 2009), those endophytes utilizing a FA strategy should be less constrained by dispersal limitations than the community at large. As such, the rate of beta-diversity turnover for the subset of endophytes utilizing a FA strategy should be lower than the rate of beta-diversity turnover for the set of all endophytes. (4) The FA strategy allows fungi to escape unfavorable conditions. Since the environment within leaves is highly buffered from the environment by the host, we would expect the endophyte-phase of fungi using a FA strategy to be less sensitive to environmental gradients than their saprotroph-phase counterparts. (5) Given the diversity of ecological strategies present in the Fungi, we expect the FA strategy to be a specialized survival/dispersal mechanism utilized by a subset of fungi in any given group.

The fifth and final prediction is, however, related to the concept of phylogenetic niche conservatism (PNC), which is the theory that more closely related taxa are more

likely to be ecologically similar than distantly related tax (Harvey and Pagel 1991). This stems from multiple processes, including an increased likelihood for unused niches to be filled by the most ecologically similar species, followed by divergence; stabilizing selection; and habitat selection (Losos 2008). From PNC, we expect the FA strategy to be common within certain fungal lineages and uncommon in others, rather than being randomly distributed across all potential endophytic lineages.

For this reason, we have chosen to focus on a particular lineage, the Ascomycete family Xylariaceae. Fungi in this family are well known to be common foliar endophytes in the tropics (Bayman et al. 1998, Arnold et al. 2000, Davis and Shaw 2008) without any apparent host preference (Murali et al. 2007, Davis and Shaw 2008, Okane et al. 2012, Ikeda et al. 2014), and there is evidence that the genus *Xylaria* may utilize a FA strategy (Thomas & Vandegrift et al. 2015). The Xylariaceae are an ancient lineage of predominantly wood decay fungi, which likely diversified heavily coincident with the radiation of angiosperms, ca. 100 mya (Rogers 1979a, 2000, Ju and Rogers 1996). The fruiting structures of most xylariaceous fungi are macroscopic perithecial stromata occurring on wood—or occasionally other plant-derived substrates—and are readily identified to family in the field (Ju and Rogers 1999). Endophytic and saprotrophic examples of the same taxa within Xylariaceae have been observed by both molecular and culture-based techniques (Okane et al. 2008, Thomas & Vandegrift et al. 2016).

To test the predictions of the FA hypothesis with the Xylariaceae, we utilized a spatially explicit sampling scheme designed to test turnover effects (Rodrigues et al. 2013) in an evergreen subtropical forest in northeastern Taiwan. We coupled traditional specimen-based collection with Illumina next-generation sequencing (NGS) metabarcode

microbial survey techniques (Taberlet et al. 2012, Blaalid et al. 2013, Meadow et al. 2013, Schmidt et al. 2013). This sensitive, culture-independent detection technique allowed us to accurately determine presence of fungi in the endophytic community based on sequences generated from specimens collected. Furthermore, the spatial relationships between fungal fruiting bodies and occurrence in the endophytic community could be examined across the site. Lastly, trends in diversity and turnover were examined with respect to environmental gradients across the site.

METHODS

Field.—All field work for this study was conducted in July and August of 2013, at the Fushan Forest Dynamics Plot (FDP), a 25 ha (500 x 500 m) permanent plot in northeastern Taiwan (24º 45' 40" N, 121º 33' 28" E), part of the worldwide network of forest monitoring plots organized by the Smithsonian Tropical Research Institute's Center for Tropical Forest Studies (CTFS) (Losos and Leigh 2004). Fushan FDP is within an old-growth submontane rainforest, classified as part of the *Machilus-Castanopsis* zone of broad-leaved forests in Taiwan (Su 1984). A 2003-2004 census by Taiwan Forestry Research Institute, Forestry Bureau, and National Taiwan University recorded 110 woody species at the site (Su et al. 2007).

The elevation of the site ranges from 600 to 733 m above sea level. The climate at Fushan is heavily influenced by winter monsoons and summer typhoons, with an average temperature of 18.2 ºC, a mean annual precipitation of 4271 mm, and 95.1% average relative humidity. The soils at the site are very acidic (pH 3.3~5), with low carbon content in the sublayer (<5%) and low cation exchange capacity, though soil

characteristics vary across the site: there are Hapludults on stable slopes and ridges, Dystrochrepts on hillsides, and Udipsamments and Udorthents along streambeds and very steep slopes (Su et al. 2007).

We divided the Fushan FDP into 9 sub-plots, and sampled 6 subplots using a nested logarithmic sampling scheme intended to detect dispersal limitation and community turnover (Fig. 1). A one square meter PVC density frame delimited each point within the subplot; we collected all xylariaceous stromata within the density frame; additionally, many collections were made outside of the sampling design to provide reference material for diversity and DNA work. For each sampling point, we located the tree with the largest DBH with canopy directly above the density frame and collected the three lowest healthy leaves that were safely available. Leaves were obtained using a 3m collapsible pole pruner. Identification of host-tree was supplied by survey data from ongoing ecological research at Fushan FDP (Su et al. 2007). All plant material was carried to a nearby field station and stored at 4°C for no longer than 5 days before processing. All fungal stromata were air-dried indoors at Academia Sinica before being shipped to the University of Oregon for characterization.

Stromata collections & sample processing.—In our lab at the University of Oregon, all stromatal collections were fully characterized for morphological identification concurrent with the initiation of cultures from all possible collections, which were used for culture morphology characterization and DNA extraction for ITS characterization. Cultures were started from the contents of six whole perithecia plated onto 2% water agar with antibiotics and incubated at ambient conditions in the lab for up to 6 months. Fungi were

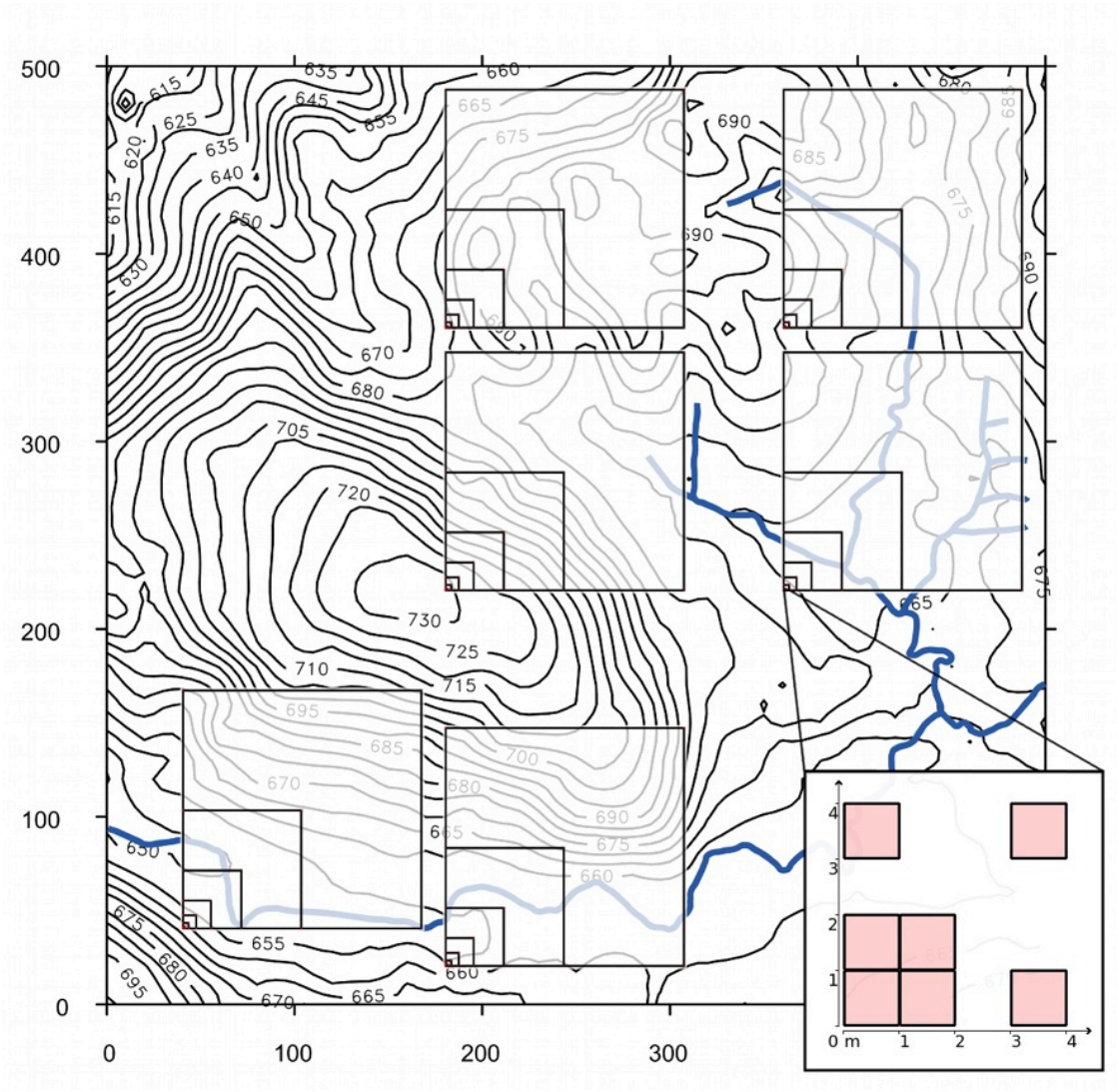


Figure 1: Schematic of the Fushan FDP in northeastern Taiwan, with sampling scheme overlaid. Nested, logarithmically increasing squares are used to maximize possibility of detecting turnover and dispersal limitation, which are theorized to be log-linear processes. Samples were collected in 1-m2 density frames at the corner of each square (detail, bottom right).

isolated onto MEA plates (2% malt, 0.1% yeast extract, 2% agar) as they grew out, generally within the first two weeks as spores germinated, though some spores took

considerably longer for germination. After isolation, all fungi were sub-cultured into liquid MEA culture in 50 mL Erlenmeyer flasks, incubated for two weeks, and then harvested for DNA extraction. Direct stromatal DNA extraction was attempted for collections from which no culture could be obtained.

For DNA extraction, fungal tissues were placed in 2 mL screw-top vials with ~0.3 mL of 0.5 mm zirconium beads, and then subjected three freeze/thaw cycles by alternation between liquid nitrogen and a 65 ˚C water bath. Lysis buffer (Qiagen buffer AP1) was then added and the tissues were disrupted via two 30-second bouts of beadbeating (Biospec Mini-Beadbeater-8) at full speed. DNA extraction then proceeded via Qiagen DNeasy Plant mini spin column kit following the manufacturer's instructions.

DNA extractions were assessed for purity and concentration with a Nanodrop 2000 Spectrophotometer (Thermo Scientific, Mass., USA) prior to PCR; template DNA was subjected to additional purification (Clean & Concentrator, Zymo, California, USA) and/or diluted to ~10 ng/μL as needed. DNA amplification was carried out using the fungal-specific ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primer sets (Gardes and Bruns 1993). DNA amplification was conducted with 12.5-μL reaction volumes (2.5 μL of template, 6.25 μL of Sigma Aldrich Jumpstart^{TM} Taq Readymix^{TM}, 2.75 μL sterile water, 0.5 μL 25 mM $MgCl_2$, and 0.25 μL of each primer).

PCR amplification was done with an MJ Research PTC-200 DNA Engine thermal cycler with the following parameters: initial denaturation at 95˚C for 2 min, five cycles of denaturation at 95˚C for 30 s, annealing at 60˚C for 30 s, and extension at 72˚C for 1 min; followed by 25 cycles of denaturation of 95˚C for 30 s, annealing at 55˚C for 30 s, and

extension at 72˚C for 1 min; a final extension at 72˚C for 10 min and a final step of indefinite duration at 4˚C. PCR products were visualized on a 1 percent agarose gel. Before sequencing, all successful PCR reactions were cleaned by adding 0.4 volumes of a master mix containing 10 percent FastAP© thermosensitive shrimp alkaline phosphatase (Thermo Scientific©) and 1 percent exonuclease I solution (New England Biolabs©) to the PCR product, and incubation for 15 min at 37˚C followed by 15 min at 85˚C. Samples were then frozen until shipping for sequencing at Functional Biosciences, Inc (Madison, WI, U.S.A.) on ABI 3730xl instruments using Big Dye V3.1. Forward and reverse sequences were aligned and curated in Geneious v6.0.3 (Biomatters, Auckland, New Zealand); where morphological species concepts contained multiple distinct groups of sequence variants, variety designations were used to distinguish sequence groups (e.g., "*Nemania bipapillata* var. 1"), which were then treated as distinct taxa in downstream analysis. Consensus sequences with maximum ambiguities were generated in Geneious from all possible sequences for a given taxon (summarizing all variation within a taxon in a single sequence) and used to query the endophyte sequence library.

Endophyte metabarcode library.—Samples of leaves were processed to allow for DNA extraction and next-generation sequencing of the ITS region of fungal endophytes. We did all leaf DNA extractions in the lab at Academia Sinica in Taipei, Taiwan. First, the surfaces of fresh leaves were washed gently with tap water to reduce epiphytes. Then, one square centimeter leaf segments were cut from each of the three leaves collected per sampling plot and surface-sterilized by immersion in 70% ethanol for 30 sec, full-strength bleach (5% sodium hypochlorite) for 1 min, an additional 30 sec in ethanol, then

rinsed thoroughly in sterile deionized water. Leaf tissues were disrupted via bead beating using three 5 mm stainless steel beads for an 80 s agitation cycle at 3450 oscillations/minute. DNA was extracted from homogenized leaf tissues using a Qiagen DNeasy 96 Plant Kit following the manufacturer's instructions.

Extracted DNA was shipped overnight on wet ice to our lab at the University of Oregon, where a metabarcode sequencing library of the fungal internal transcribed spacer (ITS) region of the rRNA gene was prepared. Library preparation followed Meadow et al. (2013) , with slight modifications. Briefly, the ITS region was amplified using a modified fungal specific ITS1F/ITS2 primer set adapted from Mueller et al. (2014) (5′-CTTGGTCATTTAGAGGAAGTAA-3′ / 5′-GCTGCGTTCTTCATCGATGC-3′) (White et al. 1990, Gardes and Bruns 1993) through a two-step custom Illumina preparation protocol. We used a split-barcode system, with unique combinations of six base pair barcodes appended to both the forward and reverse primers; this allowed for fewer total primers to be synthesized, while maintaining a large number of unique possible combinations (Gloor et al. 2010). Primer secondary structures were validated using PrimerProspector (Walters & Caporaso et al. 2011). The first PCR step used forward and reverse primers that contained barcodes and partial Illumina adapters; the second PCR step appended the rest of the Illumina adapters, and barcodes were combined into unique 12 base-pair sequences *in silico* using paired-end reads (full primer sequences are available in the Supplemental Materials, Appendix D).

All first-step PCRs were amplified in triplicate, and then pooled before second-step PCR. First-step PCR (25 µL total reaction volume) was performed using 2.5 µL 10X high fidelity PCR buffer (Thermo Fisher Scientific), 0.125 µL dNTPs (10 mM, Sigma-

Aldrich), 1.25 µL $MgCl_2$ (50 mM, Thermo Fisher Scientific), 0.25 µL Platinum™ Taq high fidelity polymerase (Thermo Fisher Scientific), 14.875 µL certified nucleic-acid free water, 0.5 µL forward primer, 0.5 µL reverse primer, and 5 µL template DNA using the following conditions: initial denaturation for 2 min at 98 °C; 20 cycles of 30 s at 98 °C, 30 s at 60 °C, and 45 s at 72 °C; and 72 °C for 5 min for final extension. The products of first-step PCR triplicates were pooled and cleaned with DNA Clean & Concentrator (Zymo Research, Irvine, CA) following the manufacturer's instructions; 10 µL of 3M NaOAc (pH 5.2) was added to decrease the pH of the pooled reactions and facilitate efficient binding to the spin column, and all samples were eluted using 10 µL of the provided elution buffer. Second-step PCR reactions used a single primer pair to add the remaining Illumina adaptor sequence to the ends of the concentrated amplicons from the first-step PCR. Second-step PCR (25 µL total reaction volume) included the same reagents as above, and used 5 µL of the pooled and concentrated first-step PCR products as template; the conditions were as follows: 2 min denaturation at 98 °C; 14 cycles of 30 s at 98 °C, 30 s at 58°C, and 45 s at 72 °C; and 3 min at 72 °C for final extension. Equal volumes of each sample were then pooled, and the library was size-selected by gel electrophoresis: the wide gel bands centered at ~275bp (175-400bp were removed, to account for the variation present at the ITS1 locus across the kingdom Fungi) were extracted and concentrated using the ZR-96 Zymoclean Gel DNA Recovery Kit (ZYMO Research, Irvine, CA), following manufacturer's instructions. DNA concentration was quantified using a Qubit Fluoromoeter (Invitrogen, NY). Samples were sent to the IBEST Genomics Resources Core at the University of Idaho (Moscow, ID;

http://www.ibest.uidaho.edu/), and sequenced on the Illumina MiSeq platform as paired-end reads after qPCR validation with Illumina-specific primers.

Sequence processing for the Illumina metabarcode library of endophyte ITS amplicons was accomplished using the FastX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit), PANDASeq (Masella et al. 2012), and QIIME (Caporaso et al. 2010). Barcodes were recombined from paired-end reads prior to quality filtering. Forward reads were trimmed to 265bp, and reversed reads were trimmed to 170bp; truncation cut-offs were set using the point of average base-pair quality decline and success downstream of paired-end read alignments. Paired-end reads were assembled using *fastq-join* as implemented in PANDASeq, and then the low quality and un-alignable reads were removed from the library. Quality filtering settings were: minimum quality score of 20 or more over at least 75% of the assembled read; one ambiguous base call allowed; 1 primer mismatch allowed. After assembly, quality filtering, and barcode assignment, the remaining 9,707,490 sequences were binned into OTUs at 95% similarity cutoff using *uclust*. The more conservative 95% similarity threshold was chosen over the more traditional 97% cutoff due to the high variability at the ITS1 locus. This cutoff was validated for our focal fungal family, the Xylariaceae, using data from a previous study (Thomas & Vandegrift et al. 2016): we quantified the intraspecies variation for the ITS1 region within several species and found that a 95% similarity cutoff more accurately clusters species in the Xylariaceae than 97%, which often split species into multiple OTUs artificially (the 97% cutoff clusters species accurately across the full ITS1-5.8S-ITS2 region, but ITS1 tends to be more variable than ITS2). OTUs represented by a

single read were removed, and read counts of OTUs present in negative control samples were removed across all samples (Nguyen et al. 2015).

Taxonomy was assigned using the most abundant read in each OTU cluster by BLAST search against the UNITE database (version 6, dynamic) (Kõljalg et al. 2013). Samples with less than 1000 reads were discarded as anomalous, and data were normalized using negative binomial variance stabilization by DESeq2 (Love et al. 2014) as implemented in the *phyloseq* package in R (McMurdie and Holmes 2013, 2014). A comparison between DESeq variance stabilized normalization and other normalization techniques—traditional rarefied counts, proportional transformation, and cumulative sum scaling (CSS) as implemented in the *metagenomeSeq* package in R (Paulson et al. 2013)—revealed that normalization technique does not appreciably affect the detection nor magnitude of any of the trends presented in this paper. For taxon-specific spatial pattern analysis, taxonomy was assigned using the set of consensus sequences generated from the stromata collections (above), again with a 95% clustering threshold, as the reference set. Sequences not attributable to one of the 44 putative species of Xylariaceae collected as stromata within the site were not considered for spatial analysis.

Statistical analysis.—Data were analyzed using R Statistical Software, version 3.1.3 (R Core Team 2014), including the *phyloseq* (McMurdie & Holmes 2013), *sp* (Pebesma & Bivand 2005), *bipartite* (Dormann *et al.* 2008), and *vegan* packages (Oksanen *et al.* 2013). All scripts are publicly available online (via FigShare).

We employed a Monte Carlo type nearest neighbor with randomization statistical technique to examine spatial relationships within taxa, which we have used previously

(Thomas & Vandegrift et al. 2016). Briefly, the observed average nearest neighbor distance ($\bar{d}_o$) for each neighbor class was compared to a randomly generated distribution of the same distance class ($\bar{d}_e$) representing a null model of complete spatial randomness (10,000 permutations). *P*-values were calculated as the proportion of random permutations where $\bar{d}_e$ values were lower/higher than $\bar{d}_o$ (Fortin and Dale 2005).

Host associations were examined using bipartite network analysis (Ikeda et al. 2014, Thomas & Vandegrift et al. 2016). We constructed species interaction matrices and calculated Blüthgen's H_2', a network-wide measure of structure in the network, to characterize the level of preference (or "specialization") among host-plants and endophytes. We then used permutations with randomization (10,000 permutations) to compare the observed networks to a null model of network assembly (Vázquez et al. 2007). We constructed and tested networks for the total endophyte community and the subset of only Xylariaceae for the six most common host species to test if xylariaceous endophytes are generally more or less likely to be host-specific than the average endophytic fungus. Networks were constructed using the rarefied data, so that meaningful read cut-off levels could be established across the samples and OTUs for inclusion in the network analysis. OTUs were only included in network analysis at 6 reads and above, so that there was the possibility for reads to be spread evenly across hosts for all included taxa. To control for the effect of sample size in the network, we also constructed networks from random samples of the total endophytic community of the same OTU abundance as the Xylariaceae; to further test the effect of taxonomic group on network specificity, we also created networks from subsets of fungal groups present in the

endophyte library in similar abundance to the Xylariaceae, including the Mycosphaerellaceae and the Basidiomycota.

In the case of different life stages clustering together (e.g., endophytes around decomposers), we take this as evidence that dispersal is occurring between these different life stages. Tests for spatial correlation ("autocorrelation") of environmental variables were conducted using a Mantel correlogram of environmental dissimilarity of plots against a physical distance matrix. Testing for community turnover, or decay of similarity in Xylariaceae species composition among plots with distance, was done using a Mantel correlogram of Xylariaceae species composition distance matrix against a physical distance matrix of all plots sampled (Fortin and Dale 2005).

Tests for grouping of species by habitat type (as defined by Su et al. (2010) as "Forest_Type"), vegetative community (as defined by Su et al. (2007) as "vegcom"), and host (for the six most common host species) were done using Permutational Multiple Analysis of Variance (PerMANOVA), with the *adonis* function in *vegan* package in R. These data were visualized with non-metric multidimensional scaling (NMDS).

RESULTS

We collected xylariaceous fungi from 48 of the 133 square-meter plots; we collected a total of 44 distinct xylariaceous taxa at the site, 32 within the plots and an additional 12 taxa collected by stochastic sampling within the FDP; these collections represented 7 genera within the Xylariaceae (Table 1). Of the 44 taxa collected as stromata, we were

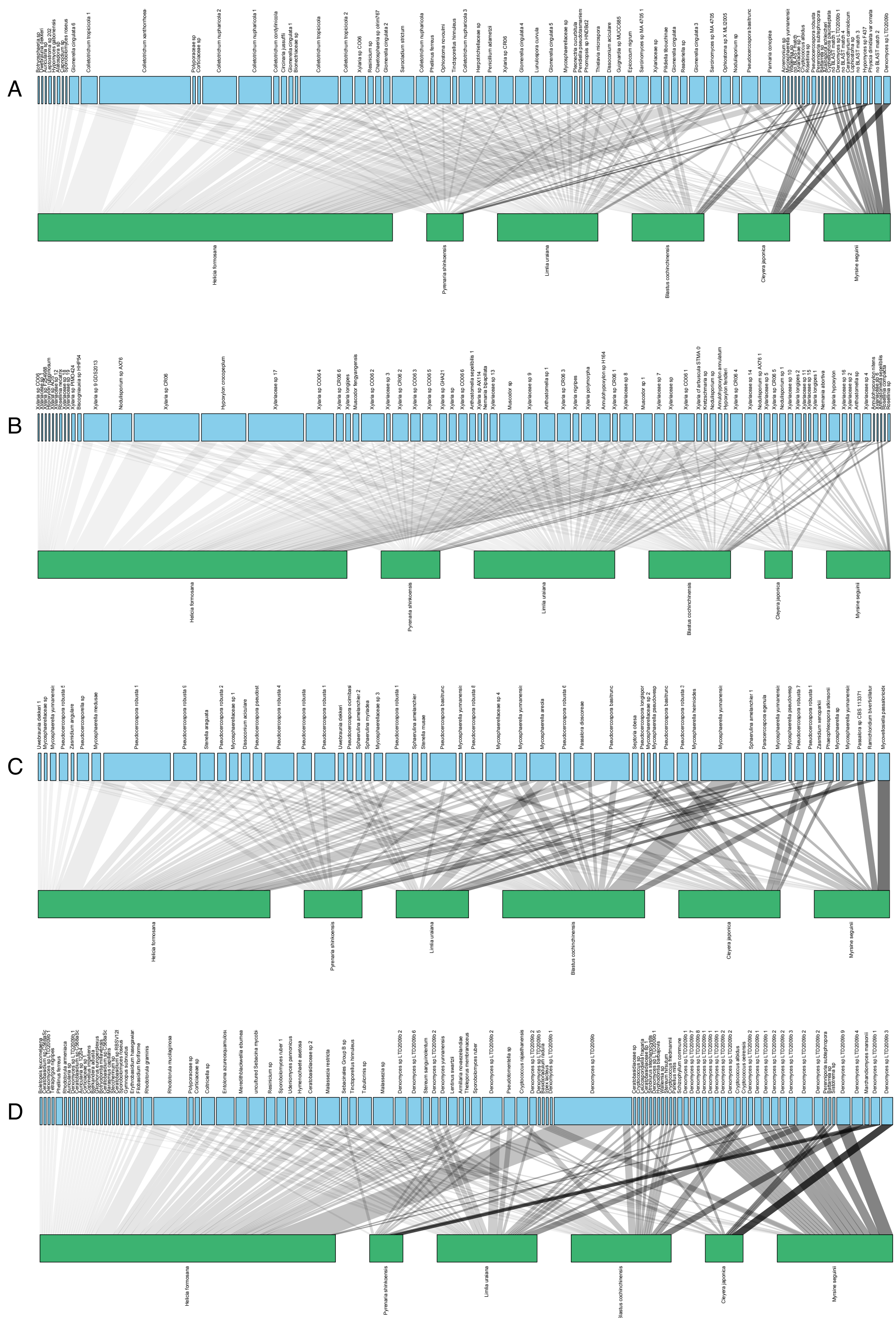
Table 1: List of all Xylariaceae taxa collected as stromata and the number of points in the study area (out of 133) from which each species was recovered in each life stage. Distinct ITS clusters in otherwise indistinguishable taxa are indicated by numbered varieties after the species epithet.

Taxa	Points with **Stromata**	Points with **Endophytes**
Annulohypoxylon atroroseum	3	13
Annulohypoxylon aff. *atroroseum*	0	17
Annulohypoxylon bovei var. *microspora*	3	2
Annulohypoxylon moriforme	1	2
Annulohypoxylon moriforme var. *microdiscus*	0	0
Annulohypoxylon nitens	0	0
Annulohypoxylon purpureonitens	1	0
Annulohypoxylon stygium	0	0
Annulohypoxylon aff. stygium	2	0
Annulohypoxylon truncatum	1	0
Annulohypoxylon sp. 1	1	0
Annulohypoxylon sp. 2	0	0
Annulohypoxylon sp. nov. 1	1	0
Biscogniauxia capnodes var. 1	1	1
Biscogniauxia capnodes var. 2	1	0
Biscogniauxia capnodes var. *rumpens*	0	0
Hypoxylon investiens	0	5
Hypoxylon munkii	0	2
Hypoxylon notatum	1	13
Hypoxylon perforatum	1	7
Kretzschmaria pavimentosa var. 1	1	8
Kretzschmaria pavimentosa var. 2	1	22
Kretzschmaria zonata	0	0
Nemania bipapillata var. "bipapillata"	2	8
Nemania bipapillata var. "macrodiscus"	1	15
Nemania bipapillata var. "mediodiscus"	0	35
Nemania diffusa	4	89
Nemania sp. 1	1	24
Whalleya microplaca	2	11
Xylaria allantoidea	1	0
Xylaria anisopleura	1	0
Xylaria aff. *anisopleura*	0	0
Xylaria atrosphaerica	2	8
Xylaria flabelliforme (penzigioid)	18	58
Xylaria flabelliforme (non-penzigioid)	1	78
Xylaria fraseri	0	1
Xylaria glebulosa	1	0
Xylaria intracolorata	0	52
Xylaria schweinitzii	4	16
Xylaria aff. *scruposa*	1	0
Xylaria telfairii	1	0
Xylaria sp. 1	1	3
Xylaria sp. nov. 1	2	7
Xylaria sp. nov. 2	0	2

able to detect 26 as endophytes in the metabarcode library (Table 1). Additionally, de novo OTU clustering and taxonomic assignment via the UNITE database indicates 2431 unique endophytic taxa (OTUs) in the Xylariaceae. Chao2 and Jackknife1 species richness estimators predicted, respectively, 61.4 (SE = 21.9) and 46.9 (SE = 4.2) Xylariaceae decomposer (stromatal) species, and 3454.5 (SE = 84.2) and 3456.5 (SE = 255.6) xylariaceous endophytes. There appears to be a taxonomic signal in the paired collection dataset: of the xylariaceous fungi we collected as stromata, the genera in the subfamily Xylarioideae (*Nemania, Kretzschmaria, Xylaria*) are over-represented (16 of 23 taxa detected as endophytes) as endophytes in the data relative to genera in the Hypoxyloideae (*Annulohypoxylon, Biscogniauxia, Hypoxylon, Whalleya*; 10 of 21 taxa detected as endophytes), though there is generic variation—for example, all collected species of *Hypoxylon* were detected as endophytes.

Host specificity.—We found significant structure in bipartite networks for host/endophyte co-occurrence across all endophytes for the six most common hosts (1215 OTUs; $H_2' =$

Figure 2 (next page): Bipartite host-association networks for (A) random subset of the total endophyte community, (B) the subset of just the Xylariaceae, (C) the subset of just the Mycosphaerellaceae, and (D) the subset of just the Basidiomycota. Networks are constructed from rarefied count data, so that meaningful read cut-offs could be utilized in filtering taxa for inclusion; samples were filtered to only include the six most common hosts (*Helicia formosana*, 30 samples; *Pyrenaria shinkoensis*, 7 samples; *Limlia uraiana*, 10 samples; *Blastus cochinchinensis*, 13 samples; *Cleyera japonica*, 6 samples; and *Myrsine seguinii*, 6 samples), and OTUs were only included that had ≥ 6 reads, such that it was possible for all taxa to be present in all included hosts. Widths of links are scaled to number of points at which endophytes were isolated from hosts; color of links is scaled by the endophyte d' value.


A
Helicia formosana
Pyrenaria shinkoensis
Limlia uraiana
Blastus cochinchinensis
Cleyera japonica
Myrsine seguinii
B
Helicia formosana
Pyrenaria shinkoensis
Limlia uraiana
Blastus cochinchinensis
Cleyera japonica
Myrsine seguinii
C
Helicia formosana
Pyrenaria shinkoensis
Limlia uraiana
Blastus cochinchinensis
Cleyera japonica
Myrsine seguinii
D
Helicia formosana
Pyrenaria shinkoensis
Limlia uraiana
Blastus cochinchinensis
Cleyera japonica
Myrsine seguinii

0.289, $p < 0.001$), indicating that some endophytes display non-random host preference. A random subset of the total endophytic community, reducing the number of reads to be equal to the Xylariaceae, was also significantly structured (78 OTUs; $H_2' = 0.365$, $p < 0.001$; Fig. 2A). Bipartite host/endophyte networks for endophytes restricted to the fungal family Xylariaceae were not significantly structured (72 OTUs; $H_2' = 0.131$, $p = 0.994$; Fig. 2B). We also tested other fungal groups present in the data at comparable frequency of reads or OTUs to the Xylariaceae: the fungal family Mycosphaerellaceae ($H_2' = 0.322$, $p = 0.043$; Fig. 2C), which had a similar read abundance to the Xylariaceae in the total dataset, though fewer apparent taxa (55 OTUs) was also significantly structured, as was the phylum Basidiomycota ($H_2' = 0.519$, $p < 0.005$; Fig. 2D), which contain a similar number of taxa to the Xylariaceae (97 OTUs).

Host was found to be a significant predictor of endophytic community via PerMANOVA analysis in the total endophyte set ($F_{5, 61} = 2.317$, $R^2 = 0.139$, $P < 0.001$; Fig. 3A). It was also significant for the subset including just the Xylariaceae ($F_{5, 60} = 1.296$, $R^2 = 0.084$, $P = 0.002$; Fig. 3B), though the variance explained is much reduced.

Spatial patterning and clustering.—We find mixed evidence of spatial linkage, indicating different spatial ecologies on a taxon-by-taxon basis (Table 2). As an example, we have pulled out *Nemania diffusa* (Pers.) Gray to examine in depth: this fungus was collected as stromata four times during our spatially explicit sampling (Fig. 4A), and was a relatively common endophyte at the site (Fig. 4B). Interestingly, we find some evidence that the

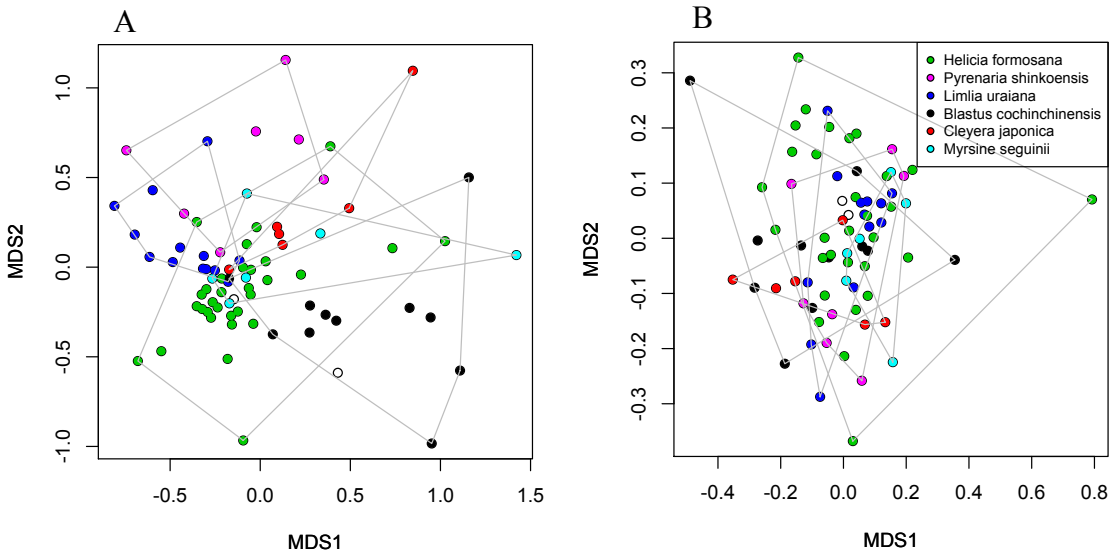


Figure 3: NMDS plots on Bray-Curtis dissimilarity of communities for (A) all endophytes, and (B) the subset of just the Xylariaceae. Samples are colored by host species for the six most common hosts sampled.

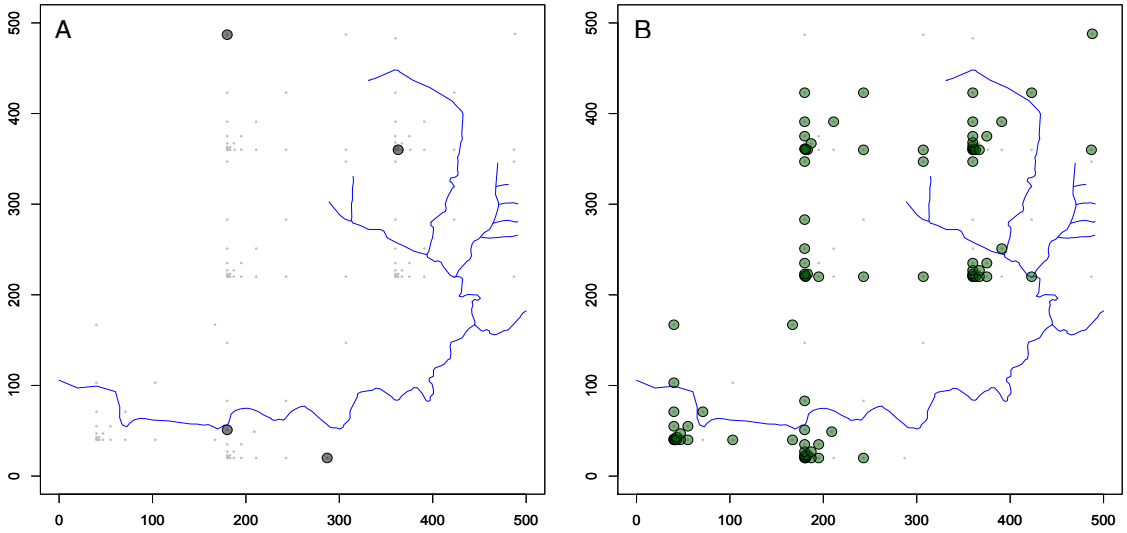


Figure 4: *Nemania diffusa* (Pers.) Gray – Location maps of our field site at the Fushan FDP for the occurrence of *N. diffusa* as both stromata (A) and endophytes (B) in the Illumina dataset. The stream that runs through the site is plotted in blue, and sampling locations are given as grey points; occurrences are plotted with transparency to make overlapping points close to the vertices of our logarithmically nested squares more apparent. Scale in meters; true north is vertical in these plots.

stromata are more dispersed than expected by chance alone (Fig. 5A). We also find evidence of increased dispersion above that expected by chance between life stages, in both directions (Fig. 5C & 5D); in other words, there is some evidence of anti-clustering, or repulsion, between the two life stages—a tendency for mutual exclusion. The rhythmic pattern to the endophyte around stromata nearest neighbor plot (Fig. 5D) reflects the presence of all four stromatal locations, and may indicate patterns from distinct dispersal

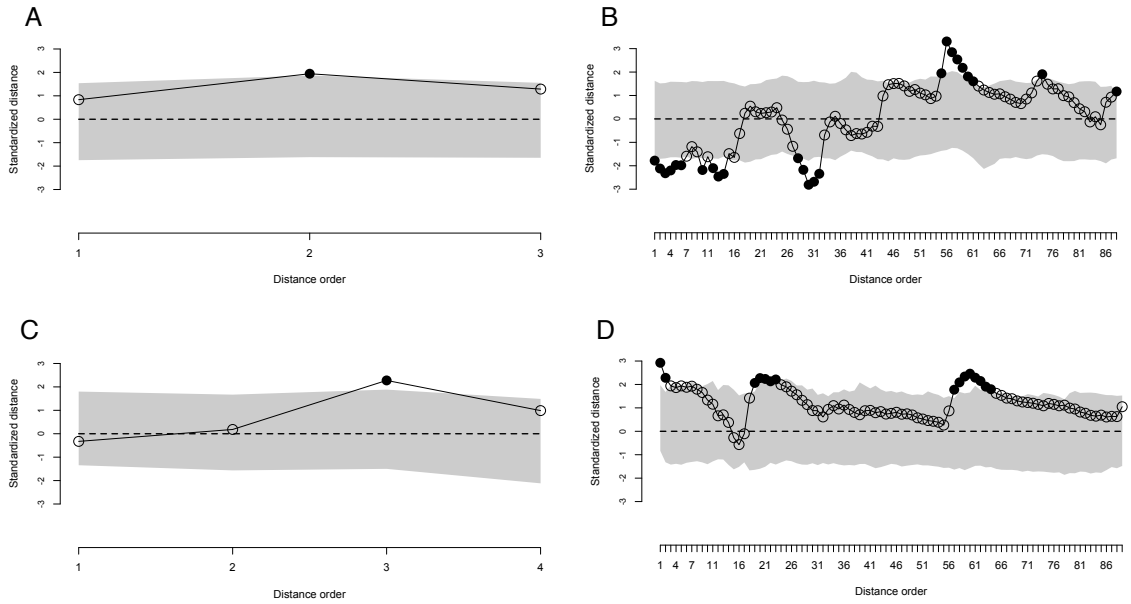


Figure 5: *Nemania diffusa* – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for clustering of different life stages of *N. diffusa*: **A**, stromata around each other; **B**, endophytes around each other; **C**, stromata around endophytes; and **D**, endophytes around stromata. The standardized mean distance to nearest neighboring point of each comparison class ($\bar{d}_o$) for all available distance classes is plotted. The dashed line represents the mean distance to point of each comparison class for the permutations on complete spatial randomness ($\bar{d}_e$), standardized to zero for all distance classes; the y-axis units represent deviation from the permutational mean (s_e). Open points are non-significant ($P > 0.05$); closed points display significant deviation from the permutational mean ($P < 0.05$ or $P > 0.95$); the grey area represents the region where $0.95 > P > 0.05$.

centers interacting with each other. The endophytes, however, appear to be clustering together (Fig. 5B); there are, again, interesting patterns at higher distance classes. The pattern here seems to be indicative of clustering within the life stage, at a cluster size of about a dozen points; however, these clusters appear to be farther apart than is expected under complete spatial randomness (and thus the evidence of dispersion in the late fifties neighbor classes).

Table 2 summarizes significant results of the nearest neighbor permutational analysis. We find evidence of clustering between life stages in four taxa within the Xylariaceae (Table 2: *A. atroroseum, K. pavimentosa* var. 2*, N. bipapillata* var. 1*,* and *X.* sp. 1); we find increased dispersion between life stages in six taxa (Table 2: *A. atroroseum, N. diffusa, W. microplaca, X. flabelliforme* (penzigioid), *X. schweinitzii,* and *X.* sp. nov. 1). In *A. atroroseum*, we find mixed evidence for clustering and increased dispersion between life stages; this likely represents small cluster size with repulsion of clusters at larger spatial scales, or the influence of multiple interacting patterns in space.

We find evidence of clustering in the endophytic stage in 8 taxa (Table 2: *A. atroroseum, K. pavimentosa* var. 1, *N. bipapillata* var. 2, *N. bipapillata* var. 2, *N. diffusa, W. microplaca, X. flabelliforme* (non-penzigioid), and *X.* sp. nov. 1). Similar to the between phase results from *A. atroroseum*, we find mixed evidence for clustering and over-dispersion in the endophytic phase of several taxa; when mixed results are present, lower neighbor classes are always clustered, while higher neighbor classes are always over-dispersed (e.g., *N. diffusa*; Fig. 5B). Again, this likely represents small cluster size with repulsion of clusters at larger spatial scales, possibly due to the influence of habitat types associated with the higher ground in the center of the Fushan plot (Fig. 1). This

trend is relatively consistent for endophytes within life phase, regardless of significance; most endophytes show early neighbor classes with observed nearest neighbor distances

Table 2: Summary of results from the permutational test of nearest neighbor distances. Arrows indicate if there were neighbor classes where $\bar{d}_o$ was above (↑) or below (↓) $\bar{d}_e$ in 95% of permutations (i.e., $P < 0.05$). Taxa without any significant clustering are omitted from the table, but see Appendix D Supplemental Fileset S1 for full results of the permutation tests. Arrows in both directions indicate that some neighbor classes were below and some above, and the order of the arrows reflects the sequence of significant deviation in neighbor classes; that is, if the first neighbor class had $\bar{d}_o$ significantly below $\bar{d}_e$, and the fifth neighbor class had $\bar{d}_o$ significantly above $\bar{d}_e$, the symbols listed would read down-up (↓↑).

	Between life stages		**Within life stage**		**Around the Stream**	
Taxa	S → E*	E → S†	S‡	E§	S	E
A. atroroseum	↓↑	↑	↓	↓	—	↓
A. aff. *atroroseum*	—	—	—	—	—	↓
A. moriforme var. *microdiscus*	—	—	—	—	↑	—
H. investiens	—	—	—	—	—	↑
H. notatum	—	—	—	↑	↑	—
H. perforatum	—	—	—	↑	↑	—
K. pavimentosa var. 1	—	—	—	↓	—	—
K. pavimentosa var. 2	↓	—	—	↑	—	↑
N. bipapillata var. 1	—	↓	—	—	—	↑
N. bipapillata var. 2	—	—	—	↓	—	↓
N. bipapillata var. 3	—	—	—	↓↑	—	↑
N. diffusa	**↑**	**↑**	**↑**	**↓↑**	**↓↑**	**↓**
N. sp. *1*	—	—	—	↑	—	↓
W. microplaca	↑	—	↑	↓	—	—
X. aff. anisopleura	—	—	—	↑	—	—
X. flabelliforme (penzigioid)	↑	↑	—	↑	—	↓
X. flabelliforme (non-penzigioid)	—	—	—	↓↑	—	↓
X. fraseri	—	—	—	—	—	↓
X. intracolorata	—	—	—	—	—	↓
X. schweinitzii	↑	↑	—	↑	—	↑
X. sp. 1	↓	—	—	↑	↓	↓
X. sp. nov. 1	↑	↑	—	↓	—	—
X. sp. nov. 2	—	—	—	—	—	↓

* Average nearest neighbor calculations from stromata to endophytes
† Average nearest neighbor calculations from endophytes to stromata
‡ Stromata
§ Endophytes

($\bar{d}_o$) below the permutational mean ($\bar{d}_e$), and later neighbor classes above $\bar{d}_e$ (Fig. 6).

Plots summarizing results from permutational nearest neighbor tests for all taxa are available in the Supplemental Materials (Figs. S1–S38 in Appendix D).

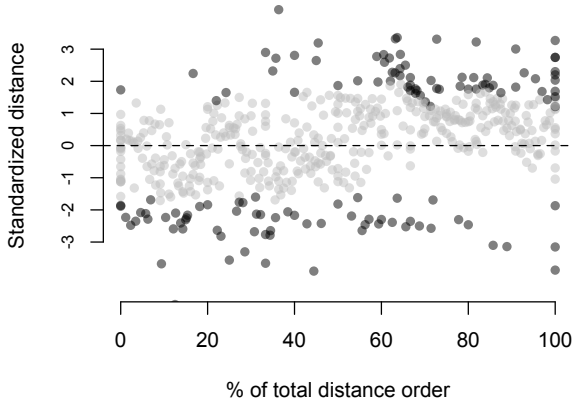


Figure 6: Plot of standardized nearest neighbor distances for all distance classes for all collected Xylariaceae detected as endophytes, plotted by percent of total available distance orders. Points are colored grey if they are within the 95% confidence window from the individual permutation tests by taxa, and black if they are outside.

Distance to stream.—It appears that there is not the widespread effect of the stream on stromata in our Taiwan dataset that we observed in our Ecuador data (Thomas & Vandegrift et al. 2015). We see evidence of clustering around the stream for the stromata in only two taxa (Table 2): *Nemania diffusa* (Fig. 7A; $P = 0.029$ for the first distance class) and *Xylaria* sp. 1 ($P < 0.005$ for the first and only distance class). There is also marginal evidence of clustering in three other taxa: *Annulohypoxylon moriforme* ($P < 0.087$ for the first and only distance class), *Nemania bipapillata* var. 2 ($P = 0.095$ for the first and only distance class), and *Whalleya microplaca* ($P = 0.092$ for the first distance class). Also of interest, and different from our Ecuadorian dataset, there appears to be some evidence of taxa occurring farther from the stream than expected by chance (Table 2). We find this with the fourth distance class of *N. diffusa* (Fig. 7A; $P = 0.030$), as well

as two species of *Hypoxylon*, *H. notatum* ($P = 0.018$ for the first and only distance class) and *H. perforatum* ($P = 0.022$ for the first and only distance class).

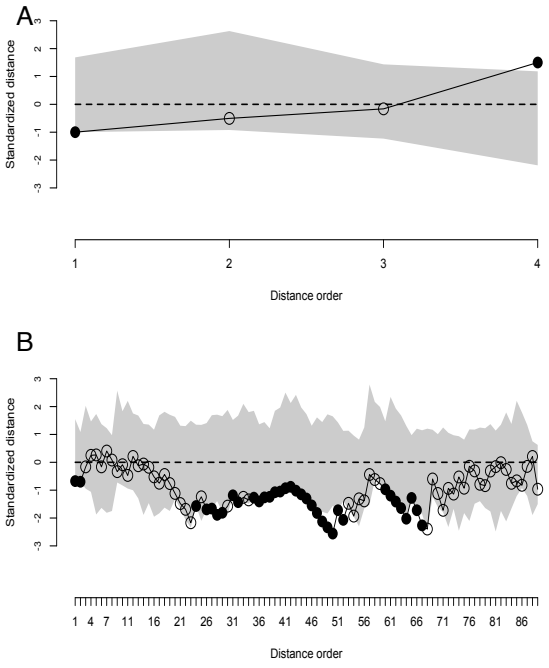


Figure 7: *Nemania diffusa* – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for distance to the stream for *N. diffusa*: **A**, stromata; and **B**, endophytes. The standardized mean distance to the stream of each distance class ($\bar{d}_o$) for all available distance classes is plotted. The dashed line represents the mean distance from occupied points to the stream of the permutations on complete spatial randomness ($\bar{d}_e$), standardized to zero for all distance classes; the y-axis units represent deviation from the permutational mean (s_e). Open points are non-significant ($0.95 > P > 0.05$); closed points display significant deviation from the permutational mean ($P < 0.05$ or $P > 0.95$); the grey area represents the region where $0.95 > P > 0.05$.

Interestingly, in our Ecuadorian data we saw no evidence of fungi in the endophytic life stage clustering around the stream, but in our Taiwanese data, we find several taxa showing either closer distributions to the stream than expected by chance, or distributions that appear to be farther from the stream than expected by chance (Table 2). The effect of distance to water sources and the associated gradients on host-plants and saprotrophic fungi may operate on different scales, or have differing effects on the fungi. When fungi are aggregated at the genus level, we find that most genera (*Annulohypoxylon, Hypoxylon, Whalleya, Nemania, Kretzschmaria, Xylaria*) display at

least some evidence of clustering to the stream, while no genera display evidence of repulsion from the stream (Fig. 8).

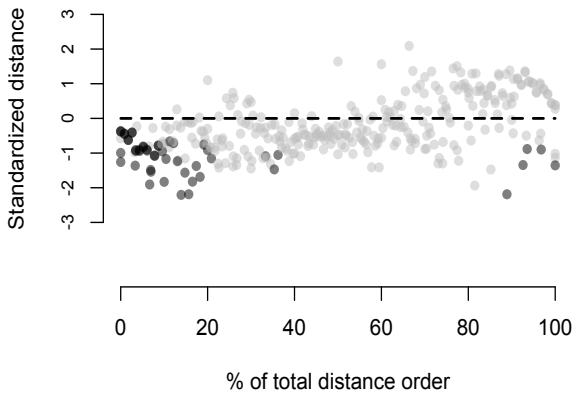


Figure 8: Plot of standardized distance to the stream for all distance classes for all collected Xylariaceae detected as endophytes, analyzed by genus, plotted by percent of total available distance classes. Points are colored grey if they are within the 95% confidence window from the individual permutation tests by taxa, and black if they are outside. Plots by individual genera are available in the Supplemental Materials (Appendix D, Figs. S39-45).

Physical and environmental factors.—We find that the vegetative community of tree species within each 20 m division of the plot (groups as defined by Su et al. 2007) is a significant predictor of turnover in endophytic community ($F_{3, 61} = 1.732$, $R^2 = 0.062$, $P < 0.001$; Fig. 9A), independent of the effect of host—that is, there is no significant interaction term. This effect is consistent for the subset of endophytes in the Xylariaceae ($F_{3, 60} = 1.333$, $R^2 = 0.052$, $P = 0.005$; Fig. 9B). The habitat type (as described in Su et al. 2010), however, is not correlated with changes in the endophytic community ($F_{6, 61} = 0.933$, $R^2 = 0.067$, $P = 0.799$), nor with changes in the subset of xylariaceous endophytes ($F_{6, 60} = 1.052$, $R^2 = 0.082$, $P = 0.230$).

There is no evidence for detectable community turnover across the site in the total endophyte community (Mantel's $r = -0.021$, $P = 0.758$), nor for the subset of xylariaceous endophytes (Mantel's $r = 0.016$, $P = 0.292$). There is also no apparent correlation between environmental dissimilarity (calculated using Bray-Curtis, from

individual site measurements of elevation, distance from the stream, slope, aspect, in its components of northern and eastern exposure, diameter at breast height of host tree, and

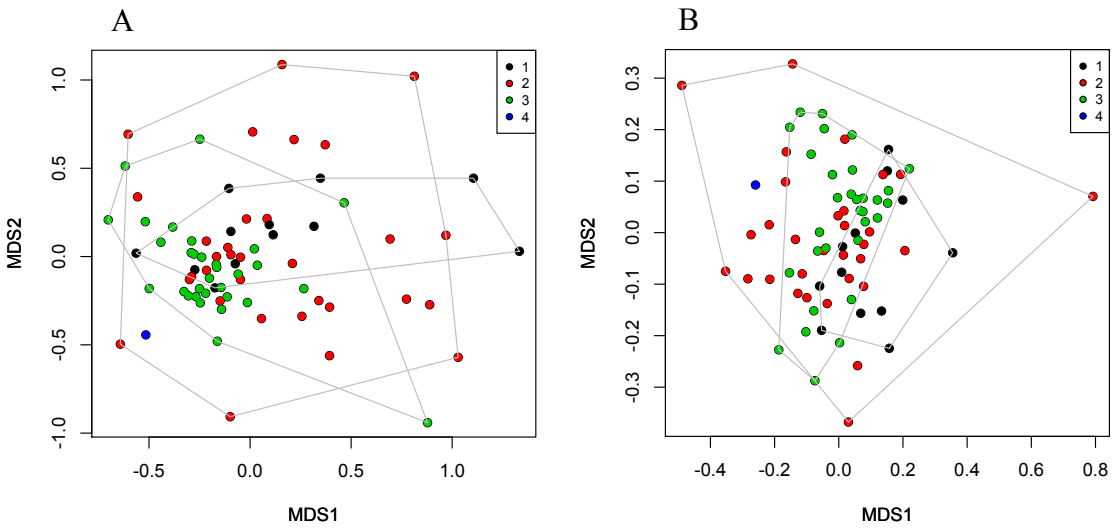


Figure 9: NMDS plots on Bray-Curtis dissimilarity of communities for (A) all endophytes, and (B) the subset of just the Xylariaceae. Samples are colored by vegetative community as defined by Su et al. (2007).

sample collecting height on host tree) and the endophyte community, either in total (Mantel's $r = 0.032$, $P = 0.277$) or just the Xylariaceae (Mantel's $r = -0.066$, $P = 0.880$). We find no evidence of spatial autocorrelation of the measured environmental variables at the scale of our sampling (Mantel's $r = 0.020$, $P = 0.729$).

Vegetative community also is a significant predictor of community turnover of xylariaceous stromata between plots ($F_{3, 60} = 16.956$, $R^2 = 0.091$, $P = 0.003$), as is the habitat type ($F_{3, 60} = 3.042$, $R^2 = 0.052$, $P = 0.005$). Additionally, there is a significant interaction between vegetation type and habitat type on the community turnover of xylariaceous stromata ($F_{3, 60} = 4.994$, $R^2 = 0.124$, $P < 0.001$). There is an effect of

physical distance on the stromatal community, with dissimilarity increasing with increasing physical distance (Mantel's $r = 0.416$, $P < 0.001$); communities of xylariaceous stromata show evidence for spatial autocorrelation under 150 m distance (Fig. 10A). Linear regression of xylariaceous community dissimilarity by distance between plots yields a significant correlation ($F_{1, 7379} = 1546$, $R^2 = 0.173$, $P < 0.001$; Fig. 10B). There is no interaction between physical distance and environmental distance (multiple regression on distance matrices (Lichstein 2007), 5000 permutations: $F = 785.108$, $R^2 = 0.175$, $P < 0.001$).

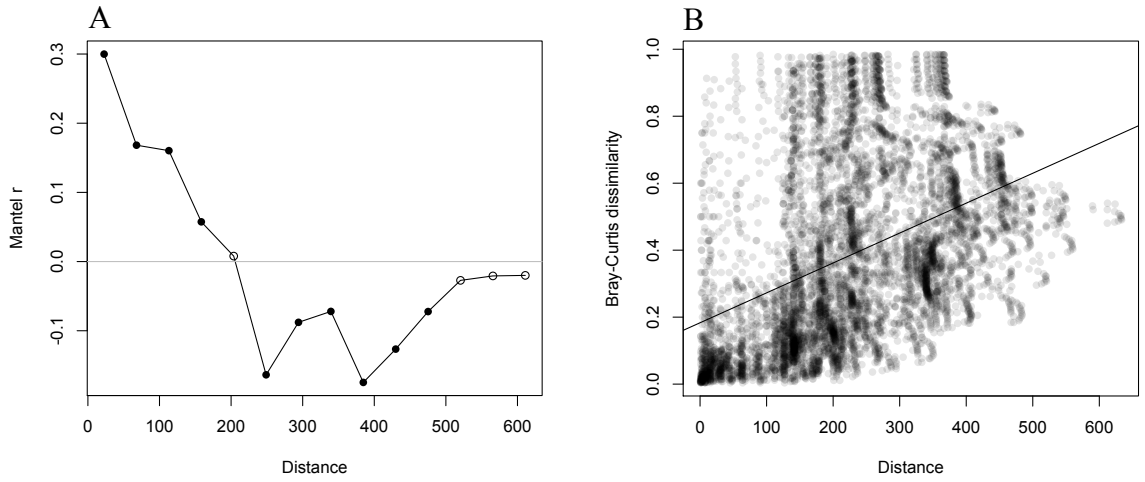


Figure 10: Effect of physical distance on community dissimilarity of Xylariaceae stromata communities: (A) Mantel correlogram, showing evidence for spatial autocorrelation below 150 m distance; and (B) plot of dissimilarity by distance, showing linear regression.

DISCUSSION

Given the lack of myco-centric views on the evolution of endophytism, we set out to investigate a promising theory for why some fungi might find it adventitious to adopt this

lifestyle, despite prolonged periods of quiescence and a lack of active reproduction. The Foraging Ascomycete (FA) hypothesis—the hypothesis that fungi may utilize an endophytic lifestyle as a means of bridging spatial and temporal gaps in appropriate substrate—goes against two classic assumptions concerning fungal dispersal: first, that fungal spores are effectively unlimited in their dispersal abilities (Baas-Becking 1934, Fenchel and Finlay 2004), and second, that the spores of decay fungi are the sole major source of inoculum in the field (Malloch and Blackwell 1992, Bayman et al. 1998, Norros et al. 2012). We are by far not the first to question Becking's assertion that "everything is everywhere, and the environment selects" (Becking 1934); many others have found evidence for dispersal limitation of fungi and other microorganisms (Roy 2001, Green and Bohannan 2006, Telford et al. 2006, Grubisha et al. 2007, Peay et al. 2010, Galante et al. 2011, Norros et al. 2012). Direct colonization of substrates from mycelium is known in many tree pathogens (Epstein 1978, Morrison et al. 1988, Smalley et al. 1993, Cruickshank et al. 1997, Hansen and Goheen 2000), as well as in arbuscular mycorrhizal fungi (Dodd et al. 2000, Hart and Reader 2002), but remains obscure in decomposer fungi.

The FA framework leads to several testable hypotheses. Logically following from the FA strategy, we would predict (1) host generalism for the endophytic phase; (2) spatial linkage between the saprotrophic and endophytic phase; (3) reduced dispersal limitation in the endophytic phase as compared to the general endophyte community; (4) endophytic phase fungi to be less sensitive to environment than saprotrophic phase fungi; and (5) that the FA strategy is an ecological strategy restricted to certain groups, constrained by lineage.

Host generalism.—The hypothesis of host generalism (1) comes from the distribution of hosts in tropical forests. In diverse tropical forests, most available hosts are relatively rare (May 1991), such that the probability of encountering any particular species within the spore-dispersal radius of a fungal fruiting body is low. In diverse forests, endophytic host generalism increases the probability of making it into the endophytic phase, and increases dispersal possibility. We found evidence for host generalism within the subset of endophytes in the Xylariaceae (Fig. 2B), though there is some evidence of host affecting compositional changes in xylariaceous endophytic communities. The effect of host on endophytic community is greatly reduced for the subset of the xylariaceous endophytes as compared to the total community, however (Fig. 3). The remaining signal of host within the Xylariaceae could possibly be influenced by several factors. The forests at Fushan are diverse, but they are not hyper-diverse equatorial forests; the five most common tree species account for more than half of the basal area (Su et al. 2007). This strong dominance component may lead to differing evolutionary constraints on host/endophyte relationships. Additionally, given the breadth of evolutionary history within the Xylariaceae, and the presumed age of divergence of various genera within (Rogers 1979a, 2000, Whalley 1996), it is quite likely that endophytes within this family represent multiple ecological strategies. This is discussed further below.

We do see, however, that vegetative community predicted turnover in both the total set and the xylariaceous endophytes, but the variance explained by vegetative community is much less in the xylariaceous endophytes (Fig. 9). This is evidence that Xylariaceae are less constrained than the totality of endophytes, and is in agreement with

the prediction of the FA hypothesis—if a FA strategy is common in the Xylariaceae, we would expect this lineage to be less sensitive to the effects of host. It is likely that part of this signal is the indirect effect of vegetative community influencing substrate, which influences fruiting of many fungi, which influences inocula present, which (finally) influences endophytic community. Some Xylariaceae are know to be relatively substrate specific (Rogers 1979b, Ju and Rogers 1996, 1999, Rogers et al. 2002, Hsieh et al. 2010, Petrini 2013), so it is not surprising that vegetative community may make some difference to the endophytic xylariaceous community—despite there being little evidence of endophytic host preference in the family—through the regulation of available inocula.

Spatial linkage.—The hypothesis of spatial linkage between life-phases (2) stems from the dispersal linkage between the two life-phases: it is presumed that fruiting decomposer fungi produce spores that lead to endophytic infections, and that senescing leaves serve as dispersal propagules, carrying fungi to new substrates to decompose. While we have found evidence of spatial linkage between life-phases of *Xylaria* in a study on a smaller spatial scale in Ecuador (Thomas & Vandegrift et al. 2016), we see very limited evidence of spatial linkage in xylariaceous taxa in this study, though there are a few taxa showing linkage (Table 2). Our sampling scheme was explicitly designed to test effects of turnover and distance decay, and as such is not ideal for detecting these sorts of spatial linkages.

We do see evidence of clustering within the endophytic phase more frequently, however (Table 2; 7 taxa). There may be temporal lag in the spatial linkage, which could complicate detection. That is, it is possible that clustering within endophytes may

represent “the ghost of stromata past”. Given the relatively long life-span of leaves in evergreen tropical forests (Reich et al. 1991, Bruijnzeel and Veneklaas 1998), it is likely that we are detecting endophytes whose source was a fruiting that has since decayed and is no longer detectable. With our larger average pixel size and varying distance window, it is also possible that we are we are observing the higher order patterns when there are multiple sources of dispersal within the plot, similar to moiré pattern. These are larger-scale patters caused by the interactions of two smaller patterns; the beat frequency heard between nearly identical pure notes is a classic example of one-dimensional moiré effects. The signal of dispersal from two point sources may overlap, and cause a more complex interaction pattern in the zone of interaction. This may explain the rythmic nature of some of our observation, such as in *N. diffusa* clustering around stromata (Fig. 5D). It will take more intensive, finer-scale sampling to elucidate such interacting patterns, however.

Dispersal limitation.—Since the FA hypothesis proposes that in taxa utilizing this strategy endophytism has specifically evolved as an aid to dispersal, we expect FA endophytes to generally display reduced dispersal limitation as compared to the set of all endophytes (3). This is because the total community of endophytes includes fungi utilizing a diversity of ecological strategies (Rodriguez et al. 2009); for many of these, endophytism will have nothing to do with dispersal—it is likely that there are many endophytes that are incidental infections, representing a subset of all inocula present at a given location (Bayman et al. 1998, Carroll, pers. comm.). However, we do not see any evidence of significant turnover with distance in the total endophytic community, nor in

the xylariaceous endophytes. The Fushan plot, at 500 × 500 m, is probably too small to observe significant beta-diversity decay over space within the diverse endophytic community.

We do see turnover with distance in the community of Xylariaceae stromata, however (Fig. 10). This evidence for spatial turnover in the stromata, but not in the endophytes is consistent with the FA hypothesis predictions; we expect the endophytic phase to be less dispersal limited through reduced sensitivity to environmental limitations; that is, the "cloud" of endophyte infections originating with particular stromata extends further than the stromata themselves can spread, in the attempt to bridge unsuitable habitat.

Sensitivity to the environment.—The prediction that fungi utilizing a FA strategy will be less sensitive to environmental conditions in the endophytic phase (4) is a logical outgrowth of the concept of endophytism as a means of bridging gaps in habitat. Since the environment within leaves is highly buffered from the environment by the host, we would expect the endophytic phase to be less sensitive to environmental gradients than their saprotroph-phase counterparts. Su and colleagues used topological and other characteristics of the Fushan FDP to categorize each 20 × 20 m plot into seven distinct habitat types, which they then demonstrated are correlated with vegetative community composition (Su et al. 2010). We found that habitat type is not related to communities of xylariaceous endophytes, but is related to communities of xylariaceous stromata. We take this as evidence that the endophytic phase is released from environmental constraints as compared to the stromata.

The nature of the landscape at the Fushan plot (Fig. 1) is likely explanatory of some of the spatial structuring we see in our data. Broadly, the plot is composed of low-lying stream habitat wrapping around the south and east of a small mountain (60 m elevation gain) in the center. Our sampling includes stream habitat on either side of this mountain, as well as a set of samples centered near the top; this arrangement, combined with habitat effects, may explain some of the spatial structuring we see in our data. For example, the total endophyte community generally shows little evidence for beta-diversity turnover with space, though there is a window (~200–300 m) where dissimilarity is significantly decreasing. This window corresponds to the distance necessary to bridge the mountain: the average distance between the centers of all stream-habitat sample sets. We see a similar (though more dramatic) decrease in dissimilarity in the Xylariaceae stromata community (Fig. 10A), combined with evidence of decreasing dissimilarity at smaller distances, indicating turnover within as well as between habitats. The significant effect of vegetative community, which changes significantly with habitat (Su et al. 2010), on endophyte turnover (Fig. 9) reflects this: communities are more similar within particular vegetative communities, even if they are on opposite sides of the mountain.

Phylogenetic niche conservatism.—It is relatively well accepted that ecological traits are conserved in phylogenetic lineages (Losos 2008); this concept is called phylogenetic niche conservatism (PNC). Given previous evidence that some *Xylaria* utilize a FA strategy (Thomas & Vandegrift et al. 2016), we hypothesized that this ecological strategy would be common in the Xylariaceae (5) because of PNC.

We do see a taxonomic signal for endophytism (Table 1). Of the stromata collected at Fushan, those that have an endophytic phase that is detectable in our NGS data are more commonly in the subfamily Xylarioideae (70% of taxa detected as endophytes) than the Hypoxyloideae (48% of taxa detected as endophytes). The FA strategy may be a basal trait, utilized by fungi in both major lineages but lost more frequently in the Hypoxyloideae; alternatively endophytism likely is the result of multiple ecological strategies, such that the simple presence of an endophytic phase may not be indicative of FA ecology. If endophytism in the Hypoxyloideae represents other ecological strategies, we might expect to see deviation from the predictions of the FA hypothesis in this lineage relative to the Xylarioideae. And indeed, in the bipartite analysis for host preference within the Xylariaceae, we see the highest *d′* values in species of *Rosellinia, Anthostomella,* and *Annulohypoxylon* (as well as several taxa that could only be identified to family). *Rosellinia* is likely in the Xylarioideae, but is a highly evolved branch of this subfamily, and includes plant pathogenic ecological strategies (Petrini 2013), which are often host-specific (Petrini 1993). *Anthostomella* is likely a heterogeneous genus, but most species assigned to *Anthostomella* seem to be basal to the two major lineages in the Xylariaceae (Daranagama et al. 2015). *Annulohypoxylon* are clear representatives of the Hypoxyloideae.

Conclusions.—We find evidence for diverse spatial ecologies in the Xylariaceae, including evidence in support of a FA strategy in some taxa, particularly in the subfamily Xylarioideae. There is evidence of coupling of life phases in several taxa (*A. atroroseum, K. pavimentosa* var. 2*, N. bipapillata* var. 1*,* and *X.* sp. 1), as well as evidence of

clustering in the endophytic phase in 8 taxa (Table 2: *A. atroroseum, K. pavimentosa* var. 1, *N. bipapillata* var. 2, *N. bipapillata* var. 2, *N. diffusa, W. microplaca, X. flabelliforme* (non-penzigioid), and *X.* sp. nov. 1) that may represent "the ghost of stromata past", a product of temporal uncoupling of life phases in the FA ecology. We find that xylariaceous endophyte communities are less sensitive to distance and environment than the community of stromata, and that xylariaceous endophytes are generally not host-specific, though some influence of host remains. While more work is clearly necessary to understand the spatial ecologies of taxa within this family, and the myriad of reasons why they are such common endophytes, we believe that this study provides support for the Foraging Ascomycete strategy in the Xylariaceae, particularly those taxa in the subfamily Xylarioideae. We also believe that the pairing of traditional mycological collection with NGS metabarcode libraries is a novel approach with much potential for elucidating the spatial patterns of these intriguing organisms. Though they produce macroscopic fruiting structures, for most of their lives they are microorganisms, growing and dispersing hidden beneath the threshold of observable size.

CHAPTER VI

CONCLUSION

The body of work that I have put together—with the aid of many helping hands along the way—for this dissertation covers a wide swath of endophyte research, contributing to multiple arms of this great beast of knowledge. We have used the host-specific relationship between an aggressive invasive grass species and its *Epichloë* endophyte to put theoretical frameworks of invasion to the test. We have expanded a grass/*Epichloë* endophyte system to include two other prominent functional groups (AMF and DSE) of symbionts, and then examined the balance of these symbiont consortia in the context of a manipulative climate change experiment. We have used tropical foliar endophytes in the family Xylariaceae to test a possible ecological explanation for some cases of endophytism in both Ecuador and Taiwan. In this chapter, I will summarize some of the important results, and discuss briefly areas of further interest

SECTION A

In this section, we examine some aspects of endophyte ecology relating to the well-studied grass/*Epichloë* system. We have focused on the intersection of host-symbiont ecology with other ecological theory, particularly invasion theory (Chapter II), and community ecology and climate change (Chapter III).

Chapter II.—In this chapter, our experimental design allowed us to assess evolutionary change in the invaded range, such that we could effectively distinguish between the ERH and the EICA hypothesis. We used plants from both the native and invasive ranges, cleared all seeds of *Epichloë*, and then re-inoculated half of each population; in previous studies, endophyte infection and range of origin were always conflated, due the ubiquity of infection in Europe and the general absence of infection in the USA.

We found significantly increased mortality of inoculated *B. sylvaticum* originating from the invaded range as compared to their native range equivalents (Chapter II, Fig. 6). We also found reduced seedling growth rates in inoculated invasive-range plants (Chapter II, Fig. 5). Both of these results show a loss of tolerance for the host-specific fungal enemy in the American populations tested. The difference in germination rates seems to point to genetic mechanisms for increased germination in the invaded range, as well as loss of other factors controlling germination in the native range, such as seed-damaging pathogens and herbivores. These facts, taken together, are strong support for the Evolution of Increased Competitive Abilities hypothesis.

Research related to this chapter also led to some other surprising findings, not included in Chapter II. Throughout our work with *Brachypodium sylvaticum* and *Epichloë sylvatica*, we noticed a tendency for some bleed-over in infection status into our control plants, despite the "fact" that this endophyte was supposedly transmitted solely vertically, through the seed. An undergraduate in our lab, Matt Davis, undertook to investigate further under my mentorship. We found that, indeed, *Epichloë sylvatica* is able to transmit horizontally via conidial nets on the leaf surfaces, and we observed conidial nets

on the surfaces of grass bladse (Fig. 1). We also found evidence that there is differential resistance to horizontal infection in the populations of *B. sylvaticum* with which we were working, though the work is still incomplete; it seems that most invasive range populations are resistant to horizontal transmission, while native range populations are highly susceptible (Davis et al., in preparation).

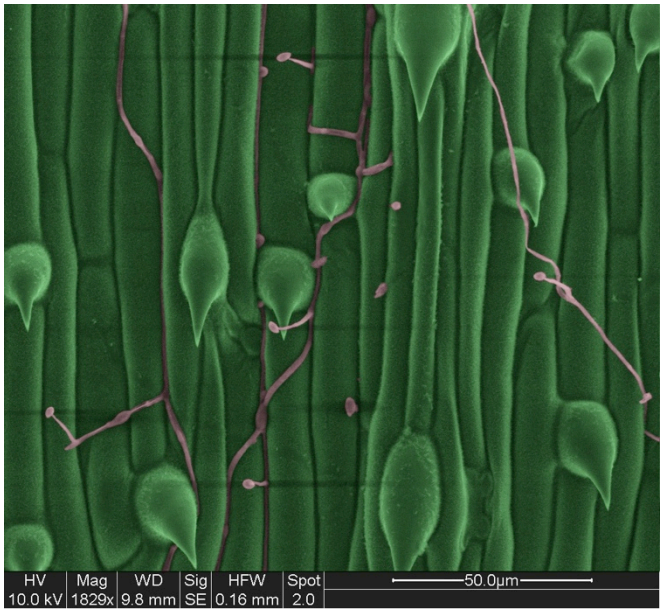


Figure 1: Hand-colored scanning electron micrograph of *Epichloë sylvatica* conidial nets on the surface of *Brachypodium sylvaticum* leaf, the putative causal agents of asexual horizontal infection.

One of the primary reasons that Clavicipitaceous endophytes are generally considered strong mutualists is the nature of the reproduction and co-evolutionary relationship between host and fungus in this group. The discovery of horizontal transmission de-couples the fitness of host and symbiont, allowing more pathogenic life-styles to exist, in theory. This finding bears follow up: how common is asexual horizontal transmission in Clavicipitaceous endophytes in nature? The answer to that question could fundamentally change the way we think about these symbioses.

Additionally, the suggestion of differential resistance to *E. sylvatica* in populations of *B. sylvatica* leads to a novel theory for the loss of the endophyte in the invasive range. Given the means of introduction—planting out of many genotypes from across the invasive range at a single location by the USDA—it is possible that genes for resistance were introduced and quickly spread to near fixation. If the endophyte were detrimental to fitness in the invasive range, endophyte-free plants would have had a selective advantage, spreading rapidly as endophyte-infected plants died out. While we had proposed that the loss of the endophyte was likely a consequence of transport conditions in the early part of the 20th century, it may have been selective, instead. This is intriguing, because it casts the ERH and the EICA hypothesis in new light: both are invasion theories that propose species' responses to loss of a specialist enemy, but what if the enemy is lost due to some selection against it in the new range? The theory is turned on its head, and selective feedback loops between invasive/enemy/environment must be considered. This intriguing system could serve as a model for invasion research for some time to come.

Chapter III.—In this chapter, we examined the relationships between multiple symbionts across a great range of climatic conditions within a large-scale manipulative climate change experiment. Our initial hypotheses centered on competition between symbionts within a shared host: we expected to find evidence that consortia of symbionts changed with environmental conditions in such a way as to minimize changes to host fitness (and maximize fitness in a given environmental context). In other words, we expected there to be interactions among environmental variables (soil temperature, moisture, and N:P

ratios) and the fitness costs/benefits of colonization by different symbionts. In addition, we expected to find evidence of competition between symbionts. Lastly, we expected the outcomes of that competition to be stabilized by the fitness benefits to the host.

What we found instead was no evidence of competition between symbionts: neither root symbiont seems to be affected by presence of *Epichloë* endophytes in the aboveground tissues of the host (Chapter III, Fig. 3). If anything, AMF and DSE appeared to have a facilitative rather than a competitive interaction (Chapter III, Fig. 4 & Fig. S1). We also did not find any effect of AMF colonization or *Epichloë* presence on plant fitness as measured by AGB (Chapter III, Fig. 4 & Figs. S10–S11). Aboveground biomass is known to be highly correlated with reproduction in *A. capillaris* (Goklany 2012), and is often used as a surrogate for overall fitness (Shipley and Dion 1992). We did find a negative effect of DSE colonization on AGB, but only in the absence of *Epichloë* endophytes (Chapter III, Fig. 4 & Fig. S2), suggesting that the presence of *Epichloë* counteracts the otherwise negative effects of DSE.

Facilitation between fungal species within a host may play a role in symbiont community determination: it has been demonstrated that both DSE and *Epichloë* derived exudates can affect the growth of AMF (Scervino et al. 2009, Novas et al. 2011), and our study supports facilitation between AMF and DSE, as well as synergistic effect of DSE colonization and *Epichloë* infection on host fitness. Indeed, facilitatory interactions need not be restricted to within single hosts: given the demonstrated movement of photosynthate between host species through common mycorrhizal networks (Martins and Read 1996, Martins and Cruz 1998, Pringle 2009), connectivity between hosts by

different species of fungi may be just as important to supporting struggling populations of fungi as it is to struggling plants.

Given this community framework, it is reasonable to expect that selective pressure on the host will favor host/symbiont relationships that structure the community of symbionts in the most beneficial way possible for the plant, not necessarily the individual symbiont that is most beneficial to plant fitness in isolation. The fitness effect of the consortium of symbionts is the integration of all fitness costs and benefits of all partners. The particular community assemblage of symbiotic fungi associated with a particular host will then be predicated upon the physiology of the host, the available inoculum, the interactions of the symbionts, and the abiotic environment's effects on both the host and the fungal partners (Schlaeppi and Bulgarelli 2014). We hope that this study will inspire others to begin to examining host/symbiont ecology from in an integrative, systems-biology perspective, thinking actively about the community of symbionts within a host, rather than just a single host/symbiont pairing.

SECTION B

In this section, we set out to test a hypothesis about a particular ecological strategy leading to endophytism: the Foraging Ascomycete (FA) hypothesis. This is the idea that endophytism may be a secondary life history strategy for some decomposer fungi to span scarcity of primary substrates and challenging environmental conditions in both time and space (Carroll 1999).

Chapter IV.—In this chapter, we utilized a spatially explicit grid-based sampling scheme over half a hectare of primary tropical cloud forest to examine spatial linkage in the distributions of endophyte and decomposer stages of *Xylaria* species. The FA hypothesis postulates dispersal linkage between life phases, which should lead to spatial linkage of distributions.

In the process, we collected 36 distinct taxa of the genus *Xylaria*, 5 of which also occurred as endophytes. Two of these five species demonstrated non-random clumping of differing life stages (Chapter IV, Table 2; Fig. 3; Figs. S2-S3). We also found that stromata of some species were restricted by distance to the stream that ran through our plot, while their endophyte-phase counterparts were not (Chapter IV, Table S5). Additionally, we found no evidence of host preference in the 5 *Xylaria* endophytes. These results are consistent with the use of a FA strategy by these five fungi.

To demonstrate the possibility of alternation of endophytic and decomposer life styles, we grew out endophytes from leaves onto sterile wood baits. Over time, a species of *Xylaria* dominated some sections of these baits and was able to utilize the wood as a carbon source and initiate fruiting (Fig. S8). This is the first conclusive demonstration that endophytic *Xylaria* and decomposer *Xylaria* can be, in fact, the same organism.

In addition to the ecologically interesting implications of this work, there are fascinating taxonomic results. Of the 36 species recovered, at least two likely represents an undescribed species, and another is likely new, as well. The data obtained will also likely help to define the boundaries of *Xylaria telfairii* and *Xylaria enterogena*, two taxa that have been synonymized and split any number of times, and around which much

confusion remains. My next major undertaking will be to assemble this taxonomic data into a useful volume, to include dichotomous keys, species descriptions, and full illustrations (e.g., Fig. 2) of all known cloud forest *Xylaria* from Ecuador.

Chapter V.—Expanding upon the work in Chapter IV—and my new interest in the ecology, taxonomy, and systematics of Xylariaceous fungi—we teamed up with *Xylaria* expert Dr. Yu-Ming Ju and went to Taiwan on an NSF East Asia and Pacific Summer Institute (EAPSI) fellowship to conduct a larger study putting the FA hypothesis to the test. After analysis of the Ecuador data in Chapter IV, we speculated that there were differential beta-diversity turnover trends expected for FA endophytes versus other ecological strategies. Given that FA endophytes use endophytism specifically as a means

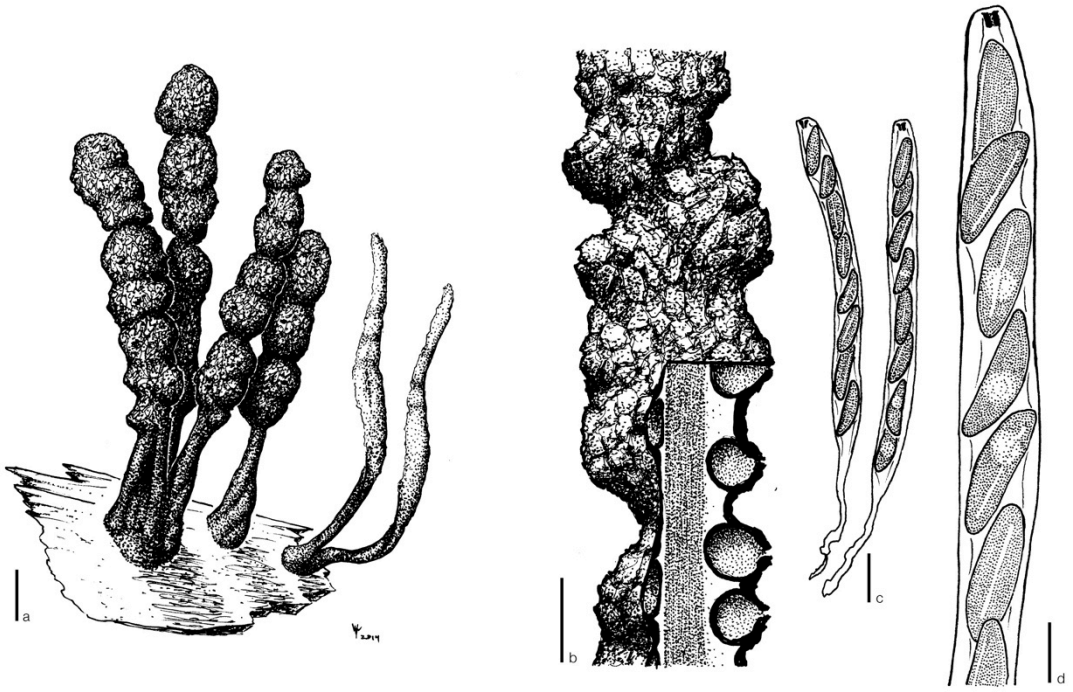


Figure 2: *Xylaria fisillis* Ces, the most abundant *Xylaria* at Reserva Los Cedros, our field site in Ecuador. This pair of illustrations is representative of the illustrations I have in progress or planned for all taxa of *Xylaria* known from cloud forests in Ecuador (~50 spp.), as part of a large taxonomic work. Scale bars: a = 2 mm, b = 1 mm, c = 10 µm, and d = 5 µm.

of dispersal, we expected these endophytes to be, in general, less dispersal limited than most endophytes, which include a diversity of ecological strategies leading to endophytism. We expanded our taxonomic focus to the entire family of Xylariaceae, based on assumptions of phylogenetic niche conservatism (PNC)—the idea that closely related taxa will share ecological traits (Harvey and Pagel 1991). We hoped that this expanded taxonomic focus, combined with more sophisticated endophyte detection methods and nested logarithmic sampling scheme across a much larger plot, would yield enough data for accurate estimates of turnover across the plot.

We collected a total of 44 distinct xylariaceous taxa at the site, 32 within the plots and an additional 12 taxa collected by stochastic sampling elsewhere within the Fushan FDP; these collections represented 7 genera within the Xylariaceae (Chapter V, Table 1). Of the 44 taxa collected as stromata, we were able to detect 26 as endophytes in the metabarcode library (Chapter V, Table 1). Additionally, de novo OTU clustering and taxonomic assignment via the UNITE database indicates 2431 unique endophytic taxa (OTUs) in the Xylariaceae. We found evidence supporting host generalism within most of the Xylariaceae (Chapter V, Fig. 2 & 3). We also found mixed evidence of spatial linkage, indicating different spatial ecologies on a taxon-by-taxon basis (Chapter V, Table 2). We found a significant effect of the local canopy vegetative community (Su et al. 2007) on both the total endophyte and Xylariaceous endophyte communities, though the effect explained much less of the variance for the Xylariaceae (Chapter V, Fig. 9). Micro-topological habitat class (Su et al. 2010) was not explanatory for endophytes, but was for Xylariaceae stromatal communities; additionally, the stromatal communities

showed significant beta-diversity decay with distance (Chapter V, Fig. 10), but the endophytic community did not. We also saw some evidence of taxonomic signal, with endophytism being more common in the Xylarioideae than the Hypoxyloideae.

While more work is clearly necessary to understand the spatial ecologies of taxa within this family, and the myriad of reasons why they are such common endophytes, we believe that this study provides support for the Foraging Ascomycete strategy in the Xylariaceae, particularly those taxa in the subfamily Xylarioideae. We believe that the pairing of traditional mycological collection with NGS metabarcode libraries is a novel approach with much potential for elucidating the spatial patterns of these organisms.

There are also taxonomic implications of this work, which I will continue working on with Dr. Ju. Most notably, the three varieties of *Nemania bipapillata* are distinguished not only via ITS sequence, but morphologically as well (Fig. 3). We believe that this taxon will need to be carefully re-examined, and likely new varieties erected, or perhaps new species, to accommodate these observations.

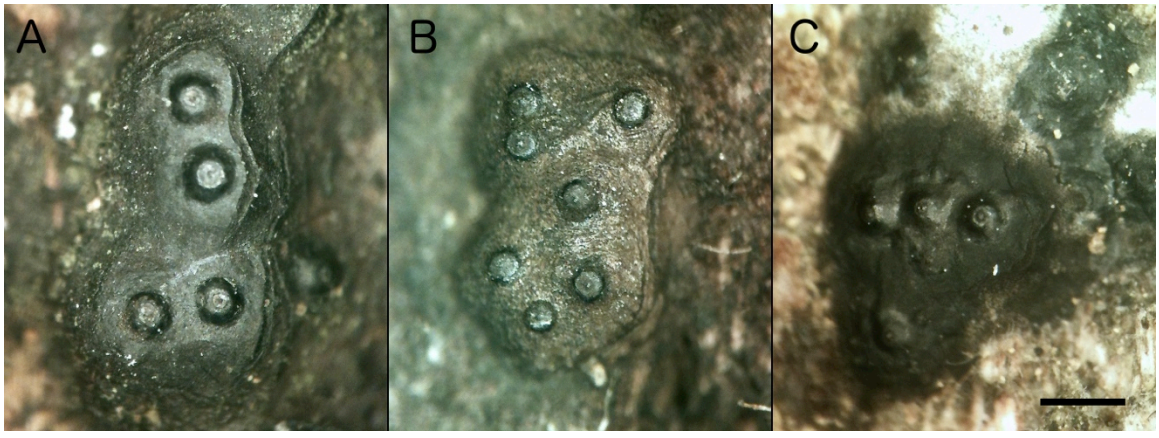


Figure 3: Examples specimens for each of the three varieties of *Nemania bipapillata* collected at Fushan. **A**, FS123 (plus one other collection) rerpresents a distinct ITS cluster from the other collections of *N. bipapillata*, and has intermediate ostiolar disk diameter; **B,** FS9 (plus one other collection) represents a distinct ITS cluster as well, and has the largest ostiolar disk diameter; and finally **C,** FS47 (plus three other collections) represents the typical variety, with the smallest ostiolar disk diameter. Scale bar = 1 mm.

APPENDIX A

SUPPLEMENTAL MATERIALS FOR CHAPTER II

Supplementary Methods:

Infection Rates

To determine whether the fungus was present in the invaded range we first used the standard morphological screen in which leaf sheaths are stained to make the fungal hyphae more evident (Latch and Christensen 1985). There were no differences among US plants, which we initially interpreted as indicative of 100% infected, like the European populations. To confirm, we screened the same populations with the Agrinostics Field Tiller immunoblot kit (Agrinostics Ltd. Co., Watkinsville, GA, USA).

We isolated *E. sylvatica* from seed with slightly modified version of the fungal culture method outlined by Mirlohi et al. (2006). Briefly, the seeds were deglumed by hand, rinsed in 100% ethanol for 30 seconds, rinsed in sterile water then sterilized by soaking in 30% hydrogen peroxide for 20 minutes, rinsed in sterile water and plated on PDA. Due to contamination, only one culture yielded *Epichloë* (from Flaesch, Switzerland 47°01'21.1" N, 009°30'03.6" E).

To verify the identity of the isolated endophyte, we sequenced the internal transcribed spacer (ITS) II region of the nuclear ribosomal DNA operon. DNA extraction was according to Winton et al. (2002), using 0.1% DDT in the buffer solution instead of 0.1% 2-mercaptoethanol. We used the standard ITS3 and ITS4 primers (White et al. 1990), with Sanger sequencing carried out by the University of Oregon Sequencing

Facility. The resultant sequence (KF225579) has 99.7% sequence similarity with the published *Epichloë sylvatica* sequence (L78304).

For the PCR screen, DNA was extracted from tillers using two different methods. We initially followed Sullivan and Faeth (2004), but shifted to a more efficient method developed for mushrooms (Dentinger et al. 2010), which yielded better quality fungal DNA. Briefly, a tiller base including a leaf was smashed into a DNA absorbing Whatman FTA card and extracted with the Sigma Extract-N-Amp Plant PCR Kit.

We used *Neotyphodium*-specific primers (IS-RS-5' and IS-NS-3') to amplify the intron region of the ß-tubulin (*tub2*) gene (Dombrowski et al. 2006). The protocol of Dombrowski et al. (2006) was developed for a different set of *Epichloë*/host species combinations, and required minor optimization to work well with *E. sylvatica* in *B. sylvaticum*. We utilized Sigma JumpStart Taq ReadyMix for our PCR reactions according to the manufacturer's instructions, with a slightly modified PCR mixture: 5µL JumpStart Taq ReadyMix, 0.4µL $MgCl_2$ (25mM), 0.2µL forward primer, 0.2µL reverse primer, 2.2µL water and 2µL DNA template in a 1:1 dilution. The positive control for these reactions was the *E. sylvatica* endophyte isolated from Swiss seed, described above. PCR conditions were as follows: Initial denaturation at 94˚C for one minute, then 18 cycles at 94˚C for 25 s, a touchdown annealing temperature starting at 73˚C and decreasing by 0.8˚C per cycle, followed by 72˚C for 3 min. The remainder of the PCR reaction was 32 cycles of 94˚C for 25 s, 58˚C for 1 min, and 72˚C for 2 min, followed by a final extension at 73˚C for 15 min.

Germination rates

It should be noted that this experiment only tests range origin of the *maternal* genotype. We did not control pollination in the greenhouse, so it is possible that up to fifty percent of the genetic compliment of the seeds may be from the other range, and that there may be effects of hybrid vigor acting to increase observed germination rates. About half of field collected *B. sylvaticum* appear to be selfed (Rosenthal et al. 2008), but selfing is more common under greenhouse conditions unless pollen is deliberately circulated with fans (M. Cruzan, pers. comm.). If there had been no admixture, we would expect steeper slopes and greater differences, increasing the significance of our results.

Table S1. *Epichloë sylvatica* immunoblot assays.

Source	Host Grass	Range	Population	Location	N	% Pos	Date
wild collected	*B. sylvaticum*	Invaded-USA	Bald Hill	44°34'23.0"N, 123°19'48.5"W	30	0	20-Jun-10
wild collected	*B. sylvaticum*	Invaded-USA	Bald Hill	44°34'23.0"N, 123°19'48.5"W	30	0	26-Aug-10
wild collected	*B. sylvaticum*	Invaded-USA	Jasper	44° 0'43.6"N, 122°53'34.1"W	30	0	20-Jun-10
wild collected	*B. sylvaticum*	Invaded-USA	Jasper	44° 0'43.6"N, 122°53'34.1"W	30	0	4-Aug-10
wild collected	*B. sylvaticum*	Invaded-USA	Jasper Park	43° 99'31.4"N, 122°89'66"W	30	0	4-Aug-10
wild collected	*B. sylvaticum*	Invaded-USA	Sweet Home	44°25'18.7"N, 122°40'32.1"W	30	0	20-Jun-10
wild collected	*B. sylvaticum*	Invaded-USA	Sweet Home	44°25'18.7"N, 122°40'32.1"W	30	0	26-Aug-10
wild collected	*B. sylvaticum*	Invaded-USA	Pisgah	44° 0'9.80"N, 122°56'59.2"W	30	0	20-Jun-10
wild collected	*B. sylvaticum*	Invaded-USA	Pisgah	44° 0'9.80"N, 122°56'59.2"W	30	0	26-Aug-10
wild collected	*B. sylvaticum*	Invaded-USA	Pisgah-BPA*	44° 0'9.80"N, 122°56'59.2"W	10	0	26-Aug-10
planted 2008	*B. sylvaticum*	Invaded-USA	MacForest	44°38'42.7"N, 123°18'37.9"W	1	0	4-Aug-10
planted 2008	*B. sylvaticum*	Invaded-USA	Sweet Home	44°25'18.7"N, 122°40'32.1"W	1	0	4-Aug-10
planted 2008	*B. sylvaticum*	Invaded-USA	Fisherman	43°58'19.5"N, 122°39'37.7"W	1	100	4-Aug-10

wild collected	*B. sylvaticum*	Invaded-USA	Fisherman	43°58'19.5"N, 122°39'37.7"W	41	100	29-Aug-11
wild collected	*B. sylvaticum*	Invaded-USA	Mill City	44°45'6.4"N, 122°29'56.7"W	30	0	29-Aug-11
planted 2008	*B. sylvaticum*	Invaded-USA	Owl	43° 59'51.6"N, 123°05'72.3"W	1	0	4-Aug-10
planted 2008	*B. sylvaticum*	Invaded-USA	Jasper	44° 0'43.6"N, 122°53'34.1"W	2	0	4-Aug-10
planted 2008	*B. sylvaticum*	Invaded-USA	Pisgah	44° 0'9.80"N, 122°56'59.2"W	1	0	4-Aug-10
planted 2008	*B. sylvaticum*	Native-CH	Albisguetli	47°21'47.4"N, 008°29'98.0"E	2	100	4-Aug-10
planted 2008	*B. sylvaticum*	Native-CH	Albisguetli	47°21'47.4"N, 008°29'98.0"E	2	100	26-Aug-10
planted 2008	*B. sylvaticum*	Native-CH	Flaach I	47°35'36.4"N, 008°36'27.4"E	2	100	4-Aug-10
planted 2008	*B. sylvaticum*	Native-CH	Flaach II	47°59'04.1"N, 008°60'74.0"E	2	100	4-Aug-10
planted 2008	*B. sylvaticum*	Native-CH	Truebbach I	47°04'04.1"N, 009°28'48.0"E	1	100	26-Aug-10
planted 2008	*B. sylvaticum*	Native-CH	Truebbach I	47°04'04.1"N, 009°28'48.0"E	1	100	4-Aug-10
planted 2008	*B. sylvaticum*	Native-CH	Truebbach II	47°04'12.2"N, 009°29'04.8"E	1	100	4-Aug-10
wild collected	*D. glomerata*†	Invaded-USA	UO campus	44°3'3.21"N, 123°4'21.8"W	1	100	20-Jun-10
wild collected	*D. glomerata*	Invaded-USA	UO campus	44°3'3.21"N, 123°4'21.8"W	3	100	4-Aug-10
wild collected	*D. glomerata*	Invaded-USA	Bald Hill	44°34'23.0"N, 123°19'48.5"W	3	100	26-Aug-10

*These ten plants were collected about 50 m away from the original population because the original had been sprayed by an herbicide, glyphosate, since we last collected in June. We were still able to collect live material at the original site, however, to be sure the herbicide had not altered infection rates, we also collected unsprayed plants from nearby.

†The *Dactylis glomerata* were not randomly sampled. They were obviously infected (showing symptoms of choke disease), and were used as positive controls.

APPENDIX B

SUPPLEMENTAL MATERIALS FOR CHAPTER III

Supplemental figures:

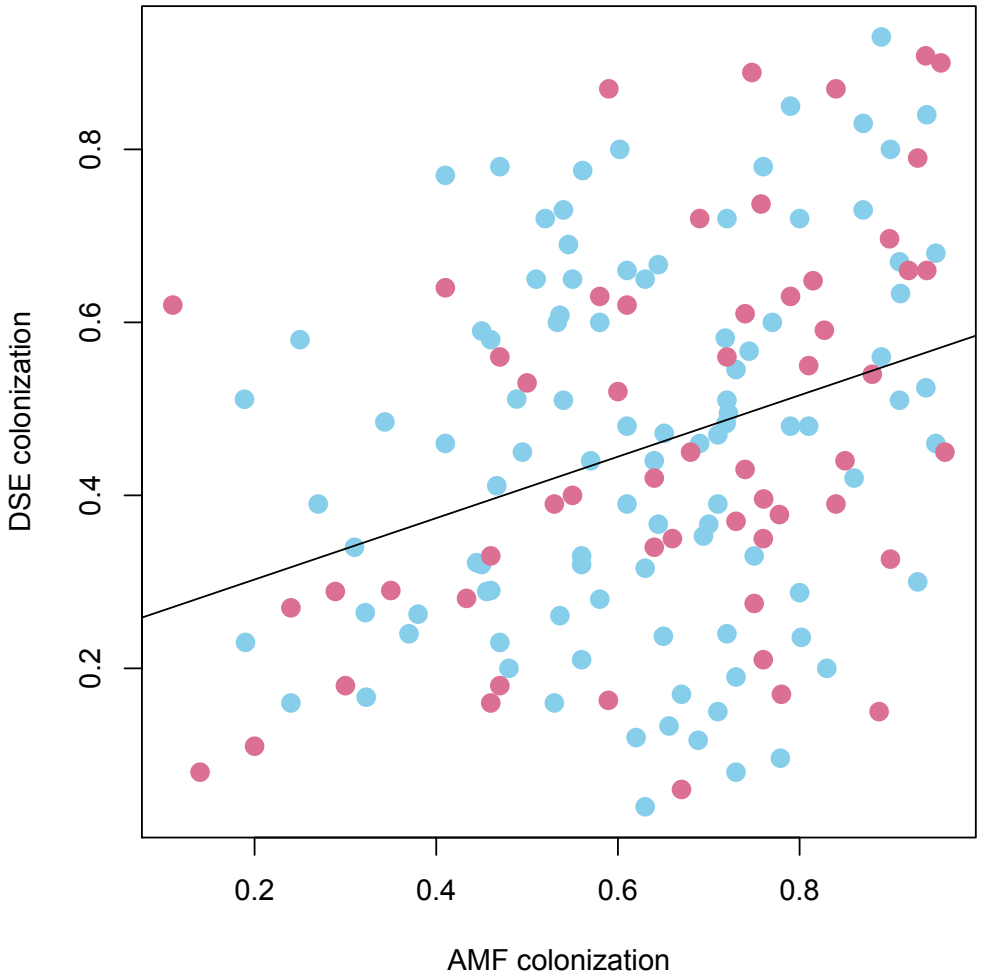


Figure S1: Percent root length colonized by AMF and DSEs were correlated positively (Adjusted $R^2 = 0.107$, $F_{1, 153} = 19.51$, $P < 0.001$), indicating facilitation rather than competition. See also Fig. 4 for SEM results.

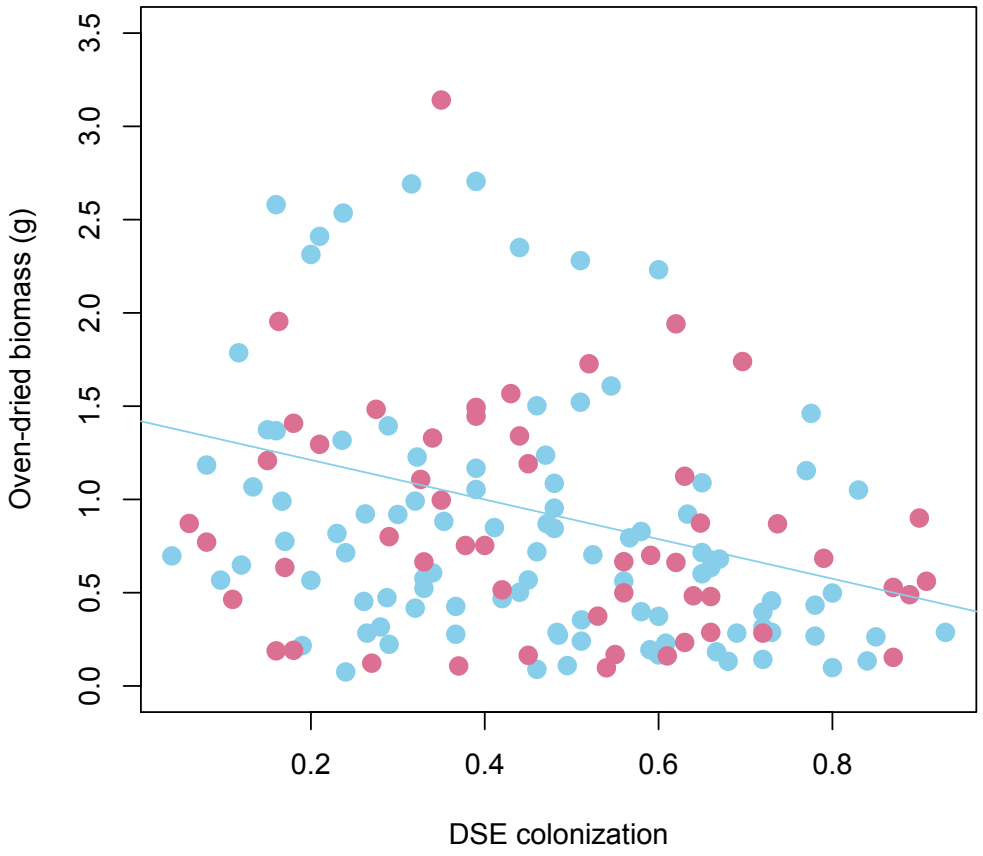


Figure S2: There was a negative correlation between DSE root length colonized and plant biomass, but only in the absence of *Epichloë* infection (E+ Adjusted $R^2 = 0.029$, $F_{1, 54} = 2.644$, $P = 0.110$; E− $R^2 = 0.053$, $F_{1, 97} = 6.437$, $P = 0.013$). See also Fig. 4 for SEM results.

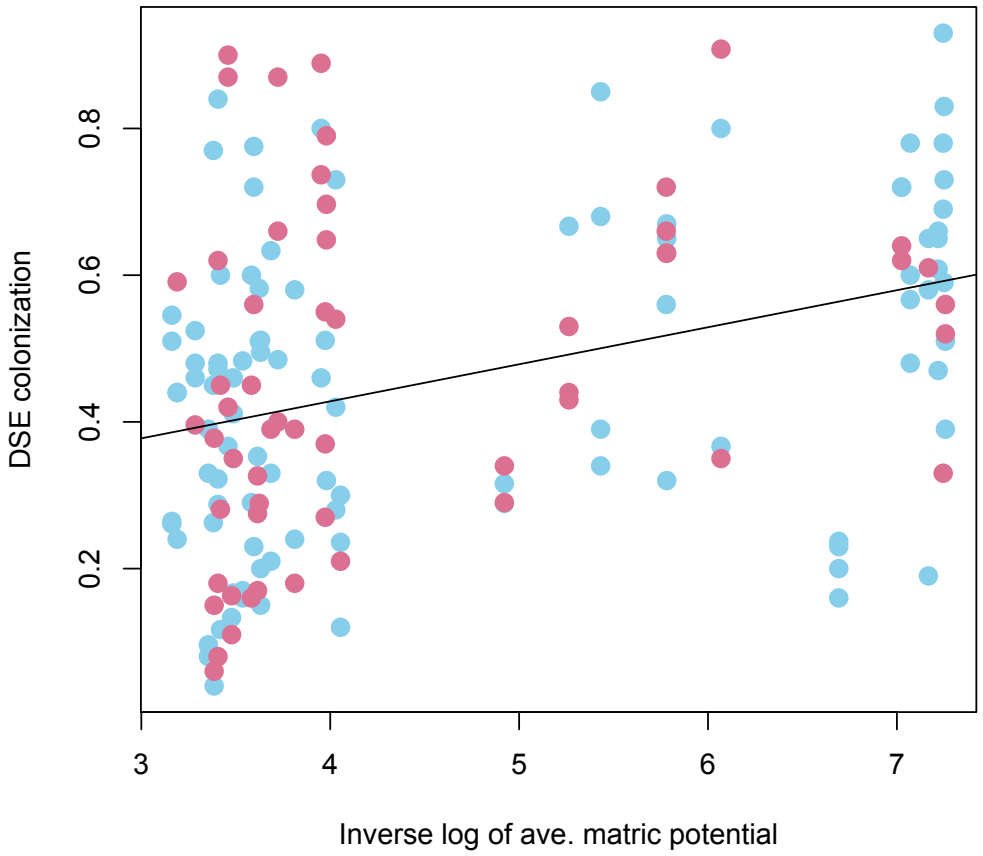


Figure S3: DSE colonization decreased as more water was available to plants (Adjusted $R^2 = 0.107$, $F_{1, 153} = 19.5$, $P < 0.001$). See also Fig. 4 for SEM results.

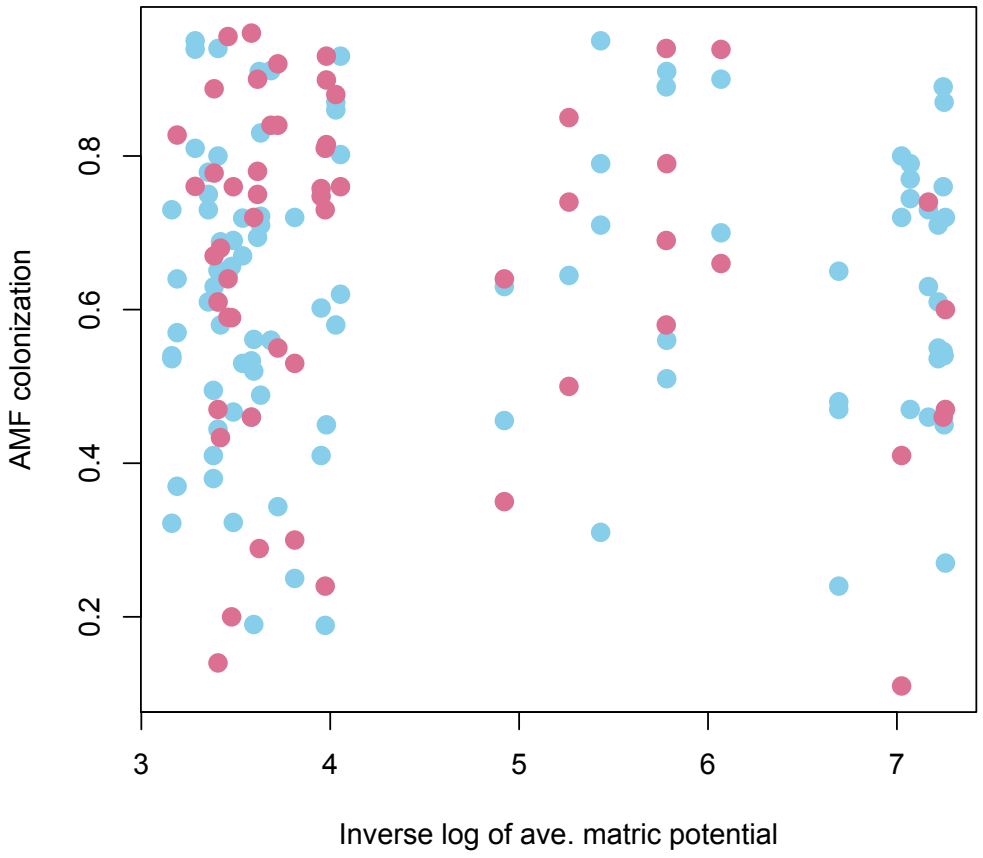


Figure S4: Neither AMF colonization nor proportion of plants hosting *Epichloë* varied significantly with measured edaphic conditions (soil moisture, soil temperature, soil N:P ratios). See also Fig. 4 for SEM results.

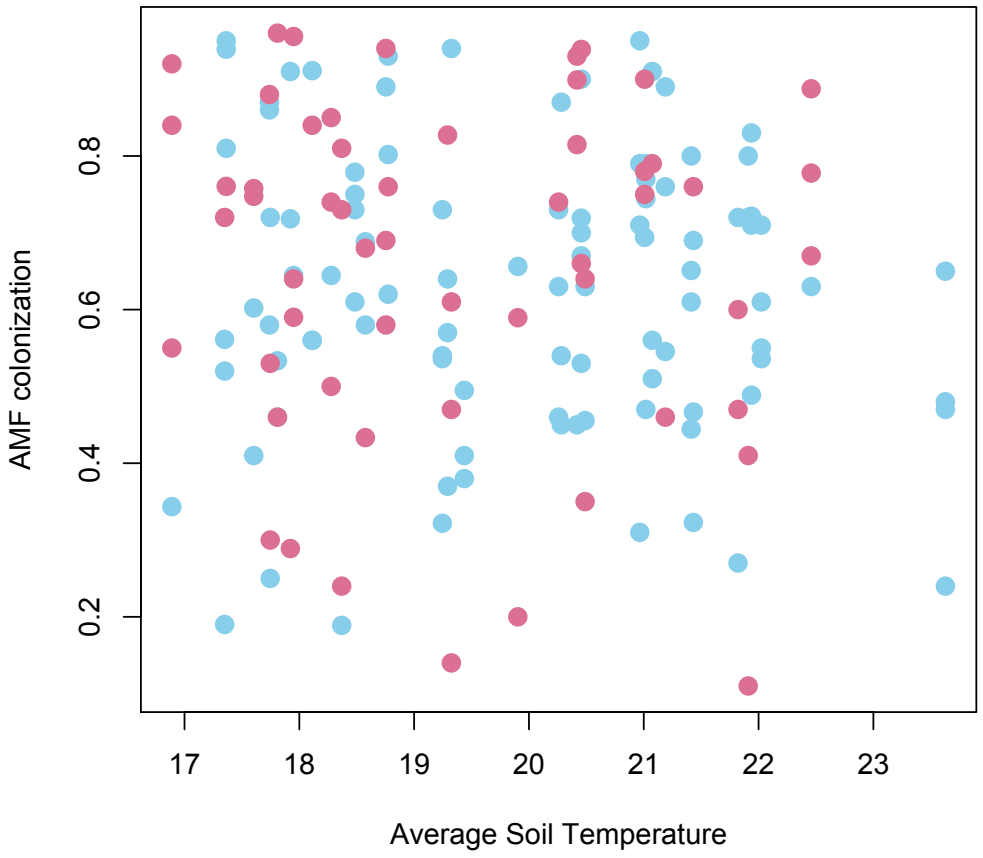


Figure S5: Neither AMF colonization nor proportion of plants hosting *Epichloë* varied significantly with measured edaphic conditions (soil moisture, soil temperature, soil N:P ratios). See also Fig. 4 for SEM results.

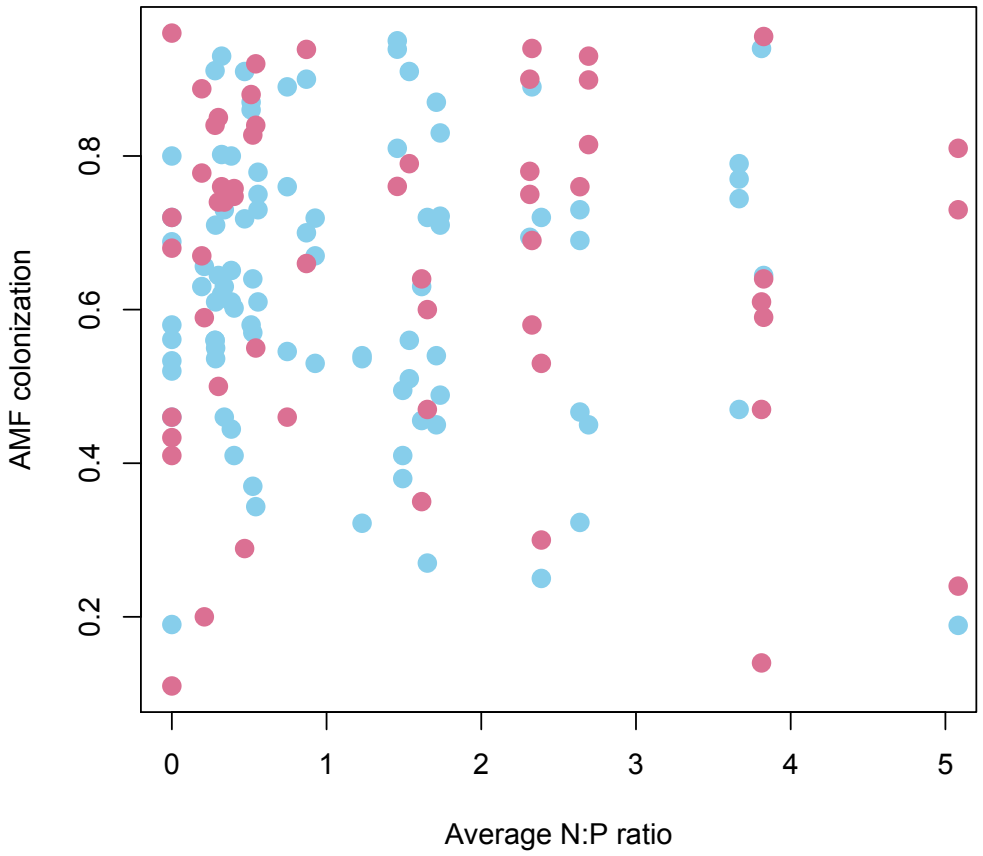


Figure S6: Neither AMF colonization nor proportion of plants hosting *Epichloë* varied significantly with measured edaphic conditions (soil moisture, soil temperature, soil N:P ratios). See also Fig. 4 for SEM results.

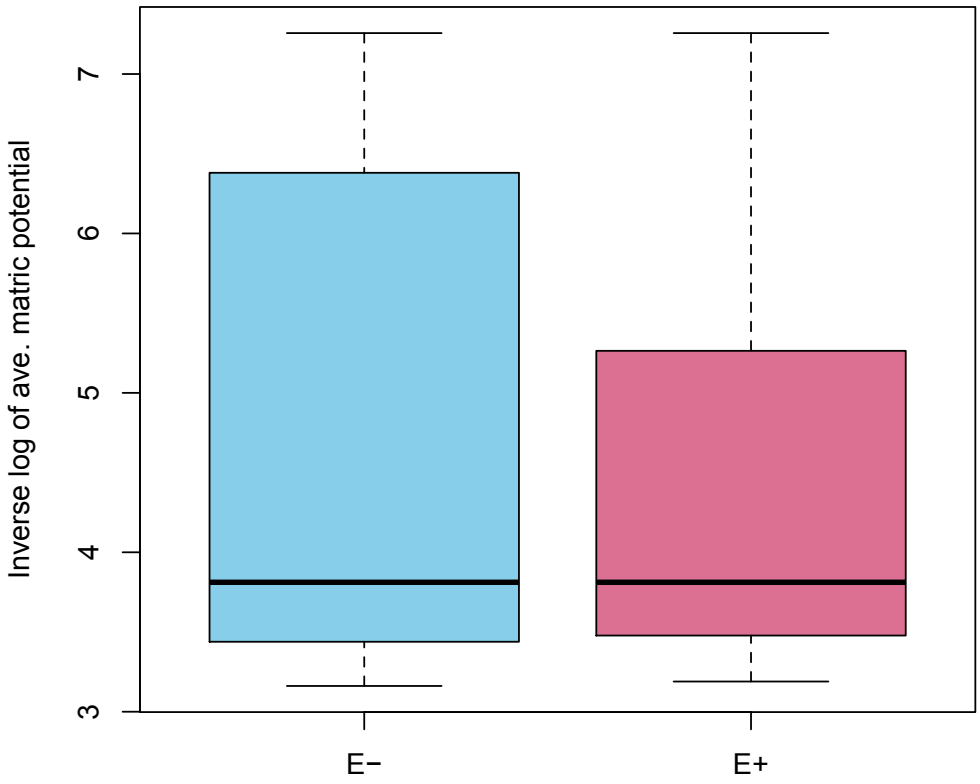


Figure S7: Neither AMF colonization nor proportion of plants hosting *Epichloë* varied significantly with measured edaphic conditions (soil moisture, soil temperature, soil N:P ratios). See also Fig. 4 for SEM results.

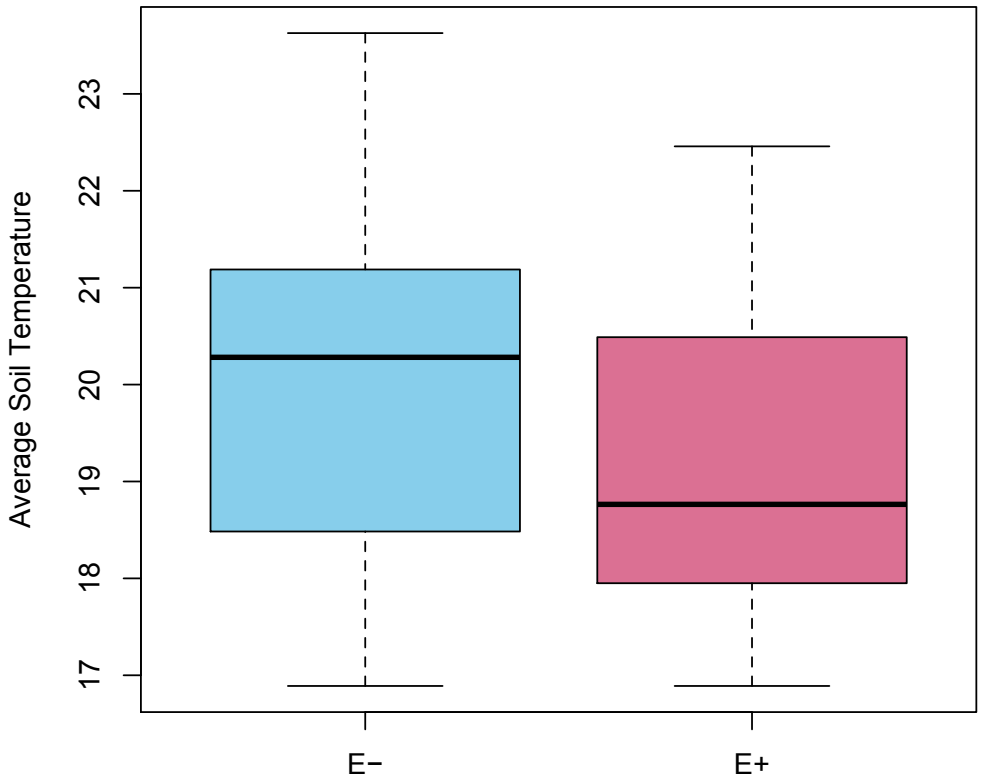


Figure S8: Neither AMF colonization nor proportion of plants hosting *Epichloë* varied significantly with measured edaphic conditions (soil moisture, soil temperature, soil N:P ratios). See also Fig. 4 for SEM results.

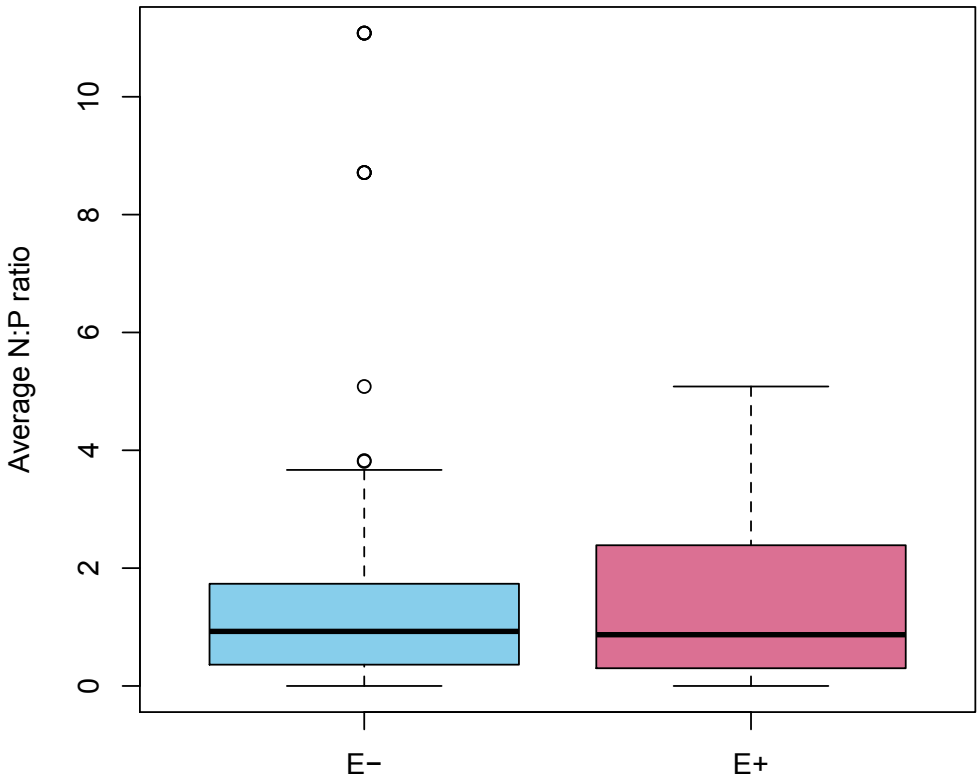


Figure S9: Neither AMF colonization nor proportion of plants hosting *Epichloë* varied significantly with measured edaphic conditions (soil moisture, soil temperature, soil N:P ratios). See also Fig. 4 for SEM results.

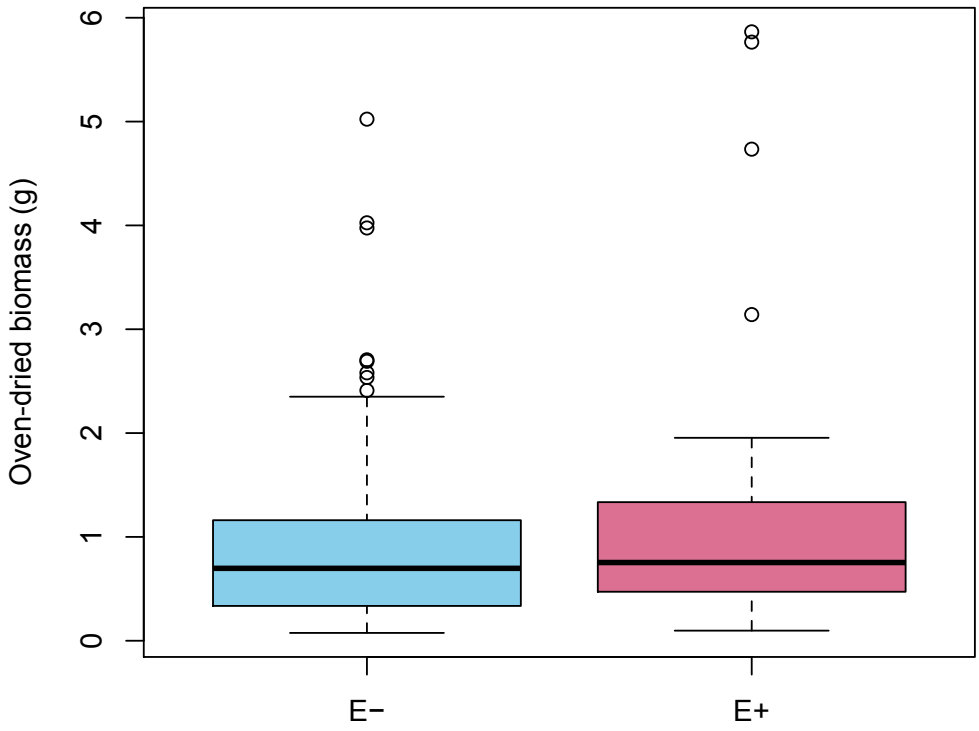


Figure S10: There was no effect of *Epichloë* presence on plant fitness as measured by aboveground biomass.

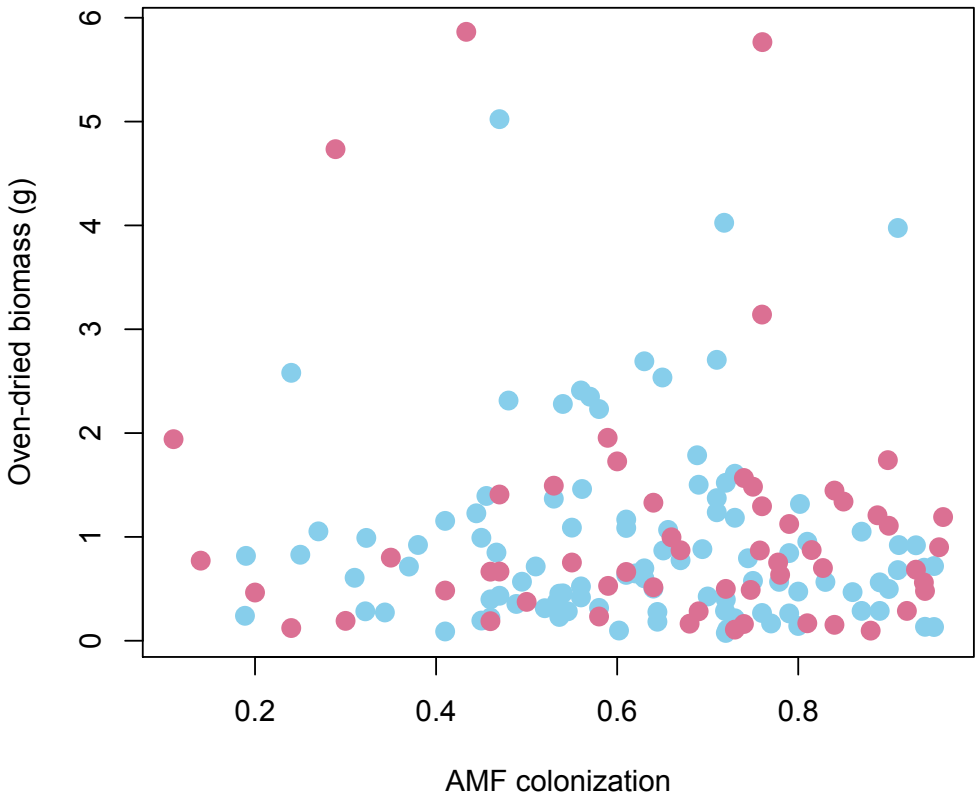


Figure S11: There was effect of AMF colonization on plant fitness as measured by aboveground biomass.

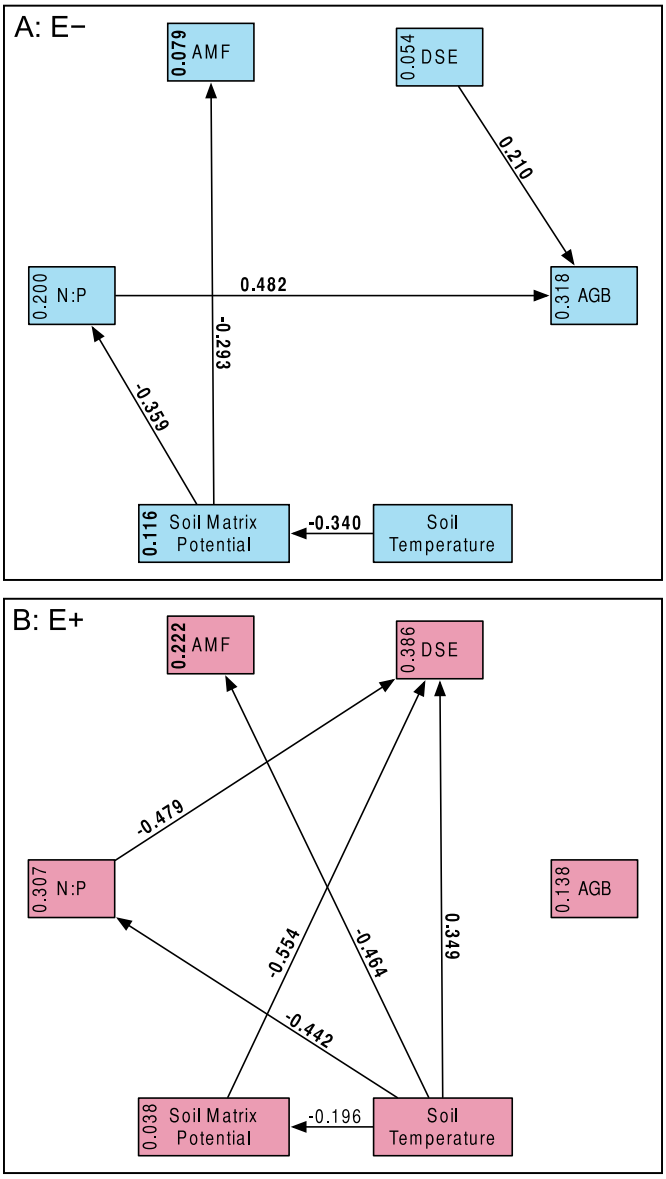


Figure S12: Overall SEMs for *Bromus hordeaceus*, with different models for those plants without *Epichloë* endophytes (A: E−, blue), and those with *Epichloë* endophytes (B: E+, red). Model fit was good for both models, though the low sample size for the E+ may potentially pose issues with interpretation (A: $\chi^2 = 2.588$, $P = 0.274$; CFI = 1.000; RMSEA = 0.000; n= 83 | B: $\chi^2 = 4.601$, $P = 0.100$; CFI = 1.000; RMSEA = 0.000; n = 19). The numbers above the arrows are the standardized path coefficients. Non-significant ($P > 0.05$) path coefficients are not shown. Numbers in the boxes are total explained variance (R^2) of each variable.

APPENDIX C

SUPPLEMENTAL MATERIALS FOR CHAPTER IV

TABLE S1. *Plant host species from which endophytes were isolated; listed by plot number. Of the 120 plots, 12 had no woody vegetation; of these, five had large ferns instead, and seven had only herbaceous plants. There were 38 tree species in 19 different families. Nomenclature follows the online version of the* Catalogue of the Vascular Plants of Ecuador*: http://www.tropicos.org/projectwebportal.aspx?pagename=Home&projectid=2, except that family names have been updated to match the* APGIII *system: http://www.mobot.org/MOBOT/research/APweb/.*

Plot	Family	Scientific Name	Guild	DBH (cm)	Notes
1	Lauraceae	*Rhodostemonodaphne* cf. *cyclops* Madriñán	Tree	8	
2	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	4	
3	Fabaceae	*Inga* sp. 1	Tree	2.5	
4	Thymelaeaceae	*Daphnopsis grandis* Nevling & Barringer	Tree, understory	4	
5	Primulaceae	*Geissanthus* cf. *longistamineus* (A.C. Sm.) Pipoly	Tree	20	sometimes placed in Myrsinaceae
6	Melastomataceae	*Miconia floribunda* (Bonpl.) DC.	Shrub	8	
7	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1	
8	Fabaceae	*Inga* aff. *sapindoides* Willd.	Tree	5	
9	Myrtaceae	*Myrcia fallax* (Rich.) DC.	Tree, understory	3	
10	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1	
11	Sabiaceae	*Meliosma* aff. *occidentalis* Cuatrec.	Tree	0.5	
12	Begoniaceae	*Begonia parviflora* Poepp. & Endl.	Herb	3	
12	Fabaceae	*Inga* aff. *sapindoides* Willd.	Tree	2	
13	Sapindaceae	*Allophylus punctatus* (Poepp.) Radlk.	Tree, mid-story	4.5	
14	Rubiaceae	*Palicourea demissa* Standl.	Shrub	3.5	
15	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	4	
16	Solanaceae	*Solanum leptopodum* Van Heurck & Müll. Arg.	Shrub	1	
17	Sapindaceae	*Allophylus punctatus* (Poepp.) Radlk.	Tree, mid-story	2.5	
18	Euphorbiaceae	*Acalypha diversifolia* Jacq.	Shrub	3	
19	Piperaceae	*Piper obliquum* Ruiz & Pav.	Shrub	4	Endangered--on an IUCN red list
20	Rubiaceae	*Pentagonia* sp.	Tree,	1	

			understory		
21	Burseraceae	*Dacryodes peruviana* (Loes.) H.J. Lam	Tree	20	
22	Burseraceae	*Dacryodes peruviana* (Loes.) H.J. Lam	Tree	7	
23	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	8	
24	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	2	
25	Rubiaceae	*Condaminea corymbosa* (Ruiz & Pav.) DC.	Shrub	1.5	
26	Fabaceae	*Inga* sp.2	Tree	2	
27	Myrtaceae	*Myrcia fallax* (Rich.) DC.	Tree, understory	3.5	
28	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1	
29	Melastomataceae	*Ossaea micrantha* (Sw.) Macfad. ex Cogn.	Shrub	1	
30	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	5	
31	Tapisciaceae	*Huertea glandulosa* Ruiz & Pav.	Tree	17	sometimes placed in Staphyleaceae
32	Melastomataceae	*Miconia* cf. *gracilis* Triana	Shrub	2	
33	Tapisciaceae	*Huertea glandulosa* Ruiz & Pav.	Tree	1	sometimes placed in Staphyleaceae
34	Olacaceae	*Heisteria concinna* Standl.	Tree, mid-story	5	sometimes placed in Erythropalaceae
35	Melastomataceae	*Meriania tomentosa* (Cogn.) Wurdack	Tree	14	IUCN vulnerable
36	Olacaceae	*Heisteria concinna* Standl.	Tree, mid-story	1	sometimes placed in Erythropalaceae
37	Lauraceae	*Persea pseudofasciculata* L.E. Kopp	Tree	17	
38	Myrtaceae	*Myrcia fallax* (Rich.) DC.	Tree, understory	9	
39	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1	
40	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	2	
41	Melastomataceae	*Miconia floribunda* (Bonpl.) DC.	Shrub	3	
42	Melastomataceae	*Ossaea micrantha* (Sw.) Macfad. ex Cogn.	Shrub	1	
43	Rubiaceae	*Pentagonia* sp.	Tree, understory	1.5	
44	Euphorbiaceae	*Acalypha diversifolia* Jacq.	Shrub	1	
45	Cardiopteridaceae	*Citronella* cf. *ilicifolia* (Sleumer) R.A. Howard	Tree	16	
46	Tapisciaceae	*Huertea glandulosa* Ruiz & Pav.	Tree	2	sometimes placed in Staphyleaceae
47	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1	
48	Lauraceae	*Rhodostemonodaphne* cf. *cyclops* Madriñán	Tree	1	

49	Melastomataceae	*Ossaea micrantha* (Sw.) Macfad. ex Cogn.	Shrub	0.8	
50	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	0.8	
51	Melastomataceae	*Miconia brevitheca* Gleason	Tree	6.5	
52	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1	
53	Olacaceae	*Heisteria concinna* Standl.	Tree, mid-story	3.5	sometimes placed in Erythropalaceae
54	Lauraceae	*Nectandra lineatifolia* (Ruiz & Pav.) Mez	Tree	3	
55	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	2	
56	Myrtaceae	*Myrcia fallax* (Rich.) DC.	Tree, understory	1.5	
57	Lauraceae	*Persea pseudofasciculata* L.E. Kopp	Tree	4	
58	Euphorbiaceae	*Acalypha diversifolia* Jacq.	Shrub	2.5	
59	Moraceae	*Clarisia biflora* Ruiz & Pav.	Tree	10	
60	Cyatheaceae	*Alsophila cuspidata* (Kunze) D.S. Conant	Fern	10	
61	Tapisciaceae	*Huertea glandulosa* Ruiz & Pav.	Tree	6	sometimes placed in Staphyleaceae
62	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	7	
63	Clusiaceae	*Garcinia madruno* (Kunth) Hammel	Tree, mid-story	7	
64	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1.5	
65	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	0.8	
66	Sapindaceae	*Allophylus punctatus* (Poepp.) Radlk.	Tree, mid-story	7	
67	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	2	
68	Sapindaceae	*Allophylus punctatus* (Poepp.) Radlk.	Tree, mid-story	0.7	
69	Rubiaceae	*Palicourea demissa* Standl.	Shrub	1	
70	Melastomataceae	*Miconia brevitheca* Gleason	Tree	1.5	
71	Heliconiaceae	*Heliconia* aff. *fragilis* Abalo & G. Morales	Herb	2	
72	Marattiaceae	*Danaea erecta* Tuomisto & R.C. Moran	Fern	1	
73	Gesneriaceae	*Gasteranthus corallinus* (Fritsch) Wiehler	Herb	2	
74	Gesneriaceae	*Gasteranthus corallinus* (Fritsch) Wiehler	Herb	1	
75	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	0.6	
76	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	0.5	
77	Lauraceae	*Caryodaphnopsis theobromifolia* (A.H. Gentry) van der Werff & H.G.	Tree	1.5	

		Richt.			
78	Moraceae	*Helicostylis tovarensis* (Klotzsch & H. Karst.) C.C. Berg	Tree	0.4	
79	Piperaceae	*Piper obliquum* Ruiz & Pav.	Shrub	0.5	Endangered: on an IUCN red list
80	Lauraceae	*Caryodaphnopsis theobromifolia* (A.H. Gentry) van der Werff & H.G. Richt.	Tree	1.5	
81	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	2	
82	Gesneriaceae	*Gasteranthus corallinus* (Fritsch) Wiehler	Herb	1.4	
83	Rubiaceae	*Palicourea demissa* Standl.	Shrub	1.3	
84	Araceae	*Anthurium* sp.	Herb	1.5	
85	Marattiaceae	*Danaea erecta* Tuomisto & R.C. Moran	Fern	1	
86	Malvaceae	*Quararibea casaseca*e Fern. Alonso & Castrov.	Tree	1	sometimes placed in Bombacaceae
87	Moraceae	*Helicostylis tovarensis* (Klotzsch & H. Karst.) C.C. Berg	Tree	0.4	
88	Solanaceae	*Solanum leptopodum* Van Heurck & Müll. Arg.	Shrub	5	
89	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	8	
90	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1	
91	Fabaceae	Inga	Tree	1	
92	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1.7	
93	Melastomataceae	*Miconia* cf. *gracilis* Triana	Shrub	0.4	
94	Clusiaceae	*Garcinia madruno* (Kunth) Hammel	Tree, mid-story	1.5	
95	Tapisciaceae	*Huertea glandulosa* Ruiz & Pav.	Tree	5	sometimes placed in Staphyleaceae
96	Sapindaceae	*Allophylus punctatus* (Poepp.) Radlk.	Tree, mid-story	1.6	
97	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	0.4	
98	Moraceae	*Helicostylis tovarensis* (Klotzsch & H. Karst.) C.C. Berg	Tree	3	
99	Solanaceae	*Solanum leptopodum* Van Heurck & Müll. Arg.	Shrub	1	
100	Rubiaceae	*Pentagonia* sp.	Tree, understory	2	
101	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	4	
102	Solanaceae	*Solanum leptopodum* Van Heurck & Müll. Arg.	Shrub	0.5	
103	Melastomataceae	*Miconia* cf. *gracilis* Triana	Shrub	1	
104	Gesneriaceae	*Gasteranthus corallinus* (Fritsch) Wiehler	Herb	0.5	
105	Moraceae	*Naucleopsis ulei* (Warb.) Ducke	Tree, mid-	3	

			story	
106	Piperaceae	*Piper spoliatum* Trel. & Yunck.	Shrub	3
107	Marattiaceae	*Danaea erecta* Tuomisto & R.C. Moran	Fern	1.4
108	Rubiaceae	*Palicourea demissa* Standl.	Shrub	0.7
109	Moraceae	*Ficus cuatrecasiana* Dugand	Tree	24
110	Melastomataceae	*Ossaea micrantha* (Sw.) Macfad. ex Cogn.	Shrub	6
111	Lauraceae	*Nectandra lineatifolia* (Ruiz & Pav.) Mez	Tree	7
112	Rubiaceae	*Faramea* aff. *oblongifolia* Standl.	Tree, understory	1
113	Lauraceae	*Nectandra lineatifolia* (Ruiz & Pav.) Mez	Tree	1
114	Myrtaceae	*Myrcia fallax* (Rich.) DC.	Tree, understory	4
115	Moraceae	*Helicostylis tovarensis* (Klotzsch & H. Karst.) C.C. Berg	Tree	0.5
116	Solanaceae	*Solanum leptopodum* Van Heurck & Müll. Arg.	Shrub	4
117	Marattiaceae	*Danaea erecta* Tuomisto & R.C. Moran	Fern	0.8
118	Euphorbiaceae	*Acalypha diversifolia* Jacq.	Shrub	0.5
119	Sapindaceae	*Allophylus* sp.	Tree, mid-story	5
120	Burseraceae	*Dacryodes peruviana* (Loes.) H.J. Lam	Tree	4.5

TABLE S2. *Herbarium (OSC) and GenBank accession numbers for specimens and cultures examined in this study. Stromata are listed by OSC accession, with the corresponding GenBank accession following in parentheses. Only DNA was allowable for export for endophyte cultures, and they are thus listed only as GenBank accessions.*

Species	**Stromata**	**Endophytes**
Xylaria adscendens (Fr.) Fr.	153227 (KP133258), 153374 (KP133295), 153447 (KP133298), 153183 (KP133281), 153197 (KP133263)	KP133253, KP133275, KP133277, KP133265, KP133259, KP133261, KP133286, KP133252, KP133299, KP133256, KP133254, KP133284, KP133300, KP133257, KP133264, KP133262, KP133282, KP133287, KP133288, KP133276, KP133292, KP133255, KP133269, KP133289, KP133293, KP133294, KP133271, KP133290, KP133280, KP133296, KP133272, KP133273, KP133291, KP133279, KP133274, KP133267, KP133285, KP133266, KP133283, KP133260, KP133268, KP133270, KP133278
Xylaria anisopleura (Mont.) Fr.	153273 (KP133317), 153407 (KP133318), 153271 (KP133319), 153276 (KP133320)	
Xylaria apiculata_1 Cooke	153267 (KP133325), 153277 (KP133329), 153281 (KP133331), 153302 (KP133330), 153347 (KP133327), 153364 (KP133333), 153389 (KP133332), 153390 (KP133321), 153394 (KP133334), 153395 (KP133335), 153415 (KP133328), 153422 (KP133323), 153464 (KP133326), 153193 (KP133322), 153178 (KP133324)	KP133336
Xylaria apiculata_2 Cooke	KP133337	
Xylaria atrosphaerica (Cooke & Massee) Callan & J.D. Rogers	153308 (KP133341), 153344 (KP133342), 153385 (KP133340), 153388 (KP133339), 153417 (KP133338)	KP133343
Xylaria aff. *comosa* (Mont.) Læssøe	153236 (KP133307), 153280 (KP133303), 153293 (KP133306), 153348 (KP133305), 153386 (KP133304), 153455 (KP133302), 153171 (KP133308)	
Xylaria cristata Speg.	153330 (KP133345)	
Xylaria cuneata Lloyd	153288 (KP133349), 153361 (KP133351), 153363 (KP133346), 153452 (KP133347), 153453 (KP133348), 153290 (KP133350)	
Xylaria aff. *curta* Fr.	153341 (KP133311), 153356 (KP133310), 153185 (KP133312)	KP133309

Xylaria curta_1 Fr.	153232 (KP133352)	
Xylaria curta_2 Fr.	153450 (KP133355), 153451 (KP133354), Inca27Jan12sn (KP133356), 153172 (KP133353)	
Xylaria enterogena Mont.	153243 (KP133364), 153251 (KP133370), 153252 (KP133361), 153263 (KP133362), 153298 (KP133366), 153322 (KP133368), 153371 (KP133360), 153382 (KP133372), 153393 (KP133367), 153396 (KP133359), 153404 (KP133357), 153420 (KP133369), 153430 (KP133358), 153475 (KP133365), 153289 (KP133363)	
Xylaria fissilis_1 Ces.	153201 (KP133386), 153202 (KP133391), 153216 (KP133379), 153221 (KP133403), 153222 (KP133408), 153223 (KP133376), 153254 (KP133399), 153255 (KP133373), 153259 (KP133392), 153262 (KP133378), 153264 (KP133394), 153328 (KP133390), 153329 (KP133375), 153336 (KP133388), 153368 (KP133395), 153376 (KP133396), 153408 (KP133393), 153410 (KP133384), 153456 (KP133401), 153457 (KP133377), 153458 (KP133374), 153459 (KP133402), 153461 (KP133404), 153462 (KP133400), 153472 (KP133397), 153189 (KP133382), 488 (KP133381), 862 (KP133389)	KP133405, KP133385, KP133398, KP133383, KP133380, KP133409, KP133406, KP133387
Xylaria fissilis_2 Ces.	153233 (KP133410), 153412 (KP133416), 153184 (KP133412), 153188 (KP133414), Sm22 (KP133413), Smp22 (KP133411), 265 (KP133415),	
Xylaria globosa (Spreng.) Mont.	153268 (KP133428), 153311 (KP133426), 153314 (KP133429), 153315 (KP133425), 153324 (KP133422), 153401 (KP133420), 153419 (KP133418), 153427 (KP133423), 153428 (KP133419), 153443 (KP133424), 153444 (KP133427), 153480 (KP133417)	
Xylaria meliacearum Læssøe	153213, 153294 (KP133434), 153272 (KP133433)	
Xylaria multiplex (Kunze ex Fr.) Fr.	153340 (KP133436), 153381 (KP133437), 153379 (KP133438), 153468 (KP133494), 153345 (KP133440), 508	
Xylaria ophiopoda Sacc.	153246 (KP133445), 153285 (KP133443), 153338 (KP133442), 153414 (KP133446), 153425 (KP133444), 153426 (KP133441), 153483 (KP133447)	
Xylaria schweinitzii Berk. & M.A. Curtis	153215 (KP133467), 153237 (KP133460), 153239 (KP133472), 153256 (KP133464), 153270 (KP133448), 153284 (KP133463), 153307 (KP133470), 153335 (KP133455), 153343 (KP133454), 153350 (KP133466), 153352 (KP133469), 153353 (KP133456), 153359 (KP133449), 153367 (KP133453), 153375 (KP133461), 153392 (KP133462),	

	153398 (KP133450), 153439 (KP133458), 153469 (KP133459), 153471 (KP133471), 153474 (KP133465), 153186 (KP133457), 153176 (KP133451), 318 (KP133452)
Xylaria scruposa_1 (Fr.) Berk.	492 (KP133476), 559 (KP133484), 153238 (KP133483), 153295 (KP133487), 153327 (KP133480), 153331 (KP133475), 153332 (KP133489), 153334 (KP133477), 153342 (KP133474), 153373, 153383 (KP133495), 153405 (KP133485), 153406 (KP133491), 153421 (KP133486), 153424 (KP133493), 153432 (KP133479), 153434 (KP133481), 153440 (KP133482), 153441 (KP133473), 153448 (KP133492), 153449 (KP133488), 153482 (KP133490), 1174 (KP133478),
Xylaria scruposa_2 (Fr.) Berk.	866 (KP133498), 153218 (KP133501), 153313 (KP133497), 153411 (KP133500), 153477 (KP133496), 153481 (KP133499)
Xylaria subtorulosa Speg.	153360 (KP133531), 153369 (KP133528), 153370 (KP133529), 153196 (KP133530)
Xylaria telfairii (Berk.) Sacc.	153229 (KP133535), 153258 (KP133536), 153261 (KP133542), 153283 (KP133539), 952 (KP133538), 153297 (KP133534), 153303 (KP133543), 153366 (KP133541), 153445 (KP133540), 153446 (KP133532), 153466 (KP133537), 153467 (KP133544), Squatter-1 (KP133533)
Xylaria xanthinovelutina (Mont.) Mont.	153325 (KP133432), 153384 (KP133431)
Xylaria sp. 01	153433 (KP133507)
Xylaria sp. 02	153214 (KP133508)
Xylaria sp. 03	153326 (KP133509)
Xylaria sp. 05	153416 (KP133511)
Xylaria sp. 06	153413 (KP133512)
Xylaria sp. 07	153316 (KP133513)
Xylaria sp. 08	153212 (KP133514)
Xylaria sp. 10	153266 (KP133516), 416 (KP133517)
Xylaria sp. 11	153210 (KP133518), 153200 (KP133519)
Xylaria sp. 12	153245 (KP133521), 153269 (KP133520)
Xylaria sp. 13	153301 (KP133522), 153372 (KP133523)
Xylaria sp. nov. 2	153228 (KP133524)

TABLE S3. *Nearest-Neighbor analysis of spatial clusters in the five species of* Xylaria *with both life stages present in our plot, for within each life stage. See also Figures S[4] & S[5]. Values shown are the observed mean nearest neighbor distance ($\bar{d}_o$), the expected mean nearest neighbor distance ($\bar{d}_e$) from a Monte Carlo simulation null model assuming complete spatial randomness (CSR), the standard deviation around the expected mean nearest neighbor distance (s_e), and the* P *values, calculated as the proportion of simulations where $\bar{d}_e < \bar{d}_o$. Bold indicates* P *< 0.05; italics indicate 0.05 < P <0.10; dashes indicate insufficient sample size to conduct the analysis at a given neighbor class.*

		Stromata around **Stromata**				**Endophytes** around **Endophytes**			
Taxa	Neighbor class	$\bar{d}_o$	$\bar{d}_e$	s_e	*P*	$\bar{d}_o$	$\bar{d}_e$	s_e	*P*
Xylaria aff. *curta*	1	22.36	44.34	23.33	0.223	—	—	—	—
Xylaria apiculata_1	1	12.23	15.82	2.80	*0.100*	—	—	—	—
	2	22.20	24.41	3.74	0.278	—	—	—	—
	3	30.83	31.99	4.57	0.398	—	—	—	—
	4	35.11	39.37	5.66	0.227	—	—	—	—
	5	44.54	47.05	8.04	0.390	—	—	—	—
Xylaria fissilis_1	1	11.87	14.18	2.20	0.148	23.27	22.39	5.76	0.563
	2	18.47	21.63	2.94	0.144	36.03	36.53	8.28	0.474
	3	22.22	28.03	3.53	*0.051*	55.49	50.77	13.57	0.672
	4	30.68	34.02	4.11	0.210	79.13	66.93	14.94	0.766
	5	42.37	39.97	4.94	0.678	—	—	—	—
Xylaria adscendens	1	51.35	31.80	10.98	0.961	8.79	9.36	0.80	0.241
	2	82.08	56.46	19.79	0.885	11.91	13.43	0.89	**0.044**
	3	—	—	—	—	17.12	16.80	1.12	0.606
	4	—	—	—	—	19.18	19.94	1.31	0.278
	5	—	—	—	—	20.83	22.82	1.46	0.088
Xylaria atrosphaerica	1	16.84	25.87	7.49	0.122	—	—	—	—
	2	29.53	43.55	13.38	0.142	—	—	—	—
	3	45.21	62.84	17.07	0.171	—	—	—	—

TABLE S4. *Nearest-Neighbor analysis of spatial clusters in the five species of* Xylaria *with both life stages present in our plot, by life stage, around the stream. See also Figures 4 & S4. Values shown are the observed mean nearest neighbor distance ($\bar{d}_o$), the expected mean nearest neighbor distance ($\bar{d}_e$) from a Monte Carlo simulation null model assuming complete spatial randomness (CSR), the standard deviation around the expected mean nearest neighbor distance (s_e), and the* P *values, calculated as the proportion of simulations where $\bar{d}_e < \bar{d}_o$. Bold indicates* P *< 0.05; italics indicate 0.05 < P <0.10; dashes indicate insufficient sample size to conduct the analysis at a given neighbor class.*

		Stromata around **Stream**				**Endophytes** around **Stream**			
Taxa	Neighbor class	$\bar{d}_o$	$\bar{d}_e$	s_e	*P*	$\bar{d}_o$	$\bar{d}_e$	s_e	*P*
Xylaria aff. *curta*	1	23.27	29.63	10.05	0.264	30.22	41.20	14.08	0.242
	2	29.67	52.75	12.81	**0.016**	—	—	—	—
Xylaria apiculata_1	1	6.85	13.49	3.50	**0.006**	49.98	41.08	14.02	0.760
	2	12.74	21.62	3.99	**0.002**	—	—	—	—
	3	18.08	28.17	4.54	**0.001**	—	—	—	—
	4	28.91	34.26	5.27	0.127	—	—	—	—
	5	32.61	40.37	6.18	*0.067*	—	—	—	—
Xylaria fissilis_1	1	15.39	12.02	3.03	0.867	13.25	18.47	5.35	0.153
	2	20.93	19.30	3.41	0.709	35.44	30.06	6.63	0.835
	3	26.71	25.01	3.80	0.708	54.42	40.61	8.29	0.924
	4	29.43	30.21	4.26	0.474	69.12	51.92	9.45	0.960
	5	36.03	35.18	4.87	0.634	72.93	64.91	8.86	0.787
Xylaria adscendens	1	24.30	24.09	7.55	0.583	5.41	7.09	1.67	0.158
	2	41.00	40.75	10.17	0.603	12.27	12.10	1.68	0.581
	3	65.18	58.54	11.28	0.660	15.48	15.38	1.91	0.549
	4	—	—	—	—	17.97	18.35	2.06	0.448
	5	—	—	—	—	20.92	21.08	2.11	0.486
Xylaria atrosphaerica	1	14.27	20.77	6.27	0.126	40.02	41.22	14.01	0.573
	2	21.11	34.23	8.02	**0.007**	—	—	—	—
	3	33.17	47.30	9.91	**0.048**	—	—	—	—
	4	50.89	62.26	9.89	0.161	—	—	—	—

TABLE S5. *Nearest-neighbor analysis of all* Xylaria *species recovered as stromata, testing for clumping within the life stage and clumping around the stream. Values shown are the observed mean nearest neighbor distance ($\bar{d}_o$), the expected mean nearest neighbor distance ($\bar{d}_e$) from a Monte Carlo simulation null model assuming complete spatial randomness (CSR), the standard deviation around the expected mean nearest neighbor distance (s_e), and the* P *values, calculated as the proportion of simulations where $\bar{d}_e < \bar{d}_o$. Bold indicates* P *< 0.05; italics indicate 0.05 < P <0.10; dashes indicate insufficient sample size to conduct the analysis at a given neighbor class.*

		Stromata around **Stream**				**Stromata** around **Stromata**			
Taxa	Neighbor class	$\bar{d}_o$	$\bar{d}_e$	s_e	*P*	$\bar{d}_o$	$\bar{d}_e$	s_e	*P*
Xylaria adscendens	1	24.3	24.1	7.6	0.583	51.4	31.8	11.0	0.961
	2	41.0	40.8	10.2	0.603	82.1	56.5	19.8	0.885
	3	65.2	58.5	11.3	0.660	—	—	—	—
Xylaria anisopleura	1	14.4	24.1	7.7	*0.058*	23.7	31.9	11.0	0.251
	2	32.7	40.7	10.1	0.225	52.9	56.7	19.7	0.452
	3	49.3	58.5	11.2	0.245	—	—	—	—
Xylaria apiculata_1	1	6.9	13.5	3.5	**0.006**	12.2	15.8	2.8	*0.100*
	2	12.7	21.6	4.0	**0.002**	22.2	24.4	3.7	0.278
	3	18.1	28.2	4.5	**0.001**	30.8	32.0	4.6	0.398
	4	28.9	34.3	5.3	0.127	35.1	39.4	5.7	0.227
	5	32.6	40.4	6.2	*0.067*	44.5	47.1	8.0	0.390
Xylaria apiculata_2	1	39.1	41.1	14.0	0.542	—	—	—	—
Xylaria atrosphaerica	1	14.3	20.8	6.3	0.126	16.8	25.9	7.5	0.122
	2	21.1	34.2	8.0	**0.007**	29.5	43.6	13.4	0.142
	3	33.2	47.3	9.9	**0.048**	45.2	62.8	17.1	0.171
	4	50.9	62.3	9.9	0.161	—	—	—	—
Xylaria aff. *comosa*	1	15.2	18.4	5.3	0.291	21.2	22.4	5.7	0.429
	2	26.0	30.0	6.6	0.281	30.5	36.5	8.2	0.237
	3	40.6	40.5	8.2	0.584	38.6	50.8	13.5	0.181
	4	44.1	51.8	9.5	0.245	51.5	66.9	14.9	0.169
	5	55.9	64.8	8.9	0.181	—	—	—	—
Xylaria cristata	1	33.7	41.2	14.1	0.410	—	—	—	—
Xylaria cuneata	1	20.3	20.8	6.2	0.522	19.4	25.9	7.6	0.206
	2	23.9	34.2	8.1	**0.041**	34.4	43.7	13.5	0.254
	3	34.5	47.3	10.0	*0.075*	53.5	63.0	17.1	0.311
	4	50.8	62.3	10.0	0.160	—	—	—	—

Xylaria curta_1	1	50.0	41.1	14.1	0.760	—	—	—	—
Xylaria curta_2	1	46.9	41.2	14.0	0.709	—	—	—	—
Xylaria aff. *curta*	1	23.3	29.6	10.1	0.264	22.4	44.3	23.3	0.223
	2	29.7	52.8	12.8	**0.016**	—	—	—	—
Xylaria enterogena	1	7.6	12.7	3.3	**0.028**	14.8	15.0	2.5	0.480
	2	13.7	20.3	3.7	**0.015**	22.3	22.9	3.3	0.423
	3	23.9	26.5	4.2	0.282	27.5	29.8	4.0	0.280
	4	26.6	32.1	4.8	*0.094*	32.5	36.4	4.7	0.208
	5	30.3	37.5	5.5	*0.057*	37.1	43.0	6.3	0.167
Xylaria fissilis_1	1	15.4	12.0	3.0	0.867	11.9	14.2	2.2	0.148
	2	20.9	19.3	3.4	0.709	18.5	21.6	2.9	0.144
	3	26.7	25.0	3.8	0.708	22.2	28.0	3.5	*0.051*
	4	29.4	30.2	4.3	0.474	30.7	34.0	4.1	0.210
	5	36.0	35.2	4.9	0.634	42.4	40.0	4.9	0.678
Xylaria fissilis_2	1	24.2	29.7	10.1	0.314	80.6	44.2	23.3	0.917
	2	63.9	52.7	12.8	0.756	—	—	—	—
Xylaria globosa	1	17.3	18.5	5.4	0.461	20.1	22.4	5.8	0.352
	2	28.8	30.0	6.6	0.490	35.6	36.6	8.2	0.455
	3	39.5	40.6	8.2	0.533	55.9	50.8	13.4	0.682
	4	46.1	51.9	9.5	0.317	66.1	67.0	14.9	0.462
	5	57.7	65.0	8.9	0.217	—	—	—	—
Xylaria meliacearum	1	29.9	24.2	7.8	0.826	35.3	31.9	11.1	0.622
	2	43.8	40.8	10.3	0.688	53.9	56.5	19.8	0.469
	3	65.5	58.5	11.3	0.664	—	—	—	—
Xylaria multiplex	1	22.7	24.1	7.6	0.479	14.0	31.8	11.0	**0.043**
	2	25.6	40.7	10.1	**0.013**	34.7	56.5	19.7	0.145
	3	35.9	58.5	11.2	**0.017**	—	—	—	—
Xylaria ophiopoda	1	18.0	18.4	5.3	0.520	12.4	22.3	5.7	**0.037**
	2	21.4	29.9	6.6	**0.050**	45.4	36.4	8.2	0.860
	3	35.3	40.4	8.2	0.303	63.0	50.6	13.4	0.828
	4	53.3	51.7	9.5	0.578	68.1	66.8	14.9	0.511
	5	58.7	64.8	9.0	0.242	—	—	—	—
Xylaria schweinitzii	1	8.8	9.6	2.3	0.393	13.4	11.7	1.4	0.877
	2	15.1	15.7	2.6	0.445	17.0	17.4	1.8	0.396
	3	18.3	20.2	2.8	0.263	23.0	22.3	2.2	0.621
	4	22.2	24.2	3.0	0.261	25.5	26.7	2.5	0.326
	5	26.1	27.9	3.2	0.296	28.2	30.7	2.8	0.186

Xylaria scruposa_1	1	15.0	11.5	2.9	0.886	11.8	13.6	2.0	0.188
	2	20.2	18.4	3.2	0.739	17.2	20.5	2.6	*0.100*
	3	25.1	23.8	3.6	0.675	22.3	26.5	3.1	*0.091*
	4	28.3	28.7	3.9	0.504	27.9	32.1	3.6	0.123
	5	32.4	33.3	4.4	0.460	32.3	37.5	4.2	0.111
Xylaria scruposa_2	1	16.0	20.8	6.2	0.226	30.4	26.0	7.6	0.715
	2	35.3	34.1	8.0	0.653	55.2	43.7	13.4	0.825
	3	63.2	47.2	9.9	0.923	79.5	63.1	17.0	0.811
	4	72.6	62.2	10.0	0.835	—	—	—	—
Xylaria subtorulosa	1	19.3	29.7	10.2	0.105	15.0	44.3	23.1	0.108
	2	28.1	52.7	12.8	**0.006**	—	—	—	—
Xylaria telfairii	1	13.3	15.4	4.2	0.342	19.9	18.3	3.8	0.674
	2	25.0	24.8	4.9	0.572	27.4	28.7	5.1	0.394
	3	35.2	32.6	5.9	0.734	34.1	38.4	6.7	0.258
	4	38.8	40.4	7.0	0.481	42.2	48.4	10.1	0.270
	5	42.9	48.6	8.0	0.272	47.3	59.5	12.2	0.165
Xylaria xanthinovelutina	1	22.4	29.7	10.1	0.213	31.6	44.0	23.3	0.366
	2	36.6	52.7	12.7	0.107	—	—	—	—
Xylaria sp. 01	1	37.6	41.1	14.0	0.510	—	—	—	—
Xylaria sp. 02	1	59.4	41.1	14.0	0.859	—	—	—	—
Xylaria sp. 03	1	33.7	41.2	14.1	0.408	—	—	—	—
Xylaria sp. 05	1	41.7	41.2	14.0	0.630	—	—	—	—
Xylaria sp. 06	1	41.7	41.1	14.0	0.638	—	—	—	—
Xylaria sp. 07	1	27.6	41.2	14.1	0.177	—	—	—	—
Xylaria sp. 08	1	60.7	41.2	14.1	0.872	—	—	—	—
Xylaria sp. 10	1	46.3	41.1	14.0	0.704	—	—	—	—
Xylaria sp. 11	1	68.2	41.1	14.0	0.934	—	—	—	—
Xylaria sp. 12	1	40.9	29.7	10.1	0.878	20.0	44.3	23.1	0.155
	2	48.9	52.6	12.8	0.433	—	—	—	—
Xylaria sp. 13	1	13.9	29.8	10.3	**0.010**	40.3	43.9	23.1	0.503
	2	42.5	52.7	12.7	0.257	—	—	—	—
Xylaria sp. nov. 2	1	59.7	41.2	14.1	0.865	—	—	—	—

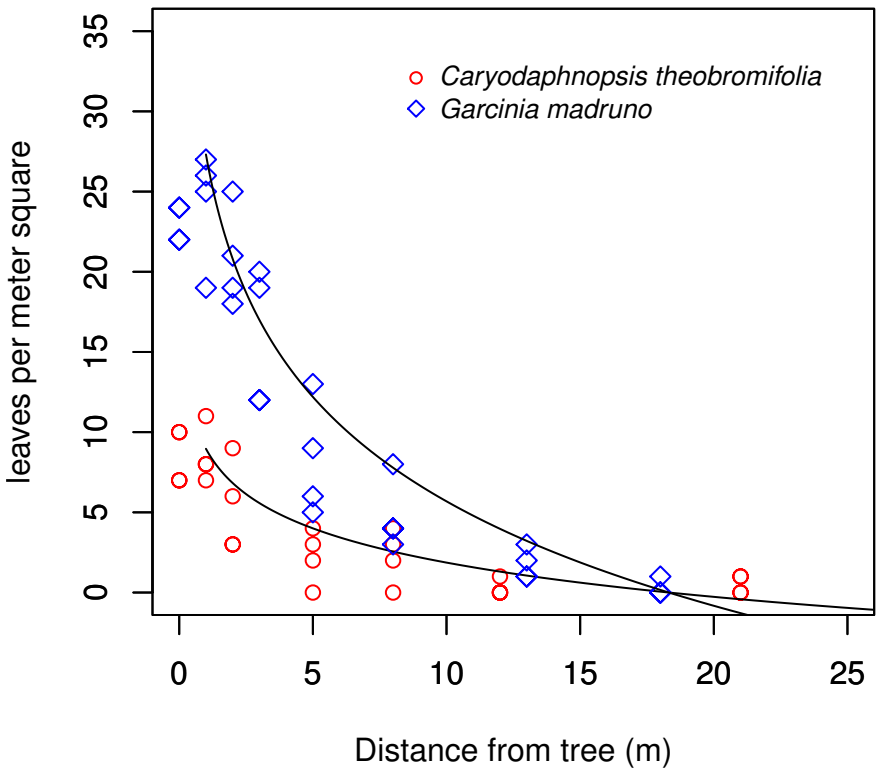


FIGURE S1. A survey of leaf fall in two trees at Los Cedros. Both trees, which vary in leaf morphology, display a negative logarithmic relationship between distance and abundance of leaf fall ($F_{1, 26} = 81.17$, $R^2 = 0.75$, $P < 0.001$ and $F_{1, 30} = 179.4$, $R^2 = 0.85$, $P < 0.001$), with most leaves deposited before 25 m. This suggests that most of the "sphere of influence" of a given host will be covered within one of our Moore-type neighborhoods of eight 5m x 10m sampling sites around a host.

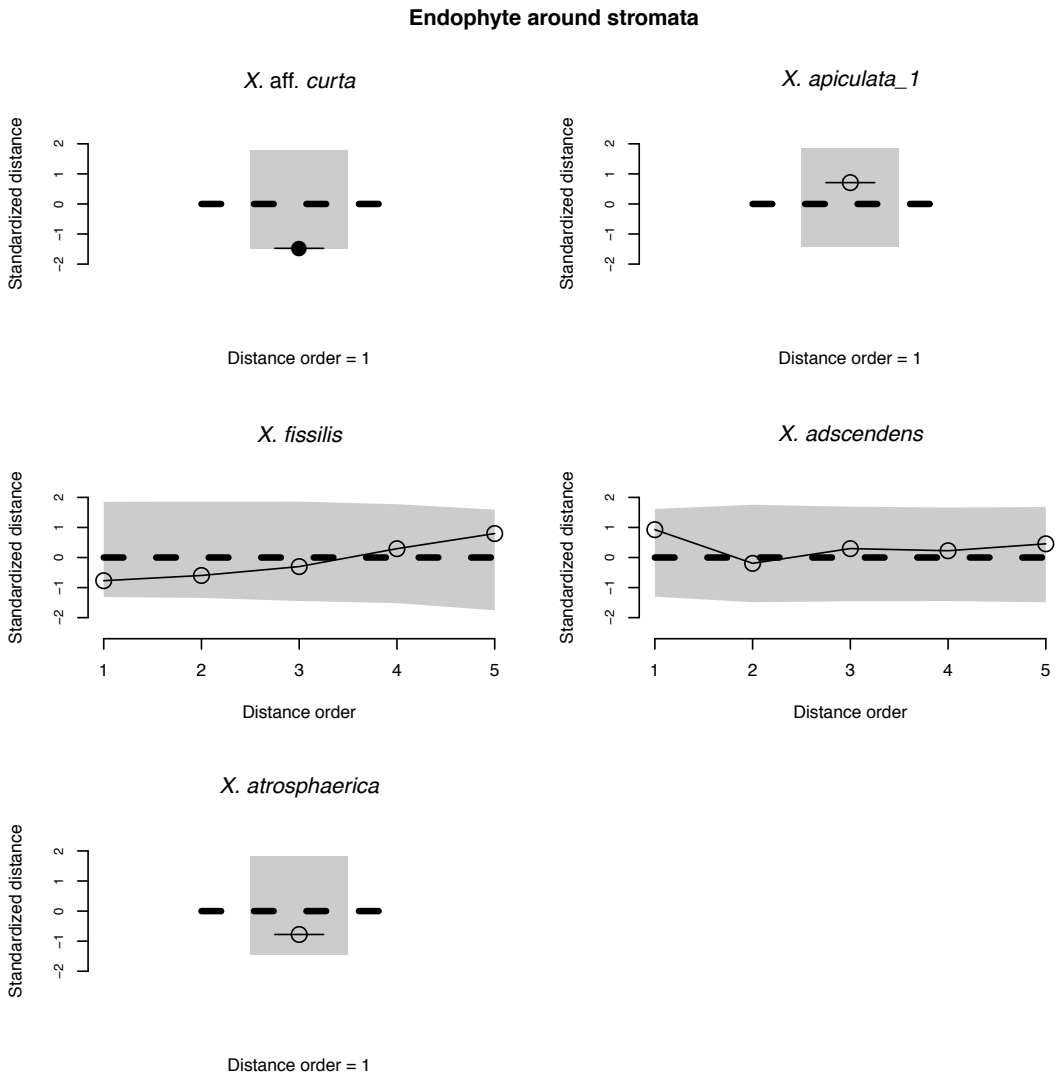


FIGURE S2. Graphical representation of results of nearest neighbor Monte Carlo-type simulations for clustering of endophytes around stromata (see also Table 2). For each species, the standardized mean distance to nearest neighboring point along the stream ($\bar{d}_o$) for all available distance classes is plotted. The dashed line represents the mean distance to points along the stream of the permutations on complete spatial randomness ($\bar{d}_e$), standardized to zero for all distance classes; the y-axis units represent deviation from the permutational mean (s_e). Open points are non-significant ($P > 0.05$); closed points are significant ($P < 0.05$); the grey area represents the region where $0.95 > P > 0.05$.

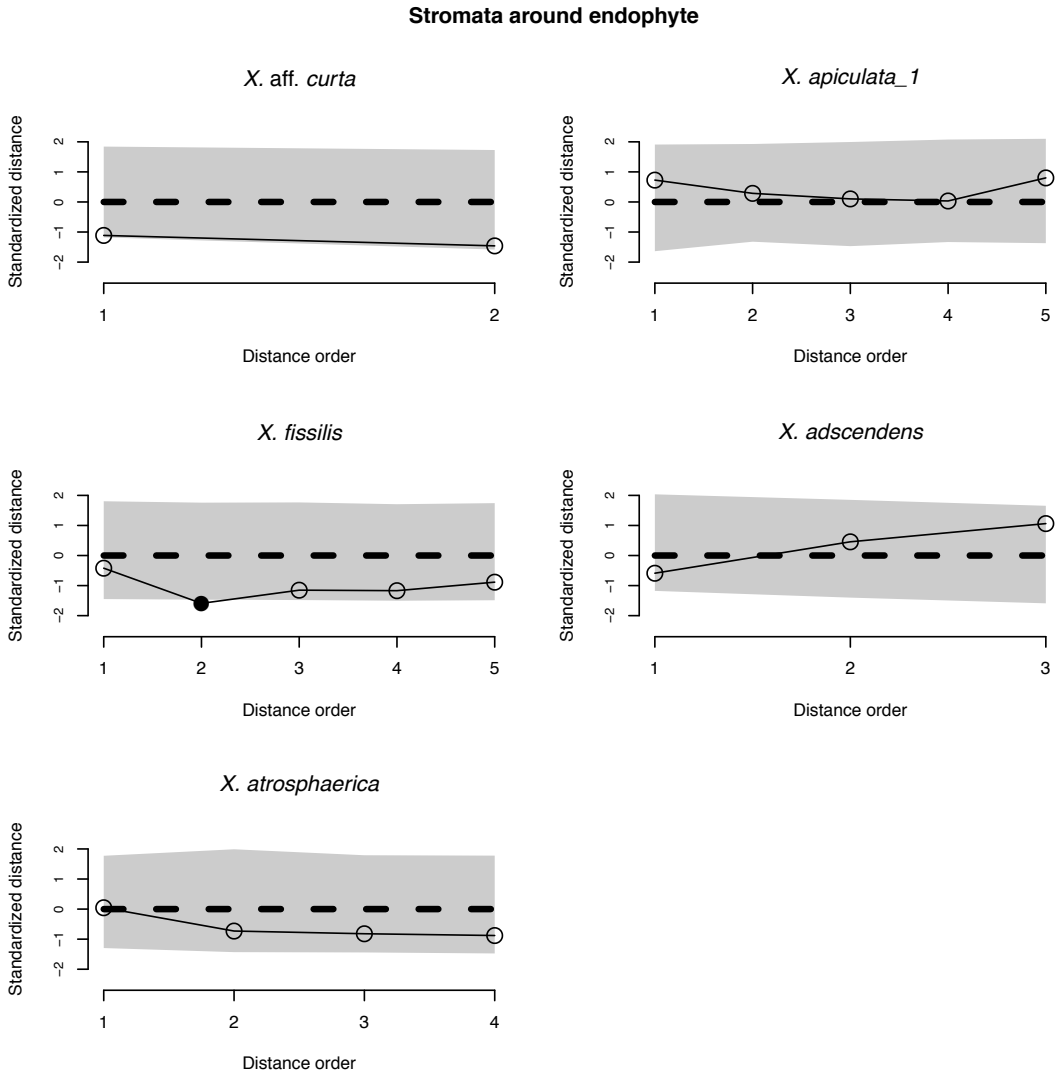


FIGURE S3. Graphical representation of results of nearest neighbor Monte Carlo-type simulations for clustering of stromata around endophytes (see also Table 2). For each species, the standardized mean distance to nearest neighboring point along the stream ($\bar{d}_o$) for all available distance classes is plotted. The dashed line represents the mean distance to points along the stream of the permutations on complete spatial randomness ($\bar{d}_e$), standardized to zero for all distance classes; the y-axis units represent deviation from the permutational mean (s_e). Open points are non-significant ($P > 0.05$); closed points are significant ($P < 0.05$); the grey area represents the region where $0.95 > P > 0.05$.

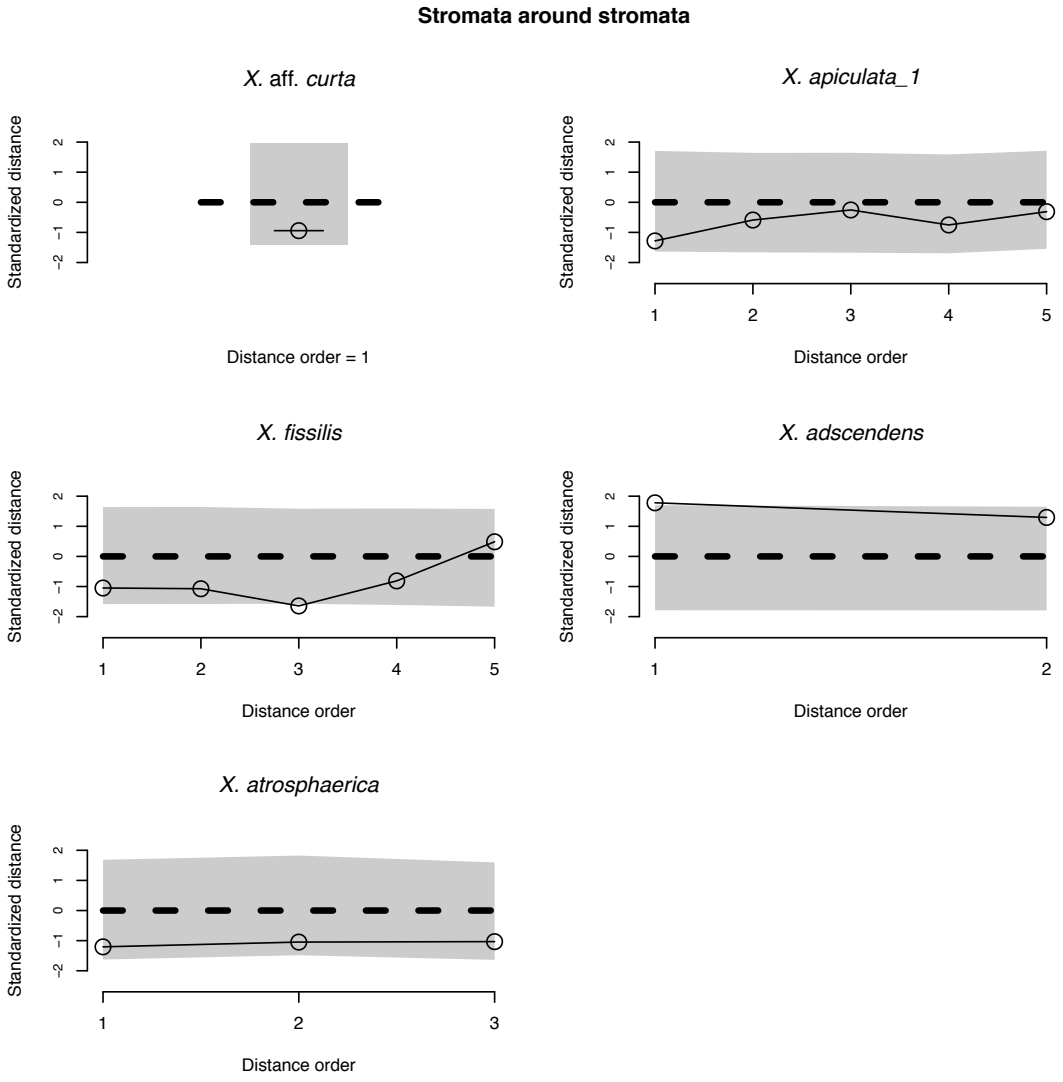


FIGURE S4. Graphical representation of results of nearest neighbor Monte Carlo-type simulations for clustering of stromata around stromata (see also Table S3). For each species, the standardized mean distance to nearest neighboring point along the stream ($\bar{d}_o$) for all available distance classes is plotted. The dashed line represents the mean distance to points along the stream of the permutations on complete spatial randomness ($\bar{d}_e$), standardized to zero for all distance classes; the y-axis units represent deviation from the permutational mean (s_e). Open points are non-significant ($P > 0.05$); closed points are significant ($P < 0.05$); the grey area represents the region where $0.95 > P > 0.05$.

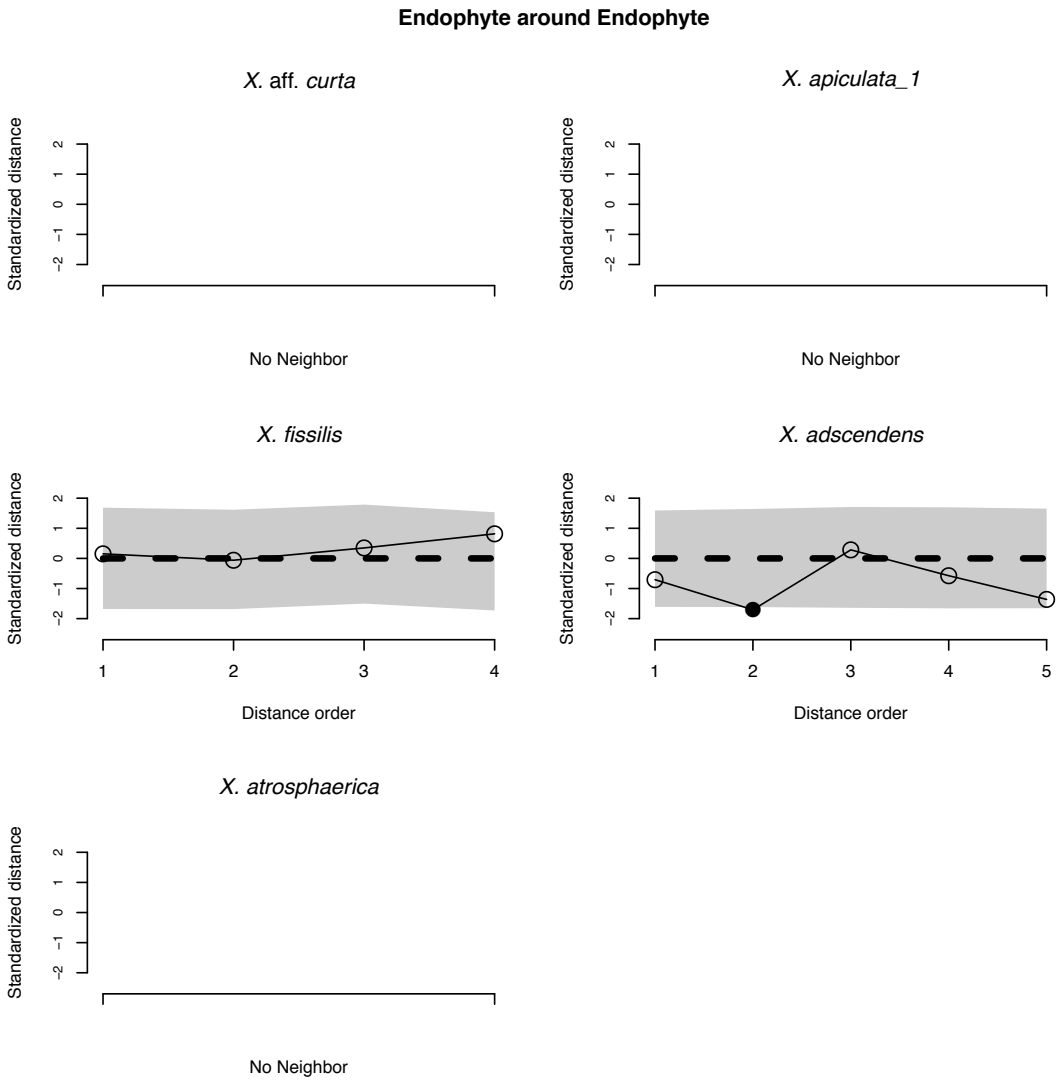


FIGURE S5. Graphical representation of results of nearest neighbor Monte Carlo-type simulations for clustering of endophytes around endophytes (see also Table S3). For each species, the standardized mean distance to nearest neighboring point along the stream ($\bar{d}_o$) for all available distance classes is plotted. The dashed line represents the mean distance to points along the stream of the permutations on complete spatial randomness ($\bar{d}_e$), standardized to zero for all distance classes; the y-axis units represent deviation from the permutational mean (s_e). Open points are non-significant ($P > 0.05$); closed points are significant ($P < 0.05$); the grey area represents the region where $0.95 > P > 0.05$.

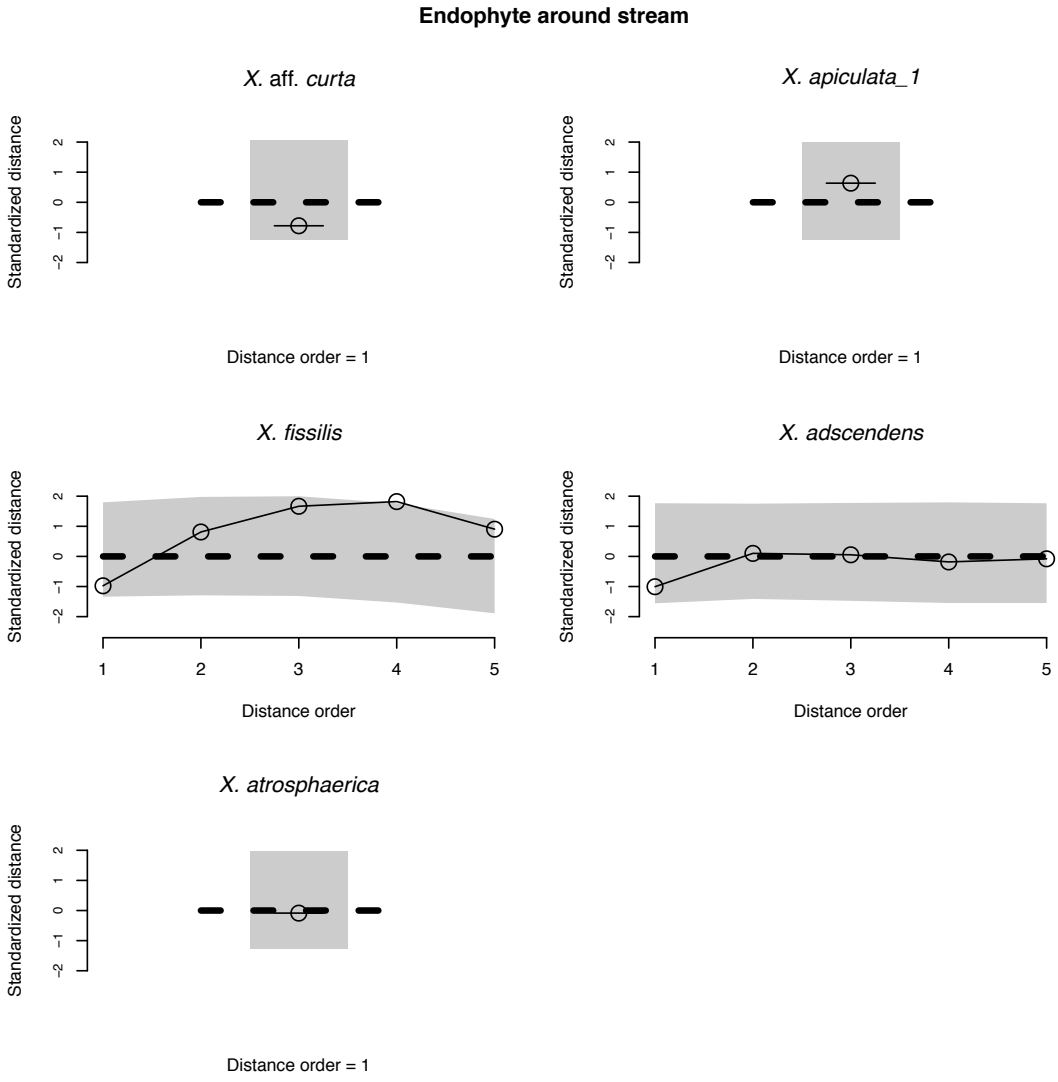


FIGURE S6. Graphical representation of results of nearest neighbor Monte Carlo-type simulations for clustering of endophytes around the stream (see also Table S4). For each species, the standardized mean distance to nearest neighboring point along the stream ($\bar{d}_o$) for all available distance classes is plotted. The dashed line represents the mean distance to points along the stream of the permutations on complete spatial randomness ($\bar{d}_e$), standardized to zero for all distance classes; the y-axis units represent deviation from the permutational mean (s_e). Open points are non-significant ($P > 0.05$); closed points are significant ($P < 0.05$); the grey area represents the region where $0.95 > P > 0.05$.

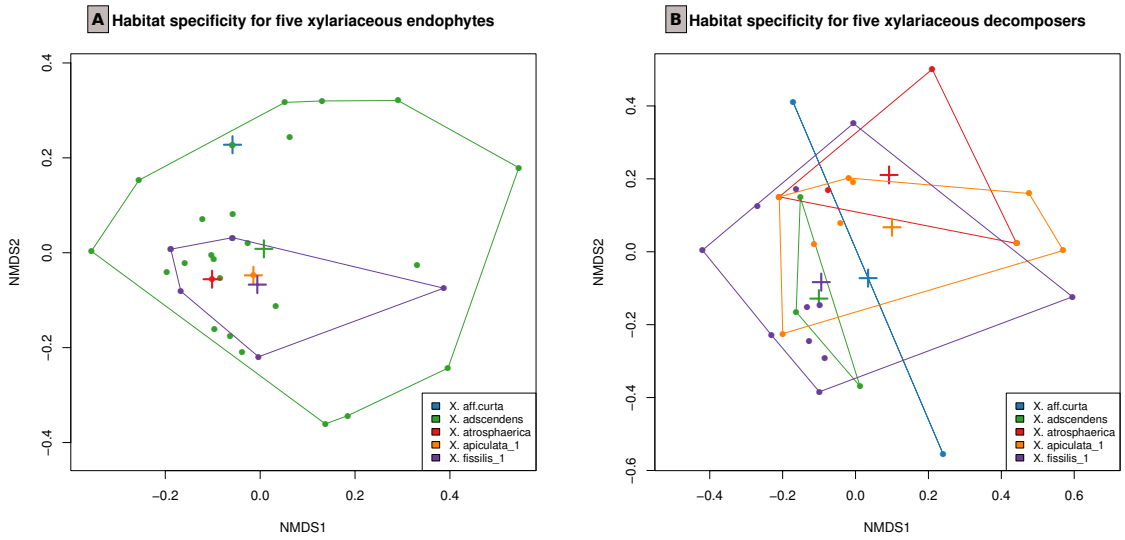


FIGURE S7. Non-metric multidimensional scaling diagram of habitat characteristics (slope, canopy cover, eastern exposure, northern exposure, and distance to water) for each point within the site from which a *Xylaria* was recovered. Each geographic point is categorized by species present (represented by color in these plots) and plotted by life stage: endophytes (A) are not differentiable by habitat (PerMANOVA, $F_{4, 29} = 0.45$, $R^2 = 0.06$, $P = 0.94$), but decomposers (B) do display some differences by habitat characteristics (PerMANOVA, $F_{4, 24} = 1.84$, $R^2 = 0.23$, $P = 0.07$; note the shifted centroids as compared to the endophytes). This trend is largely driven by proximity to water (PerMANOVA, $F_{1, 23} = 112.42$, $R^2 = 0.44$, $P = 0.001$).

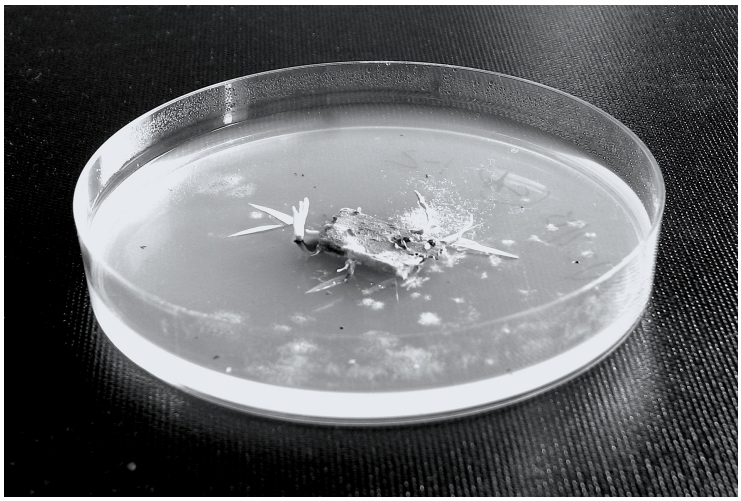


FIGURE S8. *Xylaria* sp. grown directly from leaves onto a segment of white birch tongue depressor and incubated for ~6 mo on 2% water agar. The fungus is in the conidial phase of stromatal formation (note the powdery conidia deposited on the agar to the right).

APPENDIX D

SUPPLEMENTAL MATERIALS FOR CHAPTER V

PRIMERS

ITS1F primer.—This first-step PCR primer included the 5' adapter for second step primer, a 6-mer barcode (represented by six Ns and offset by em-dashes), and the sequence of the forward primer, which binds to the template DNA.

5'-TCGGCATTCCTGCTGAACCGCTCTTCCGATCT—NNNNNN—
CTTGGTCATTTAGAGGAAGTAA-3'

ITS2 primer.—This first-step PCR primer included the 5' adapter for second step primer, a 6-mer barcode (represented by six Ns and offset by em-dashes), and the sequence of the reverse primer, which binds to the template DNA.

5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCT—NNNNNN—
GCTGCGTTCTTCATCGATGC-3'

Second-step.—These PCR primers included a region that bound to the Illumina adapters added with the first-step primers, amplifying the existing library without adding any primer affinity bias for these additional cycles. The remaining part of the primer completes the Illumina adapter for use in sequencing.

5'-AAGCAGAAGACGGCATACGAGATCGGTCTGGCATTCCTGC-3'

5'-ATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG-3'

SUPPLEMENTAL FILESET S1

Results from the permutation test are available online at:

https://dx.doi.org/10.6084/m9.figshare.3113422.v1

SUPPLEMENTAL FIGURES

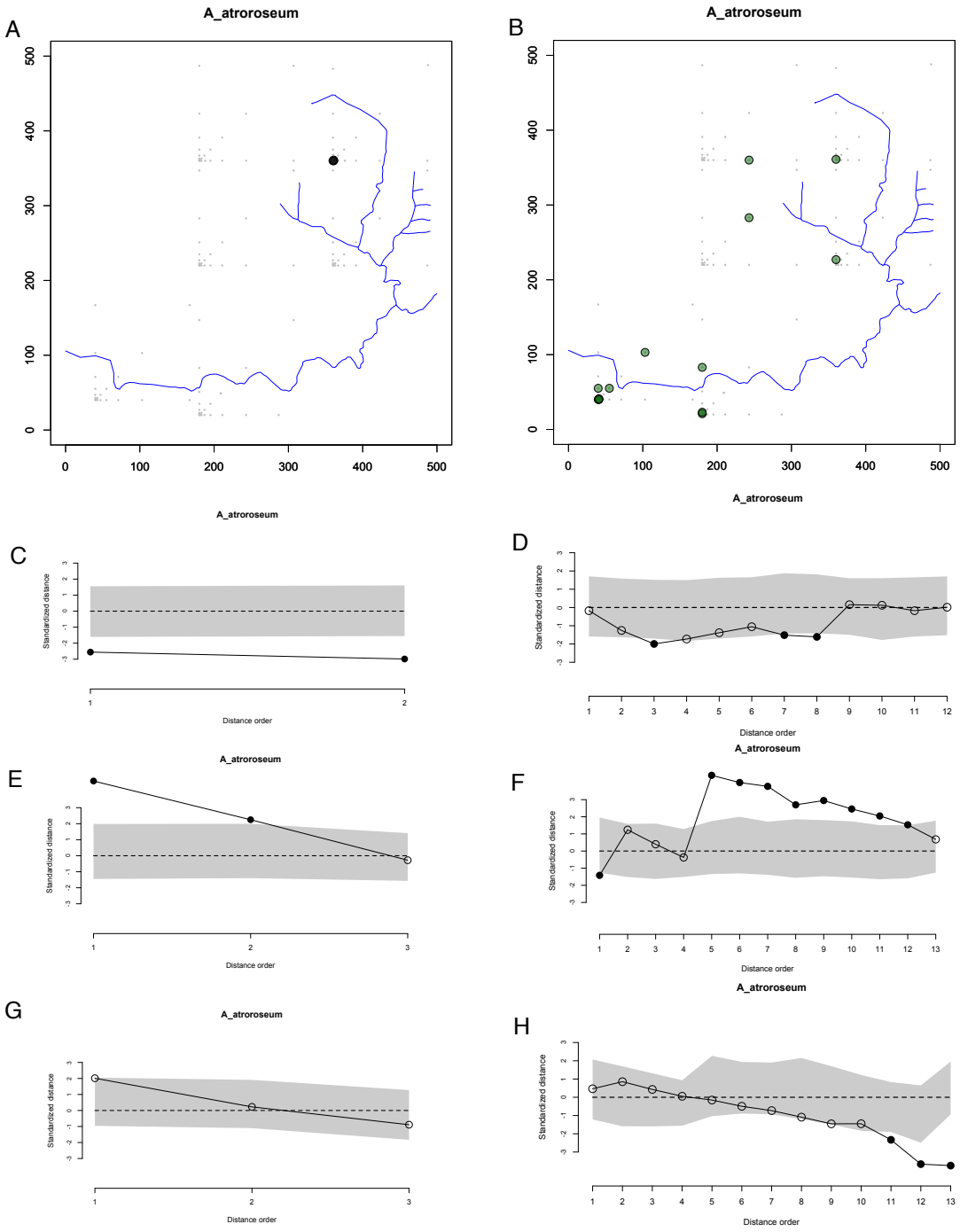


FIGURE S1: *Annulohypoxylon atroroseum* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

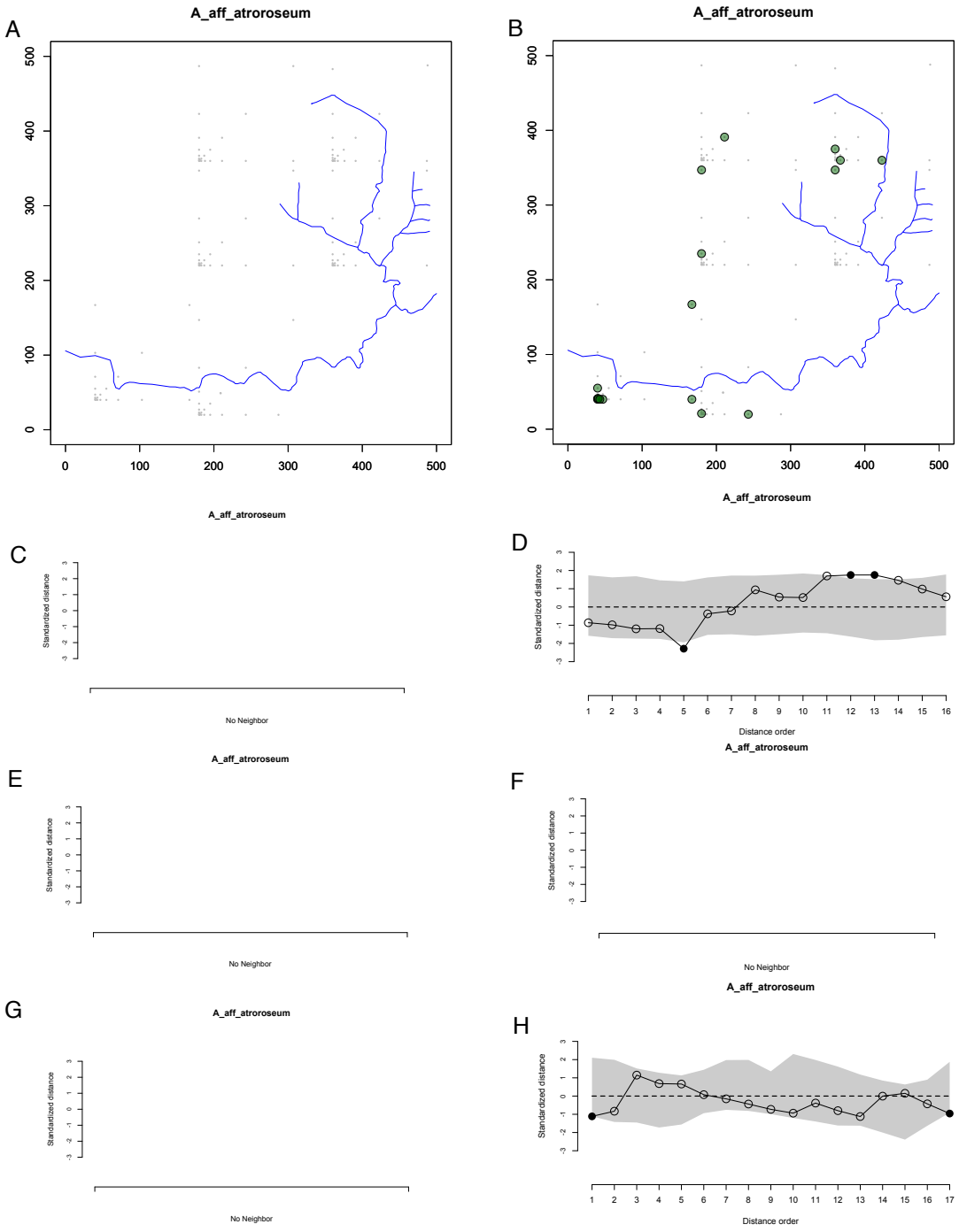


FIGURE S2: *Annulohypoxylon* aff. *atroroseum* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

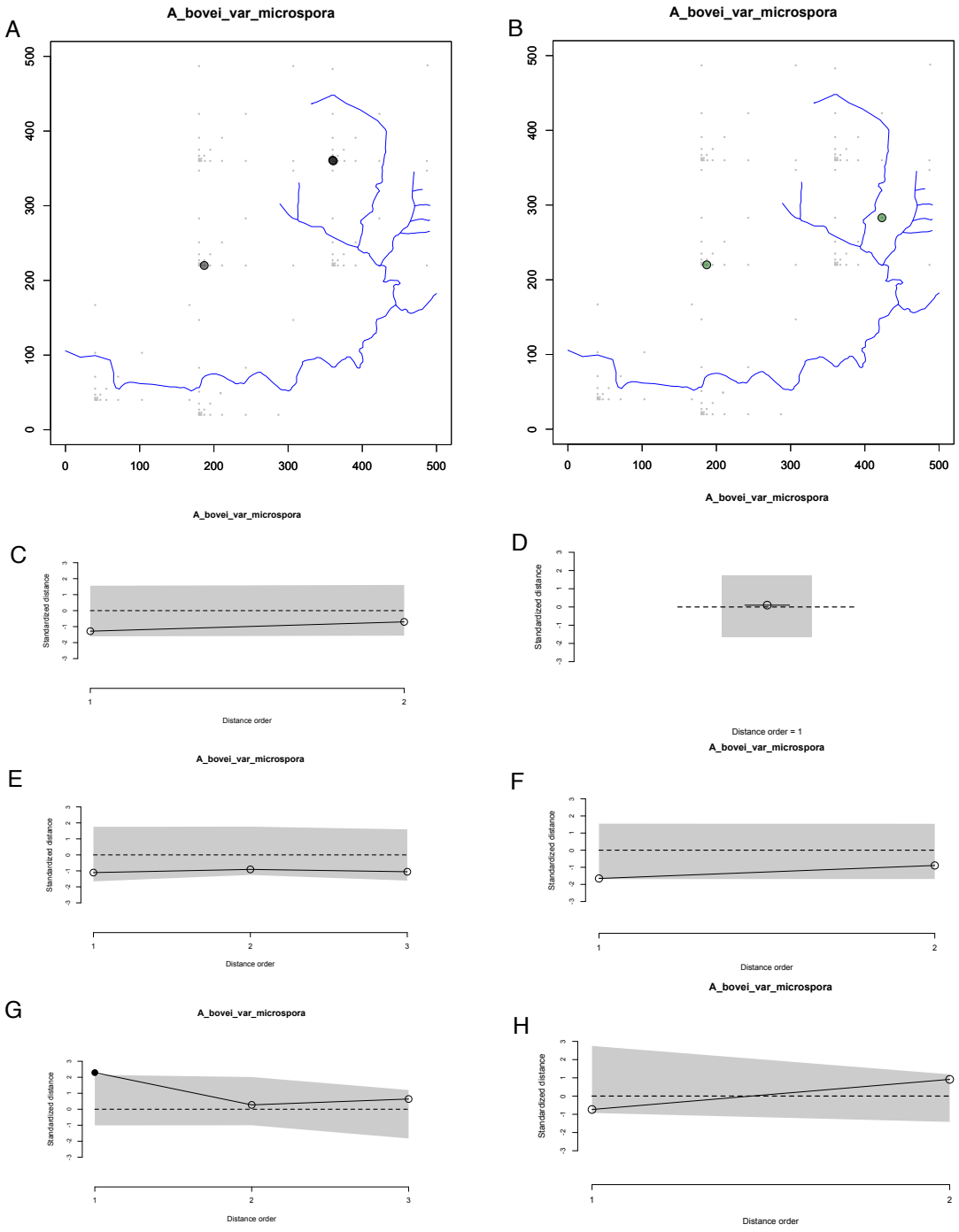


FIGURE S3: *Annulohypoxylon bovei* var. *microspora* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

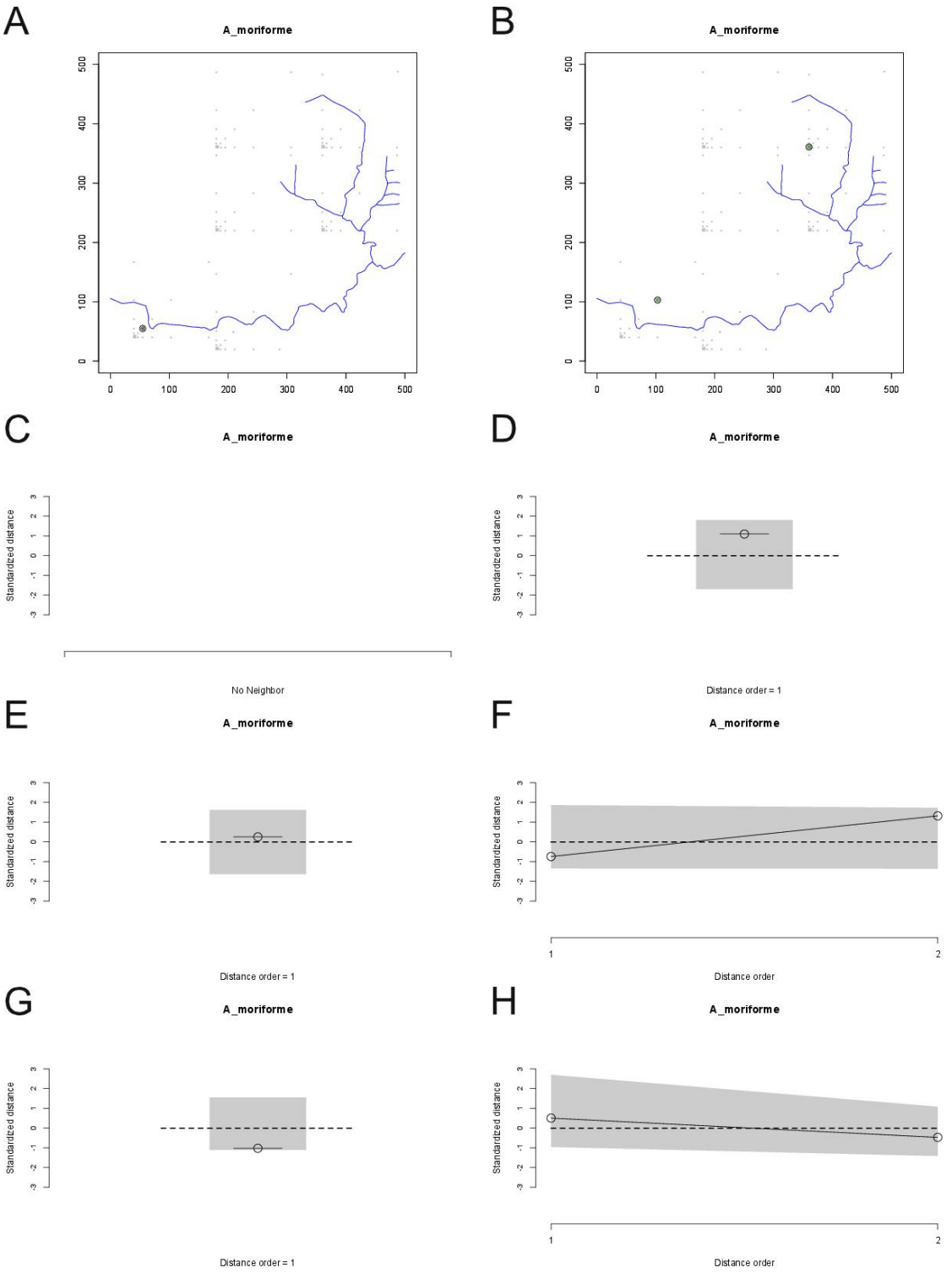


FIGURE S4: *Annulohypoxylon moriforme* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

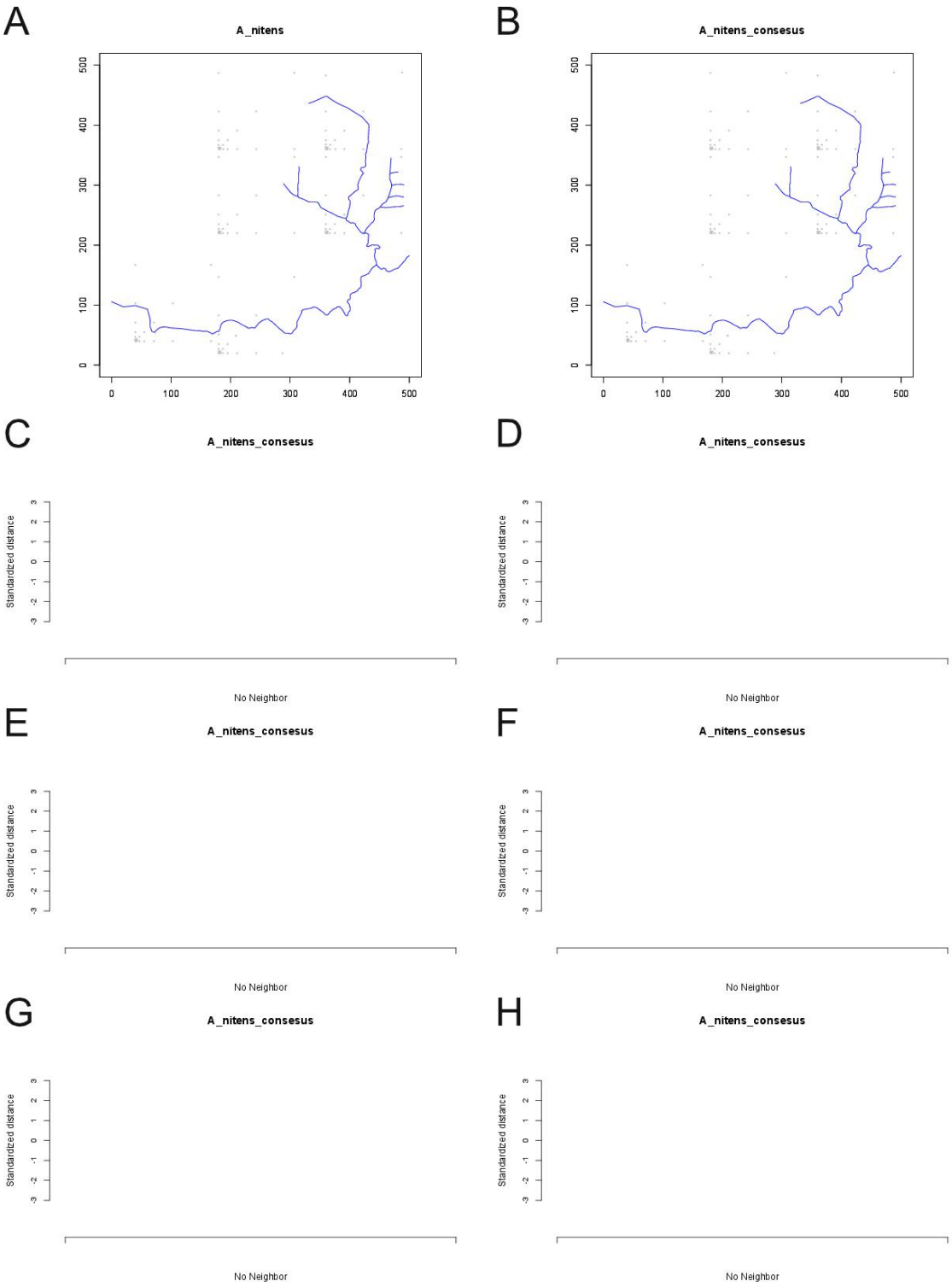


FIGURE S5: *Annulohypoxylon nitens* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

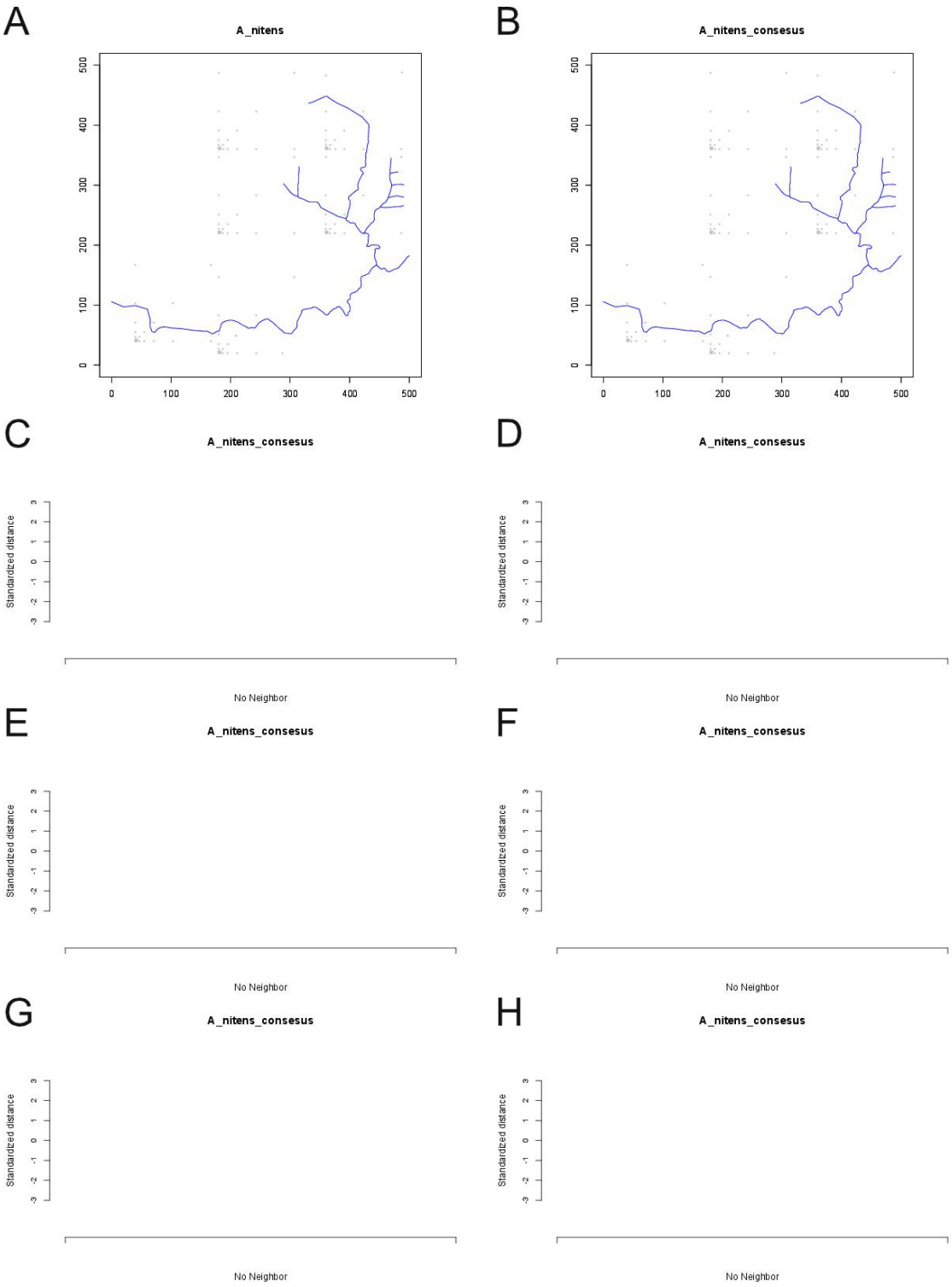


FIGURE S6: *Annulohypoxylon purpureonitens* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

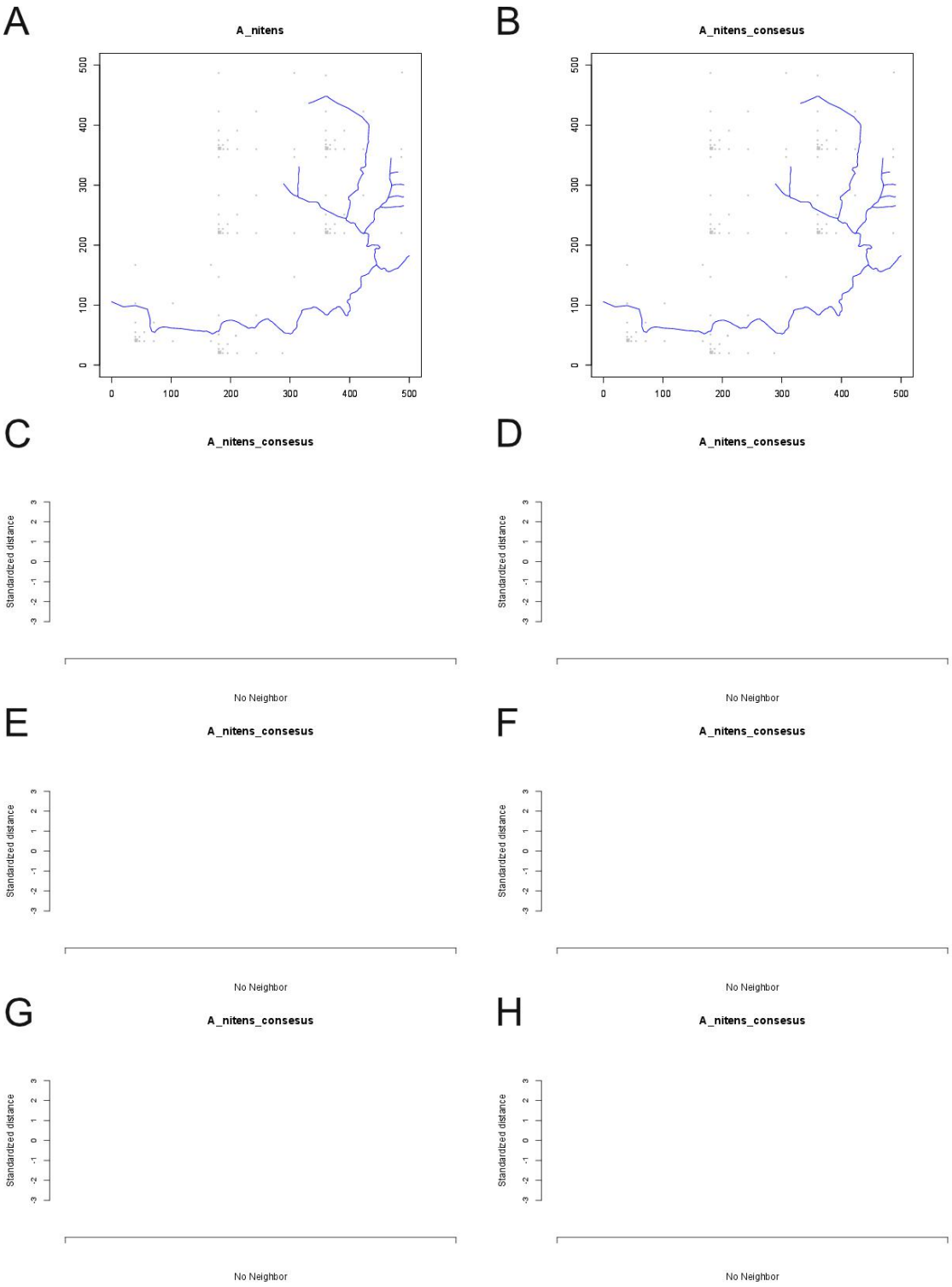


FIGURE S7: *Annulohypoxylon stygium* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

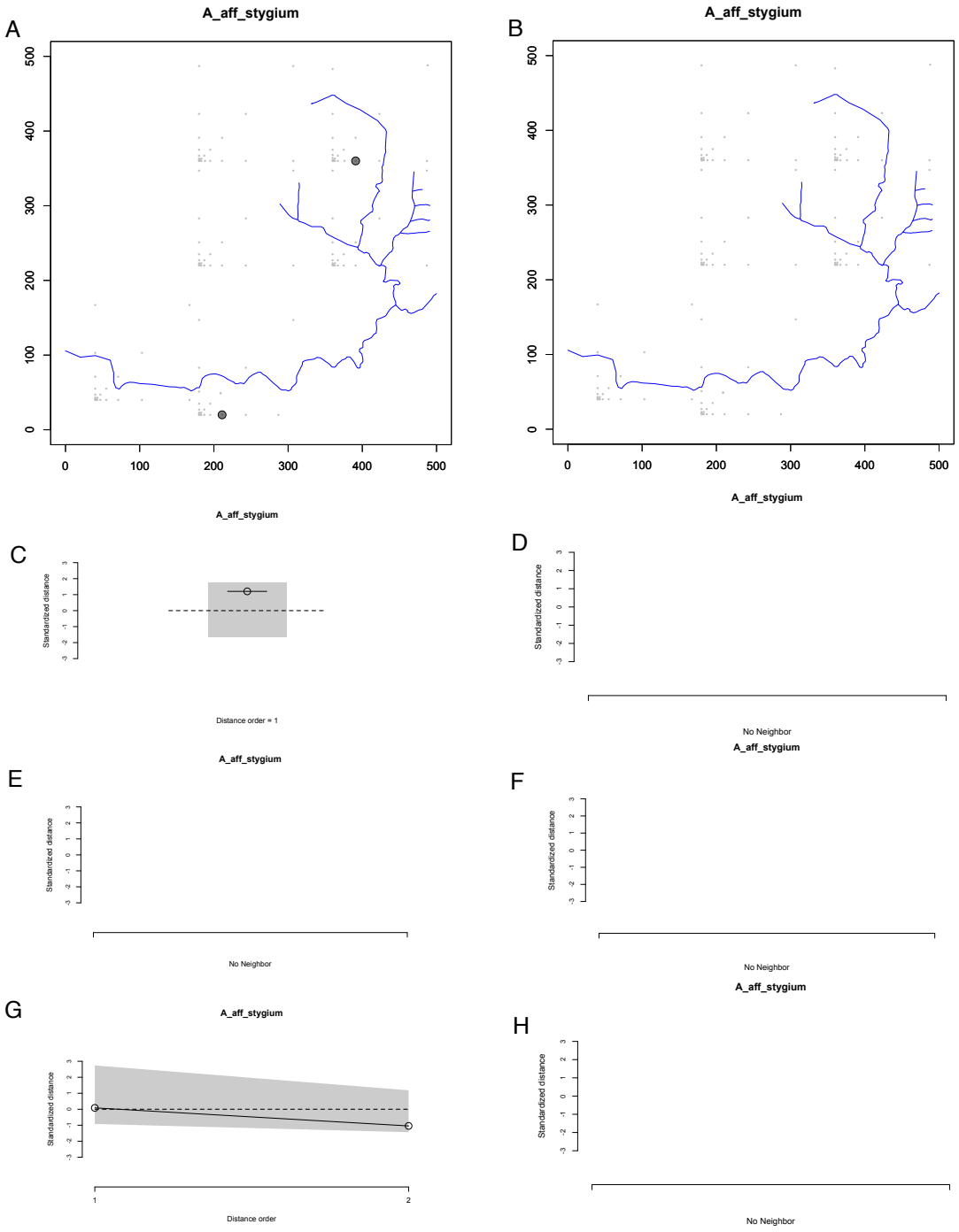


FIGURE S8: *Annulohypoxylon* aff. *stygium* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

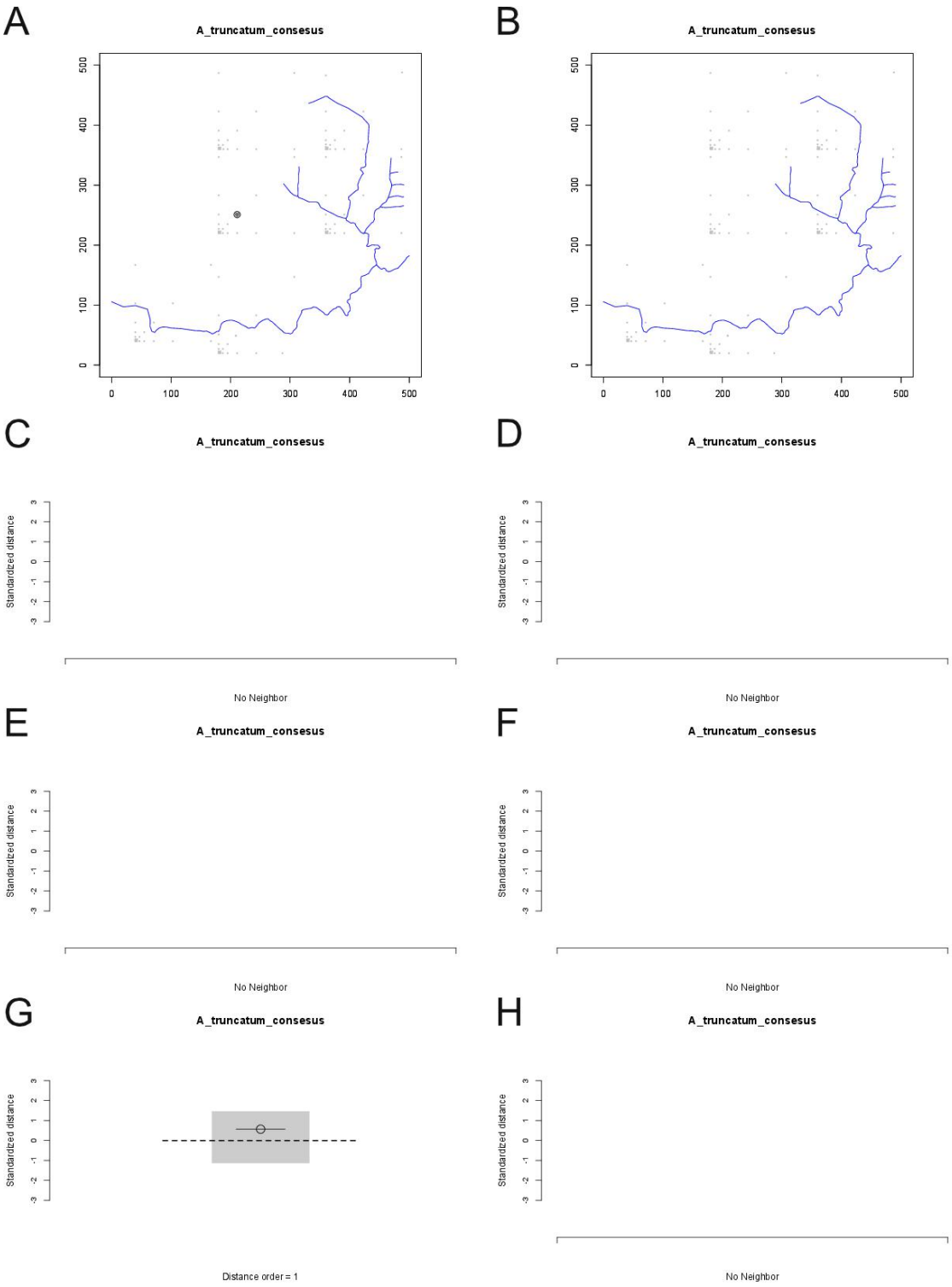


FIGURE S9: *Annulohypoxylon truncatum* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

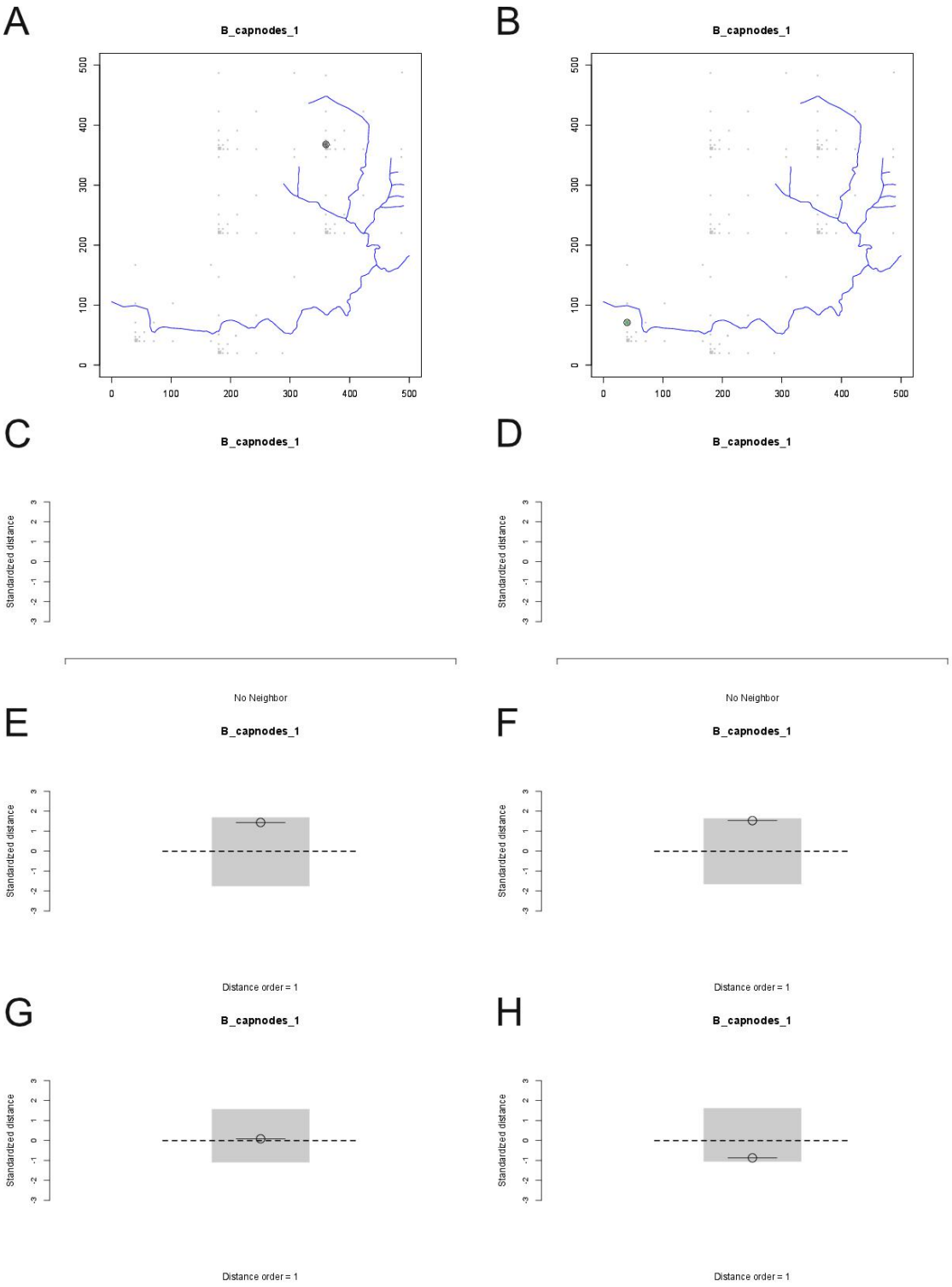


FIGURE S10: *Biscogniauxia capnodes* var. 1 – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

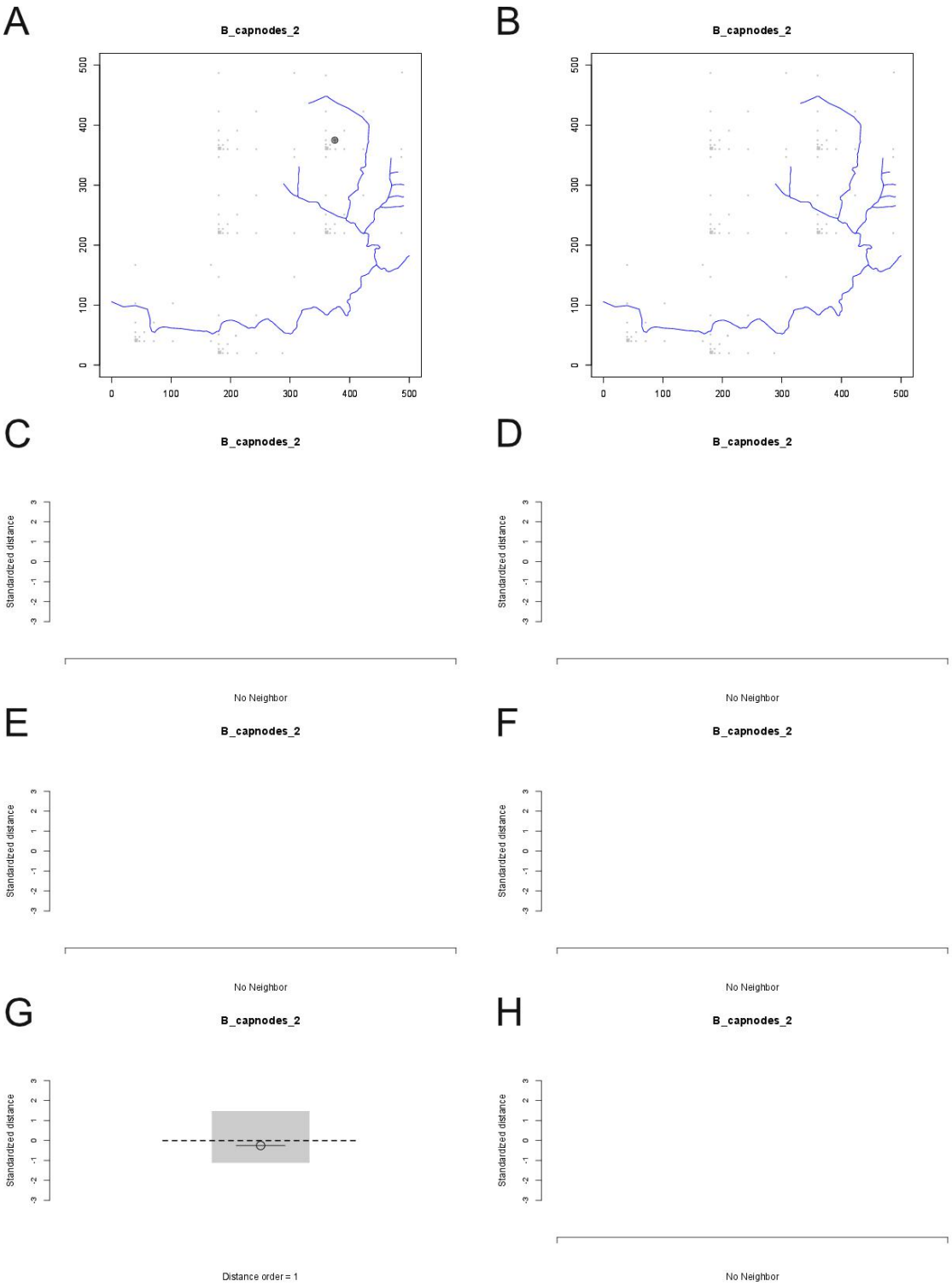


FIGURE S11: *Biscogniauxia capnodes* var. 1 – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

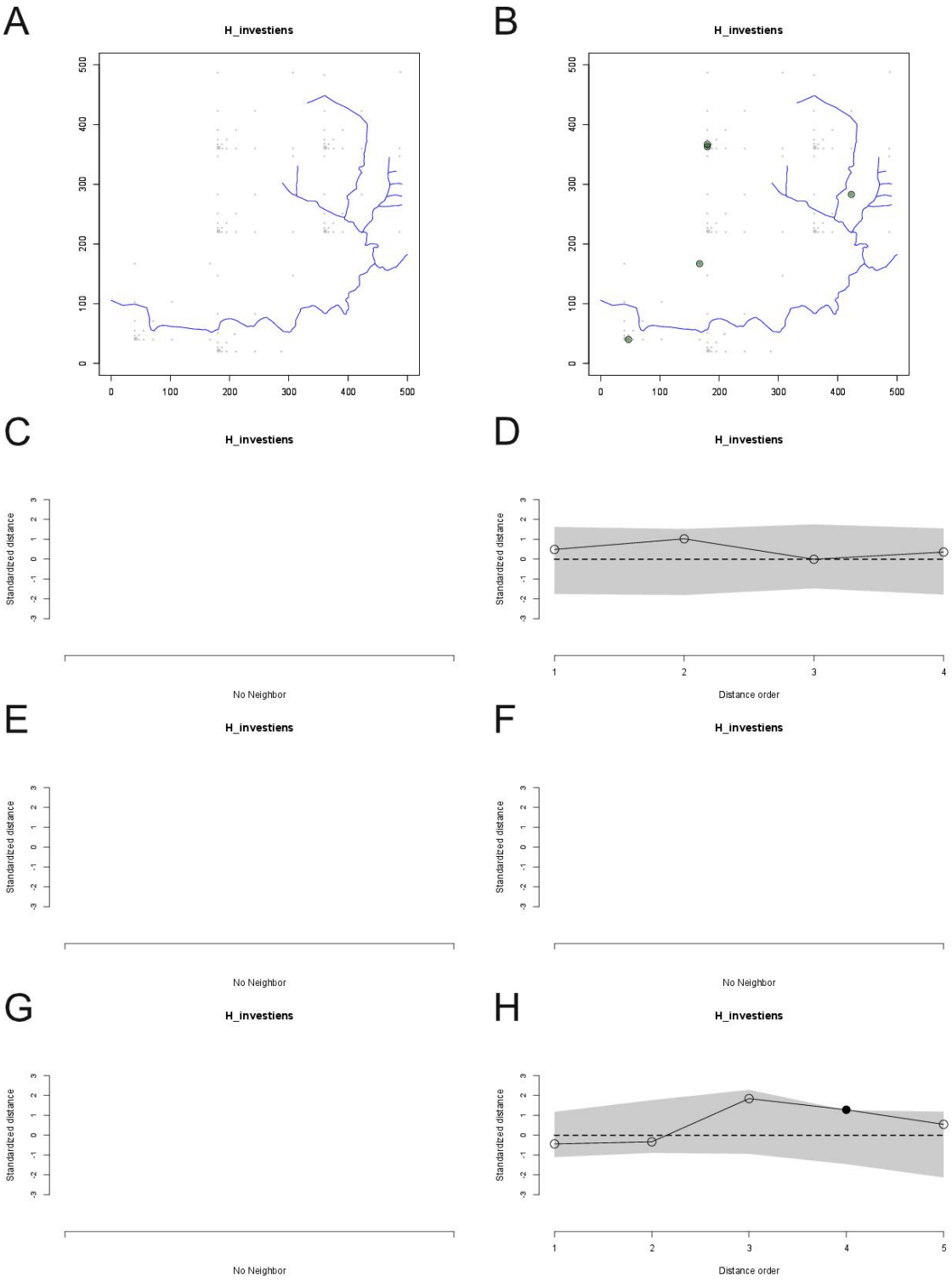


FIGURE S12: *Hypoxylon investens* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

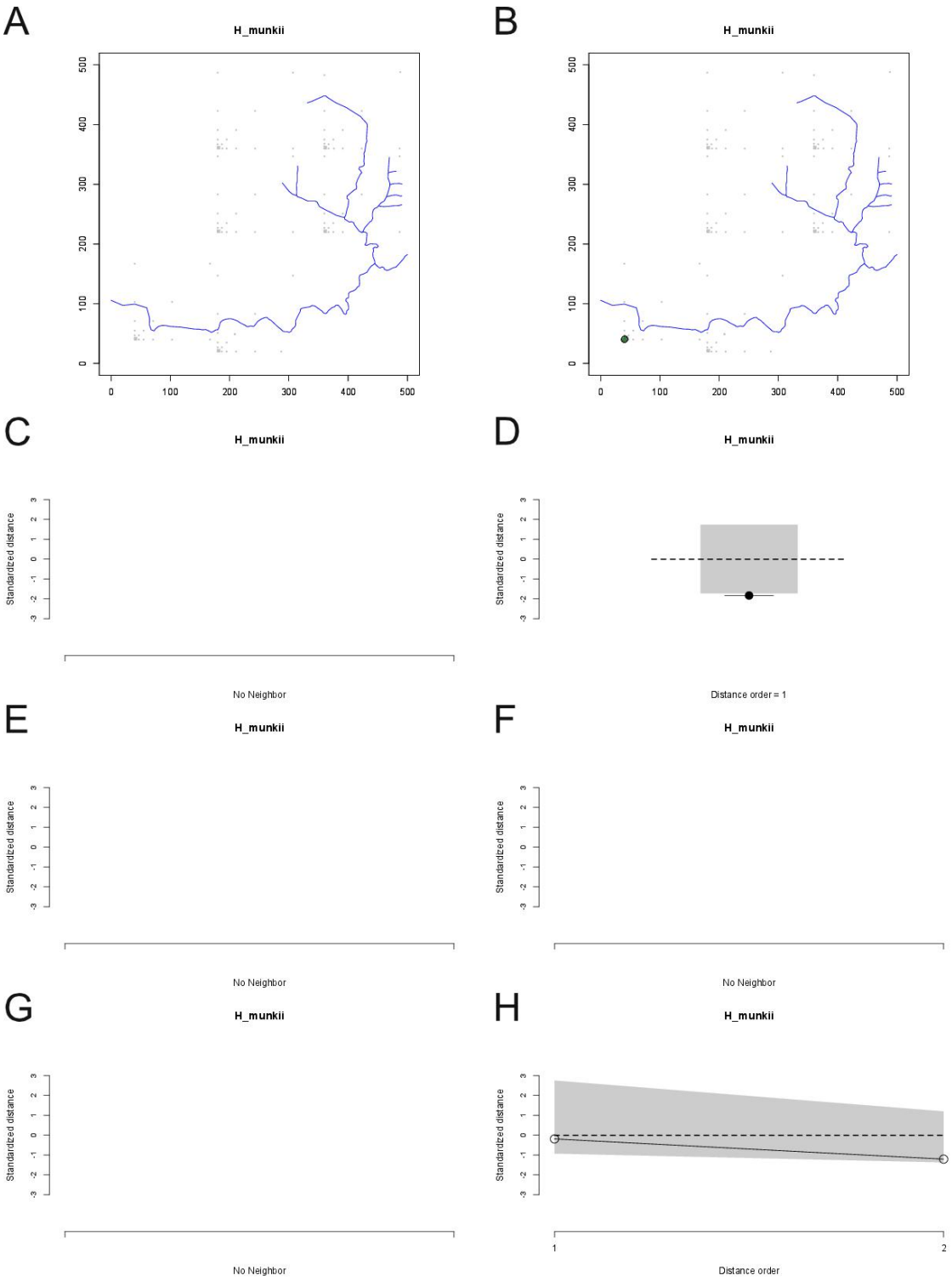


FIGURE S13: *Hypoxylon munkii* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

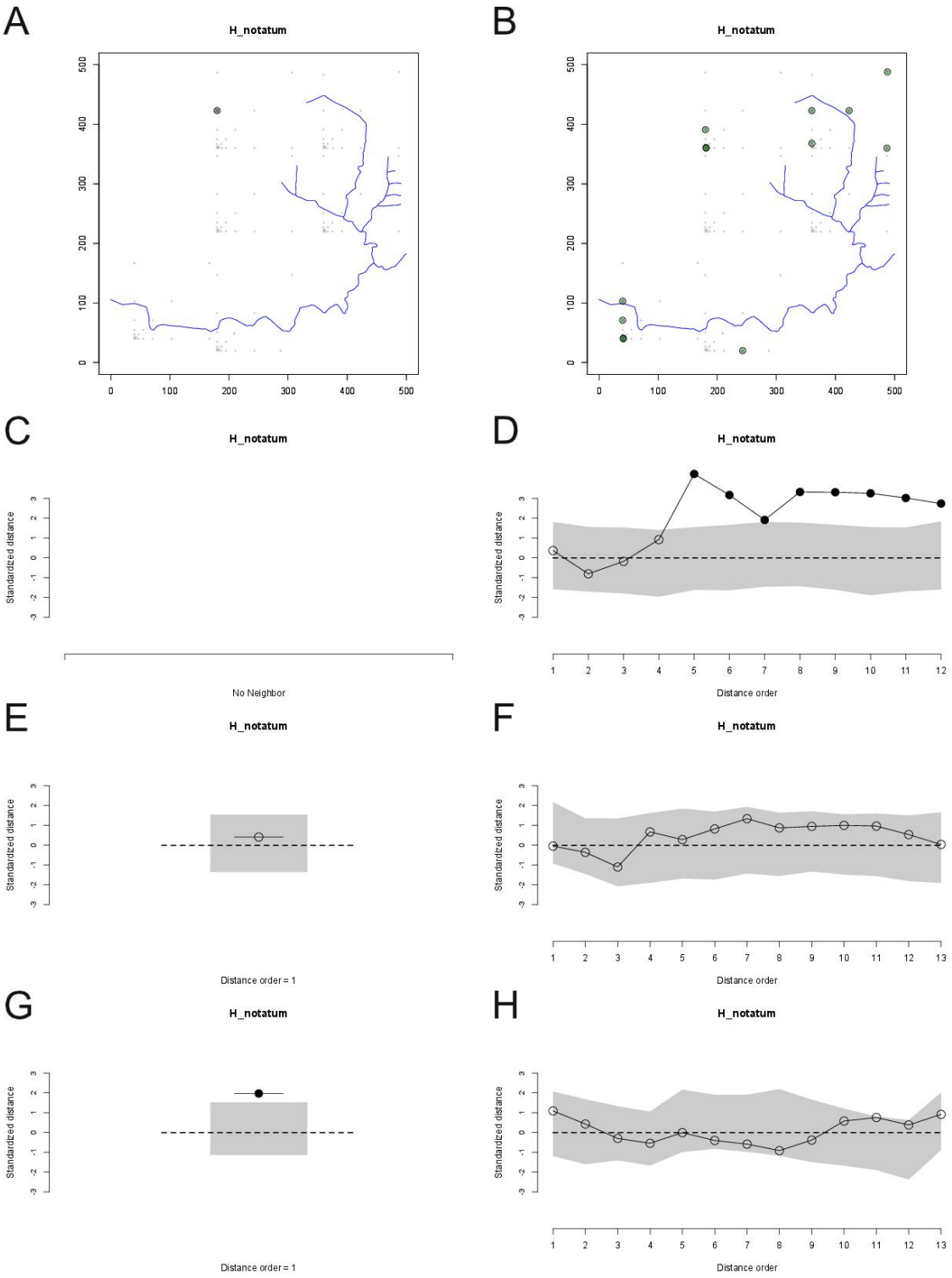


FIGURE S14: *Hypoxylon notatum* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

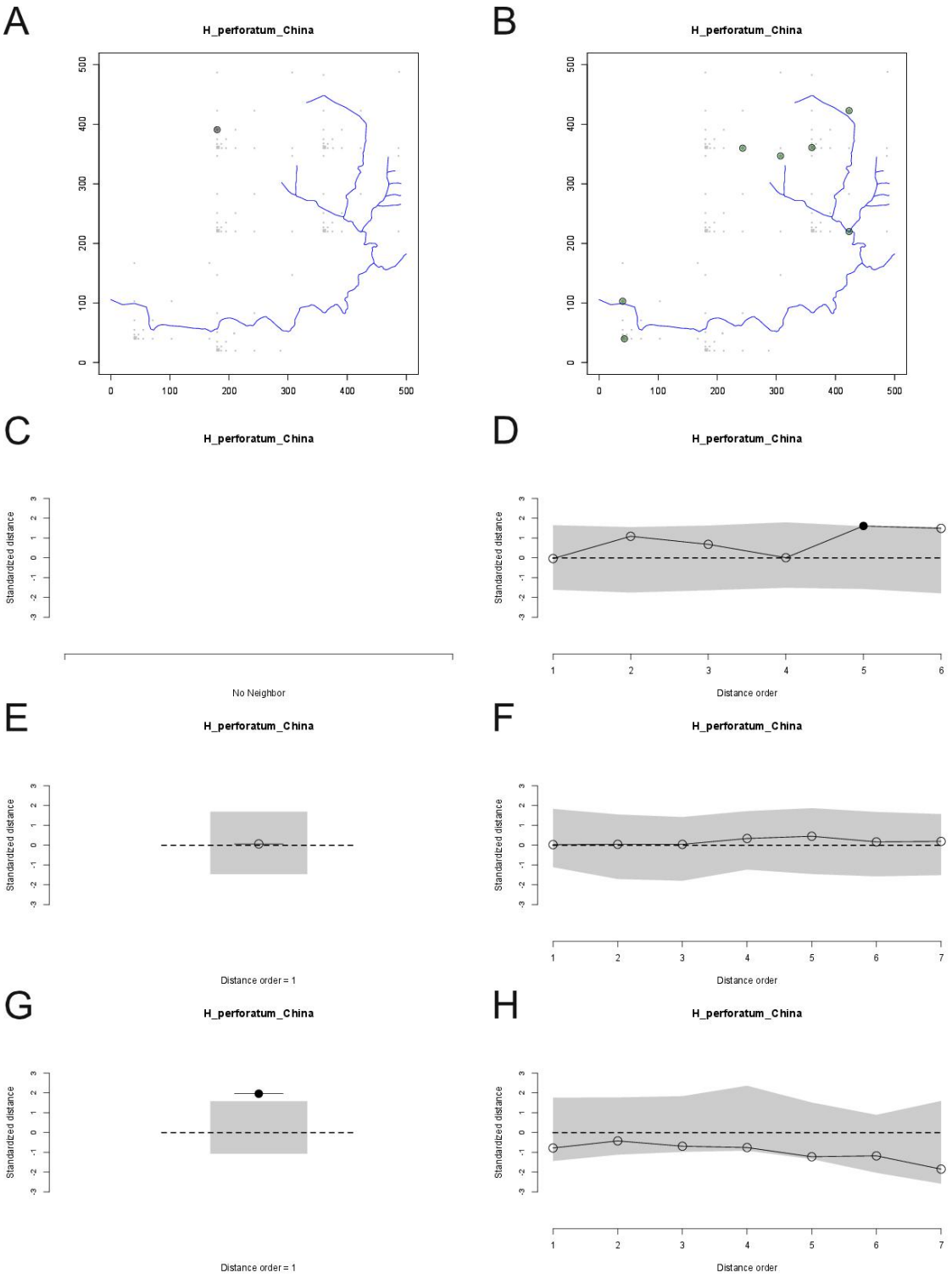


FIGURE S15: *Hypoxylon perforatum* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

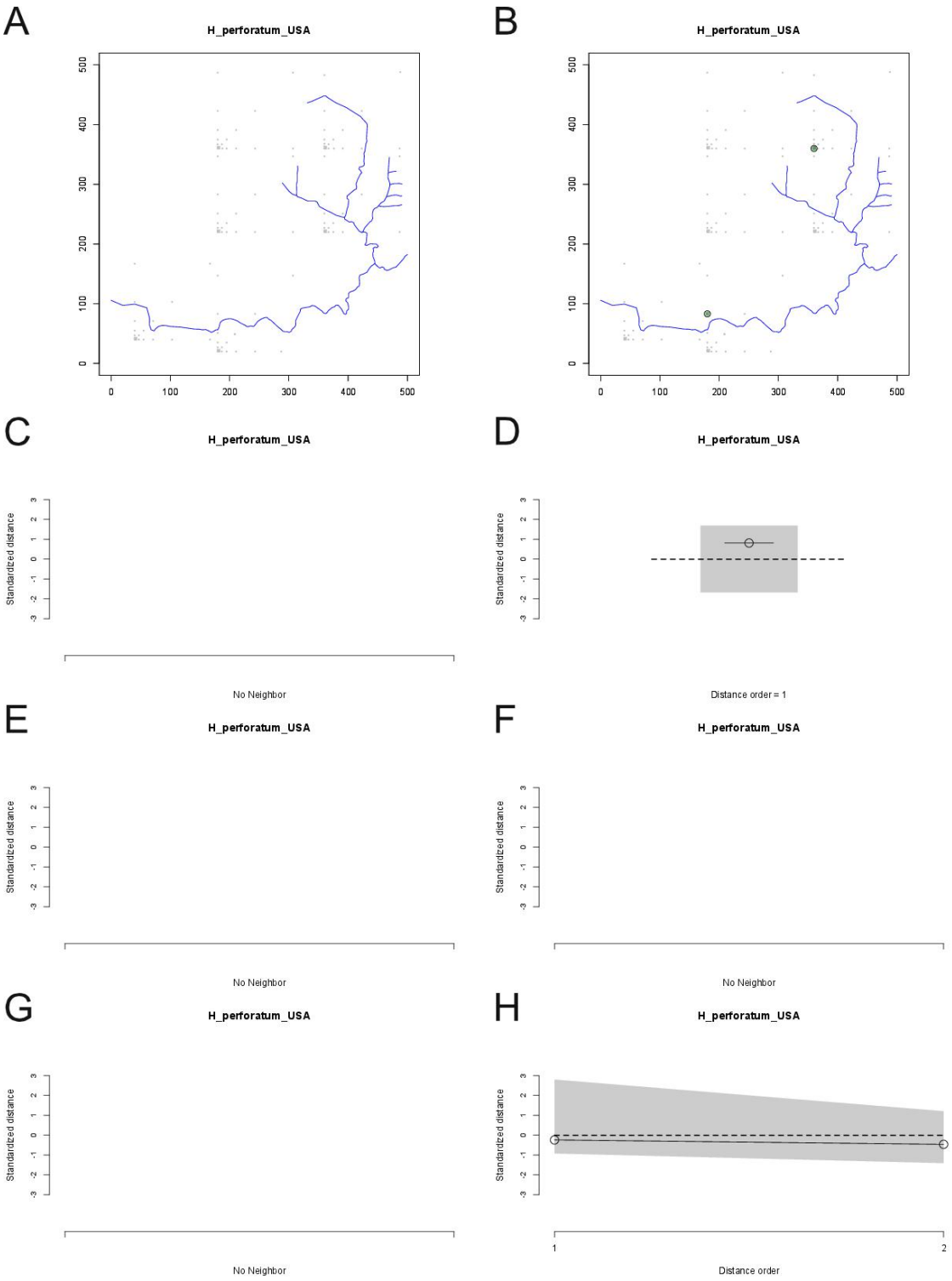


FIGURE S16: *Hypoxylon perforatum* (USA sequence variant) – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

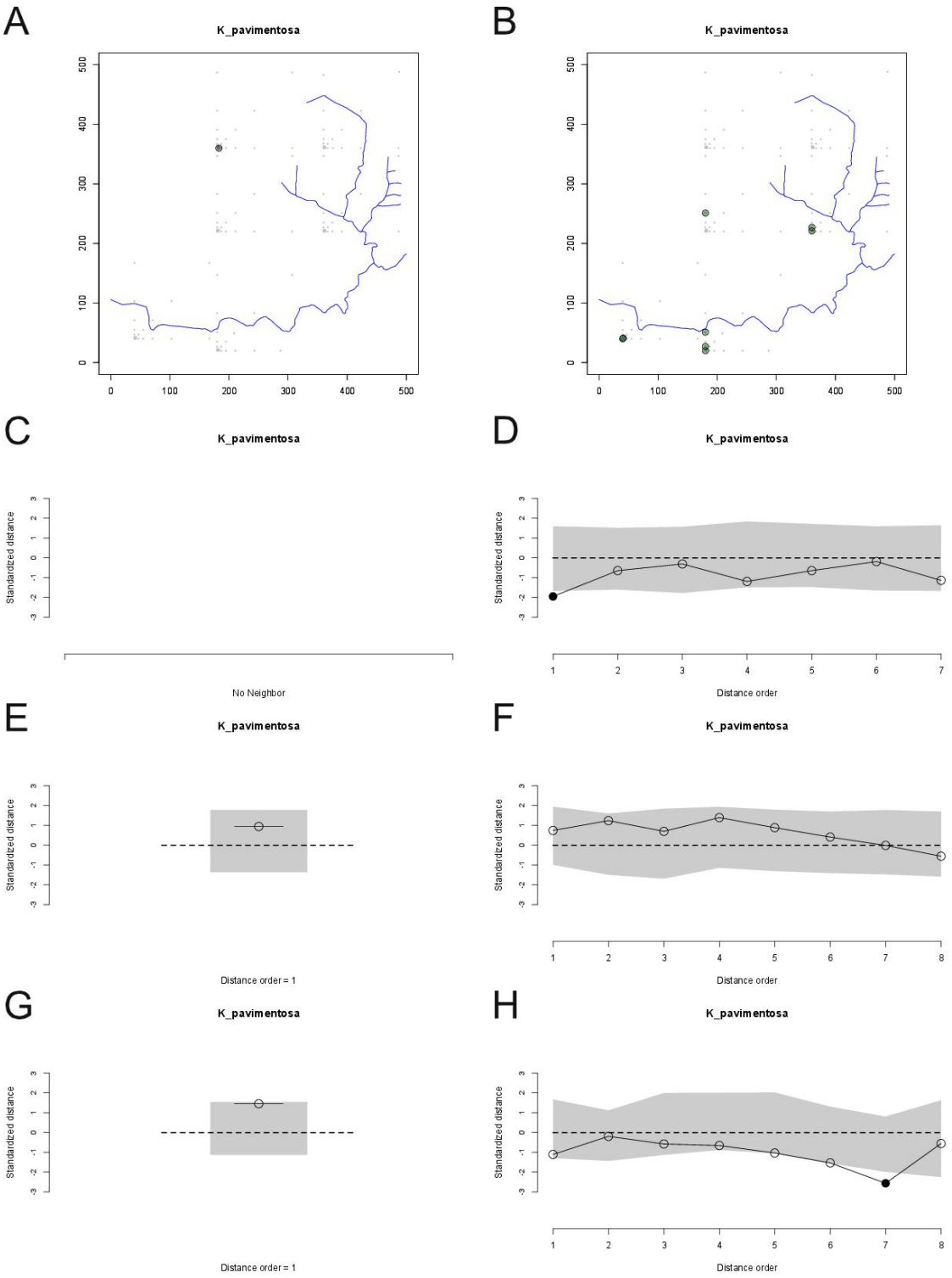


FIGURE S17: *Kretzschmaria pavimentosa* var. 1 – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

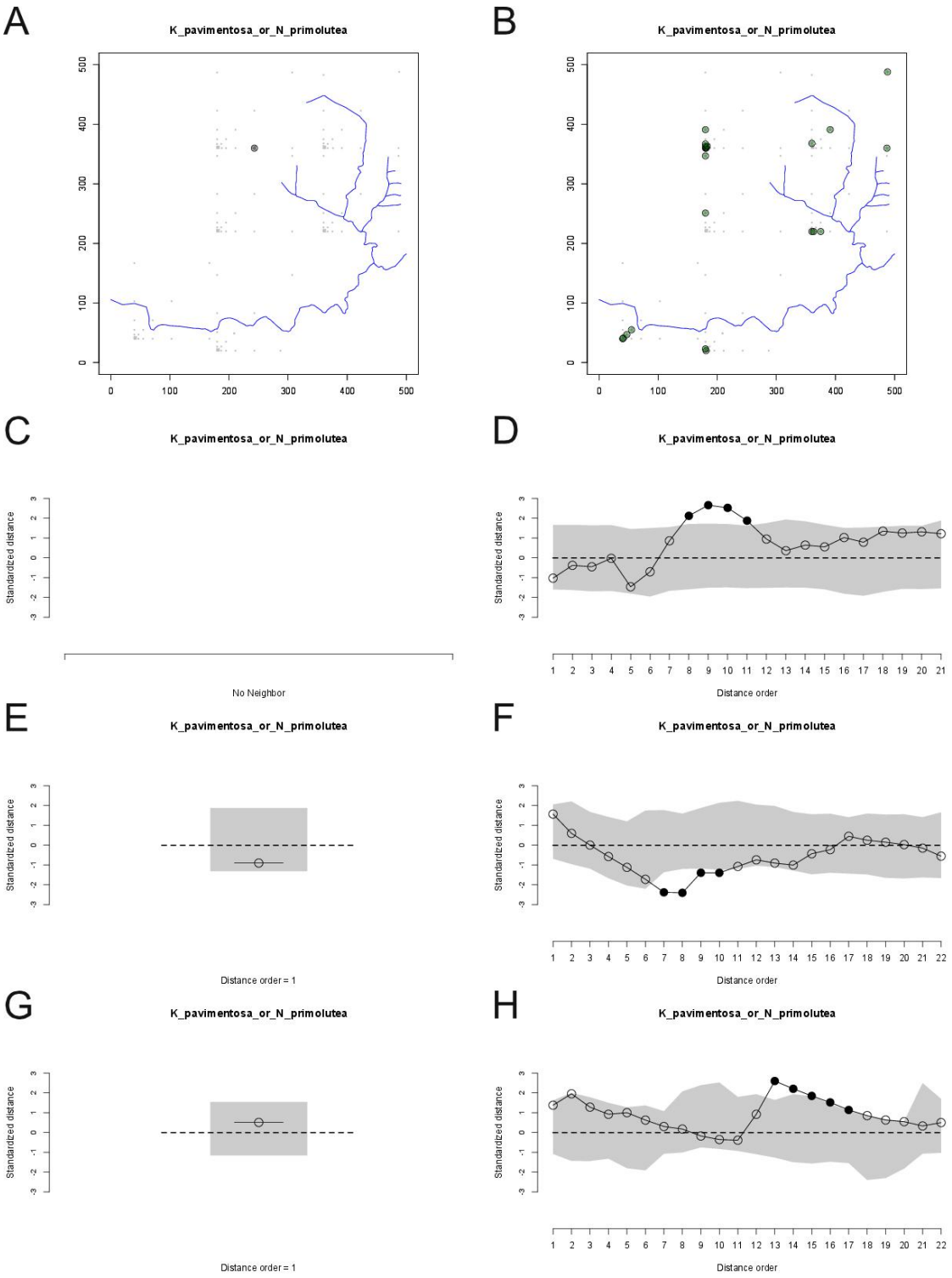


FIGURE S18: *Kretzschmaria pavimentosa* var. 2– Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

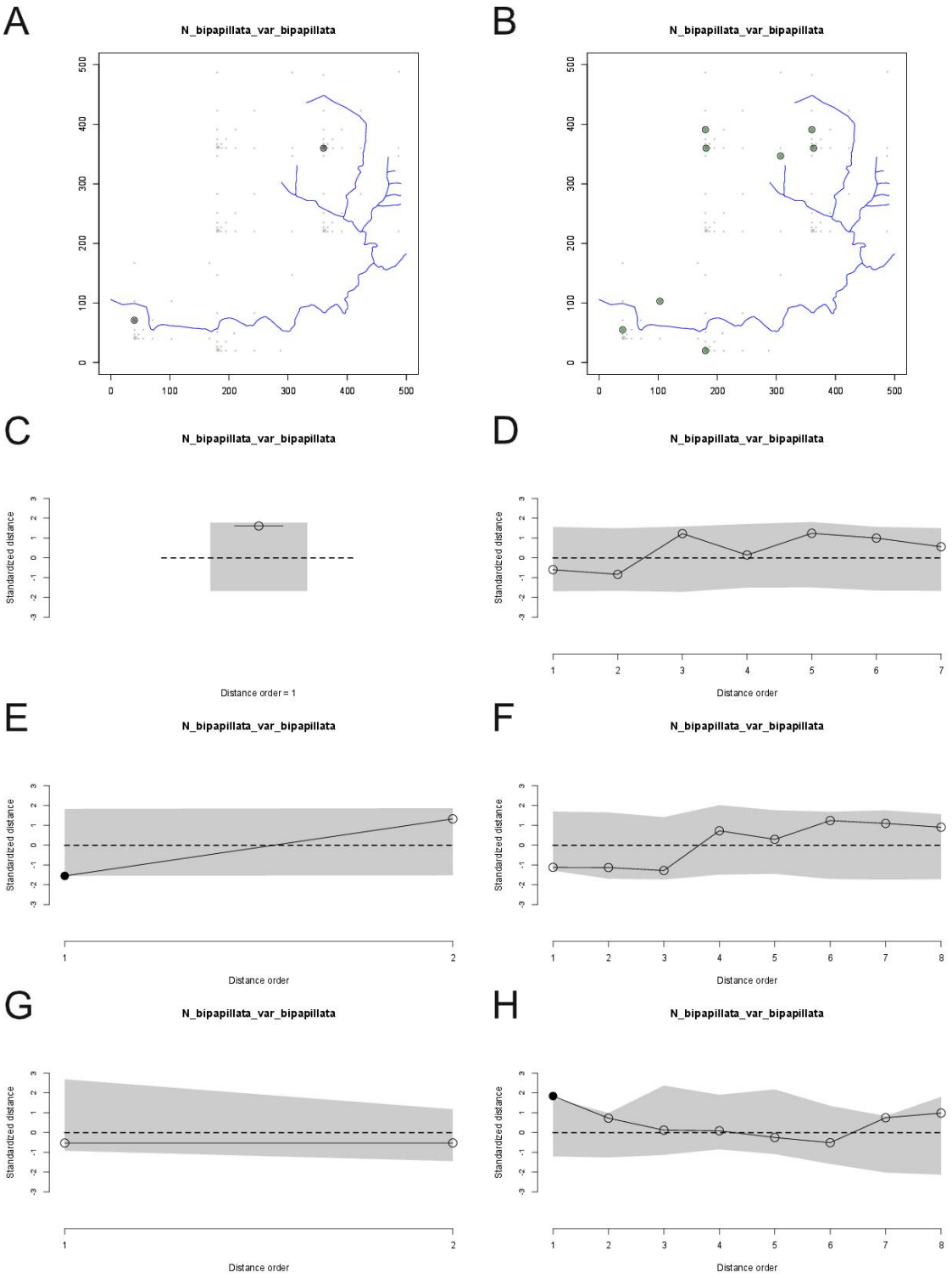


FIGURE S19: *Nemania bipapillata* var. 1 – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

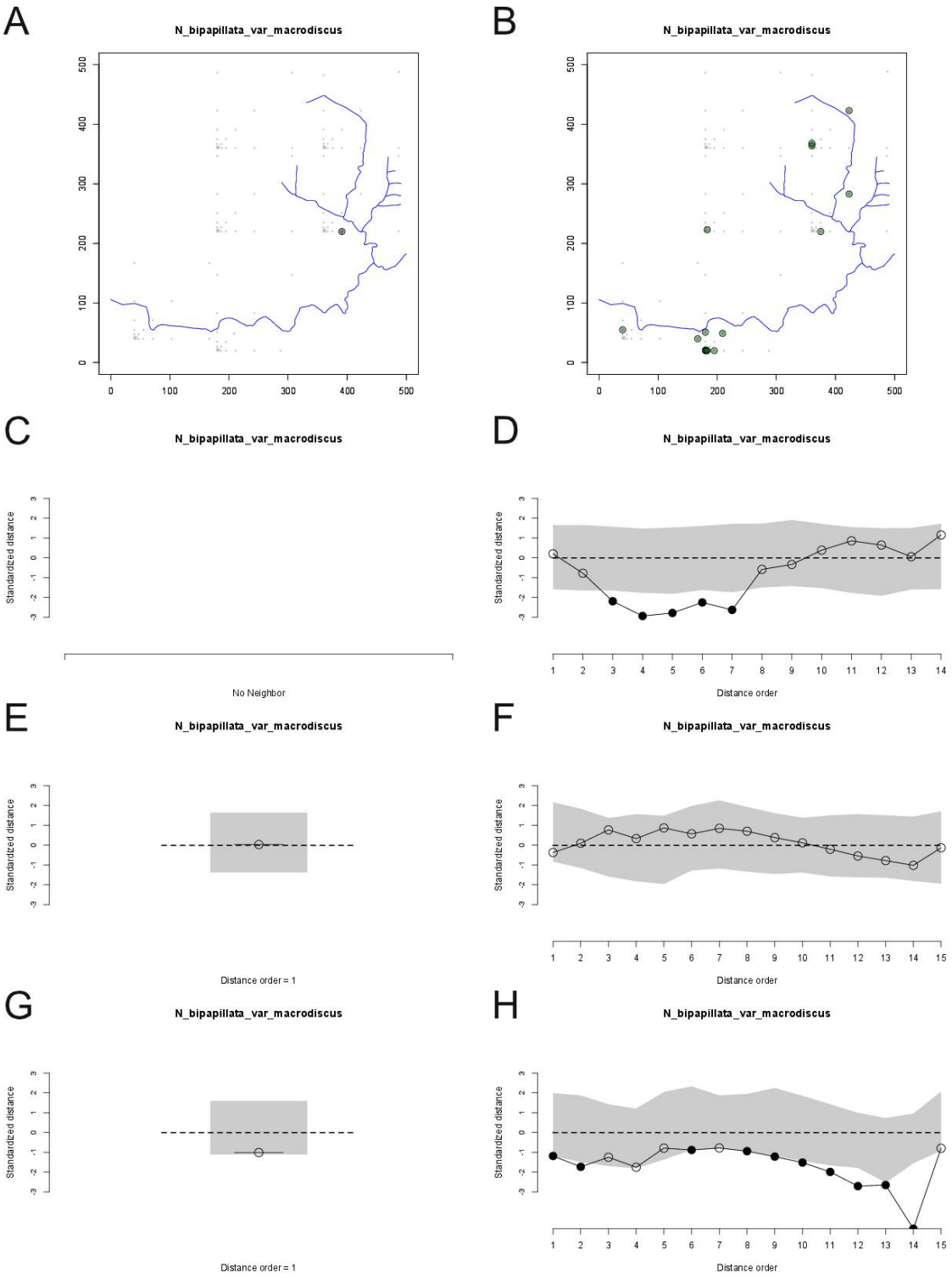


FIGURE S20: *Nemania bipapillata* var. 2 – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

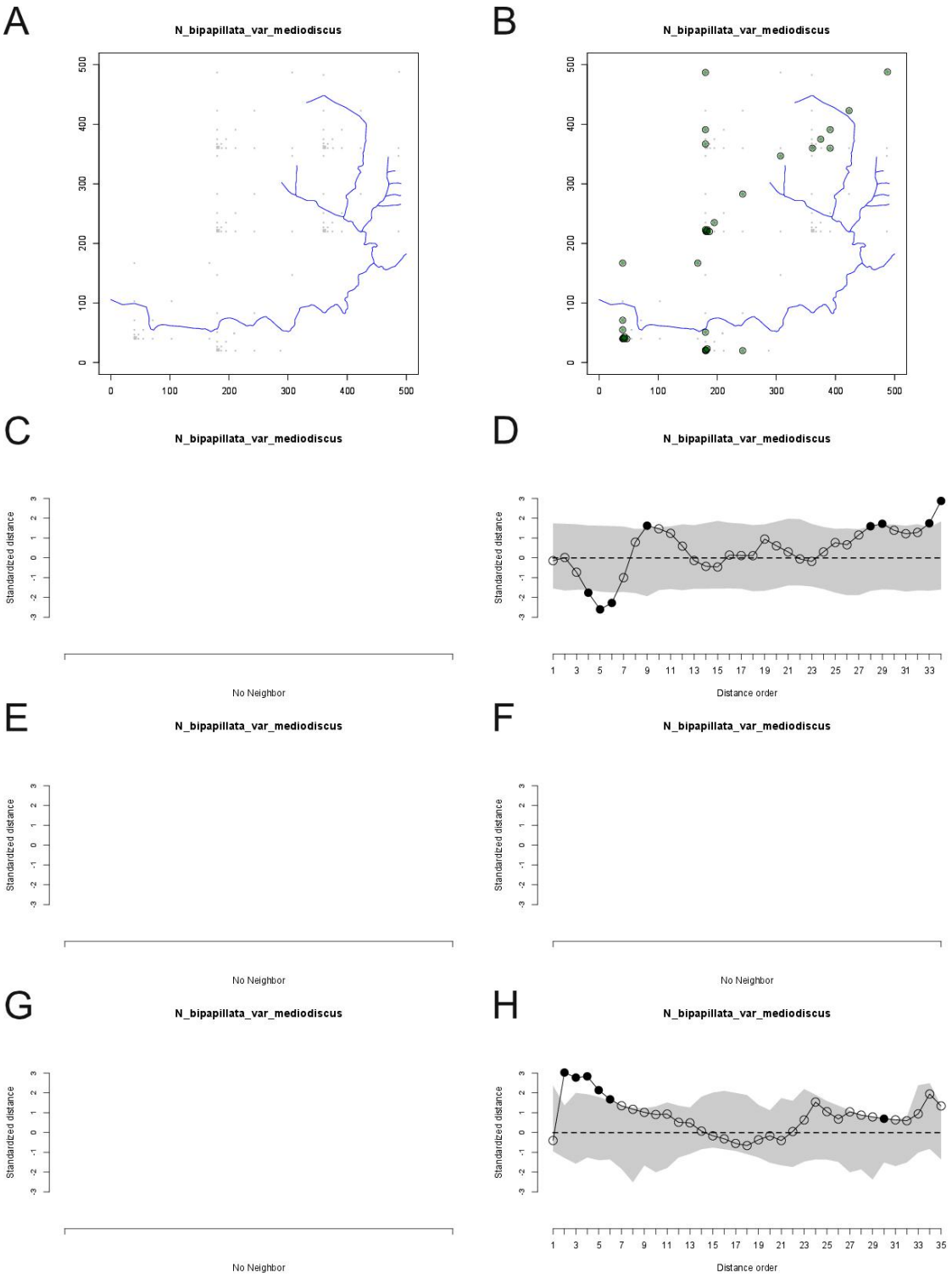


FIGURE S21: *Nemania bipapillata* var. 3– Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

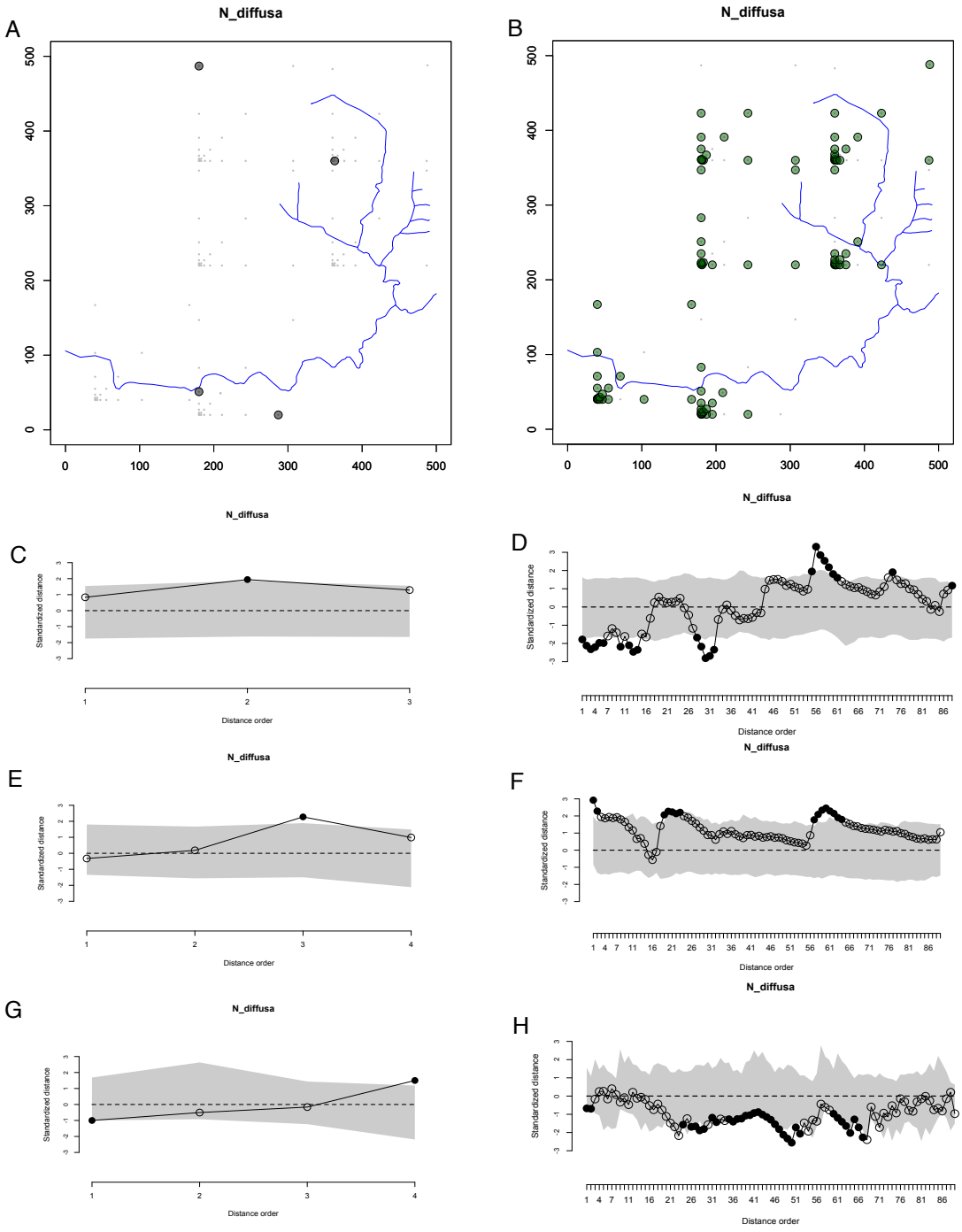


FIGURE S22: *Nemania diffusa* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

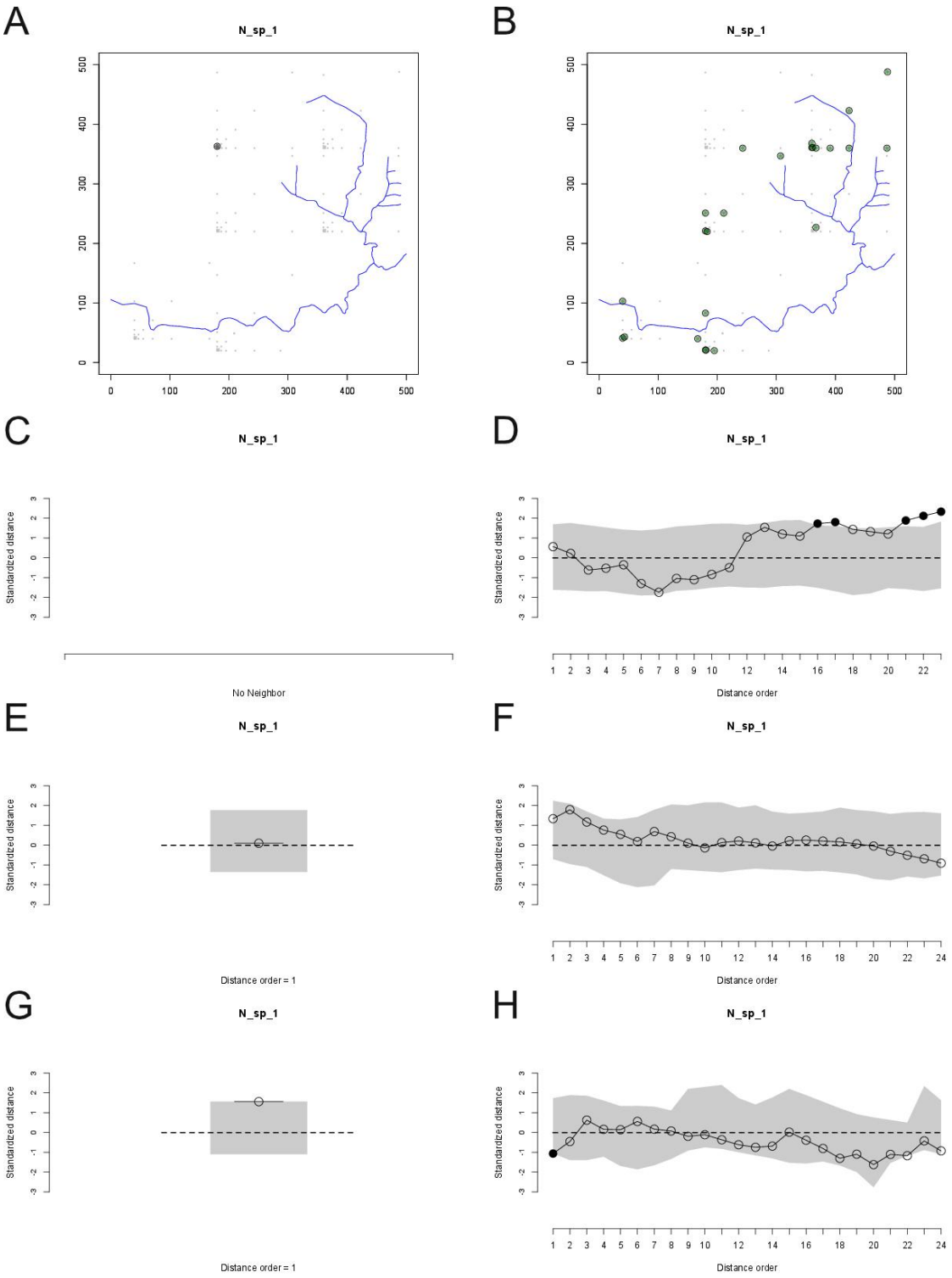


FIGURE S23: *Nemania* sp. 1 – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

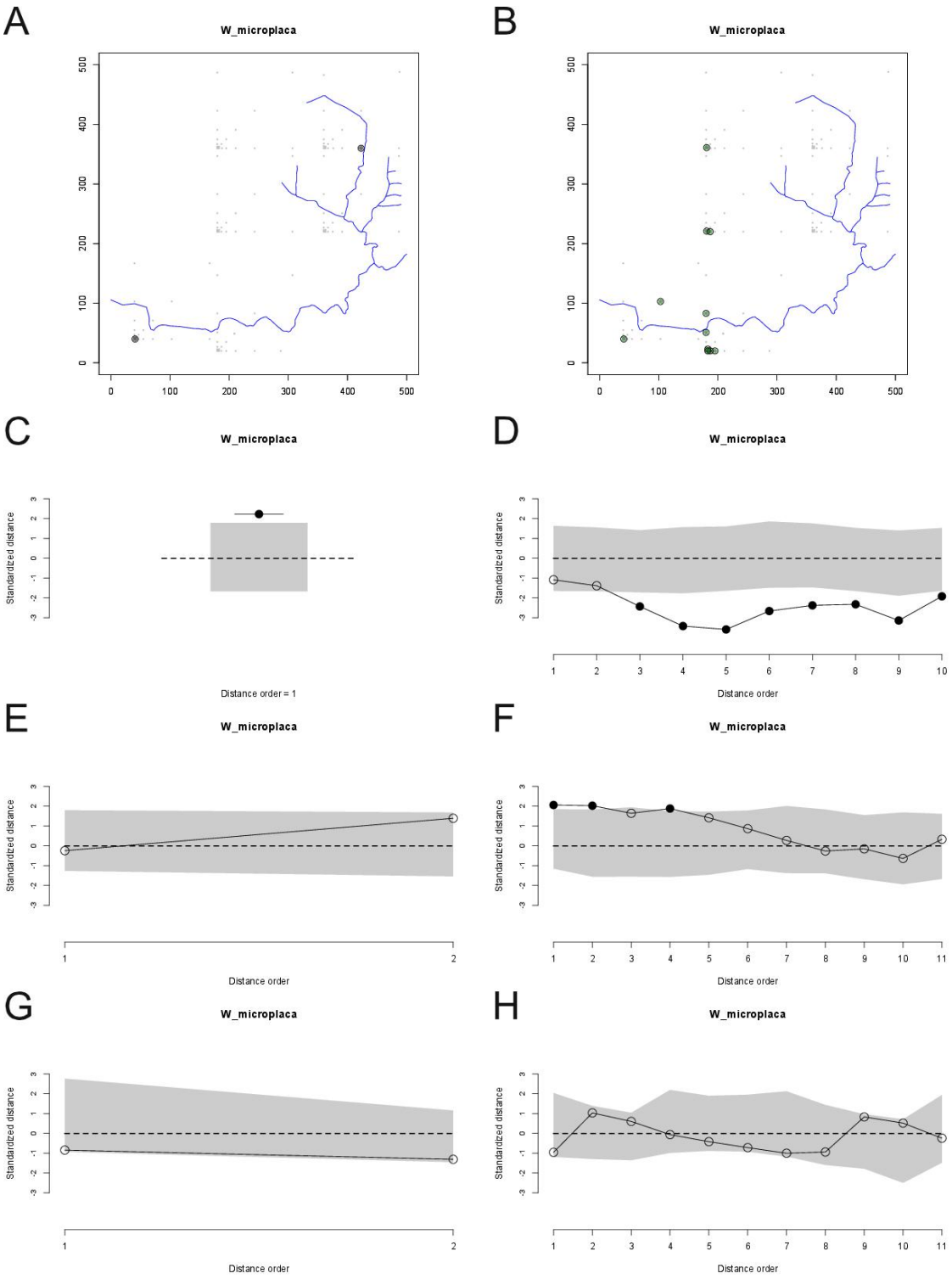


FIGURE S24: *Whalleya microplaca* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

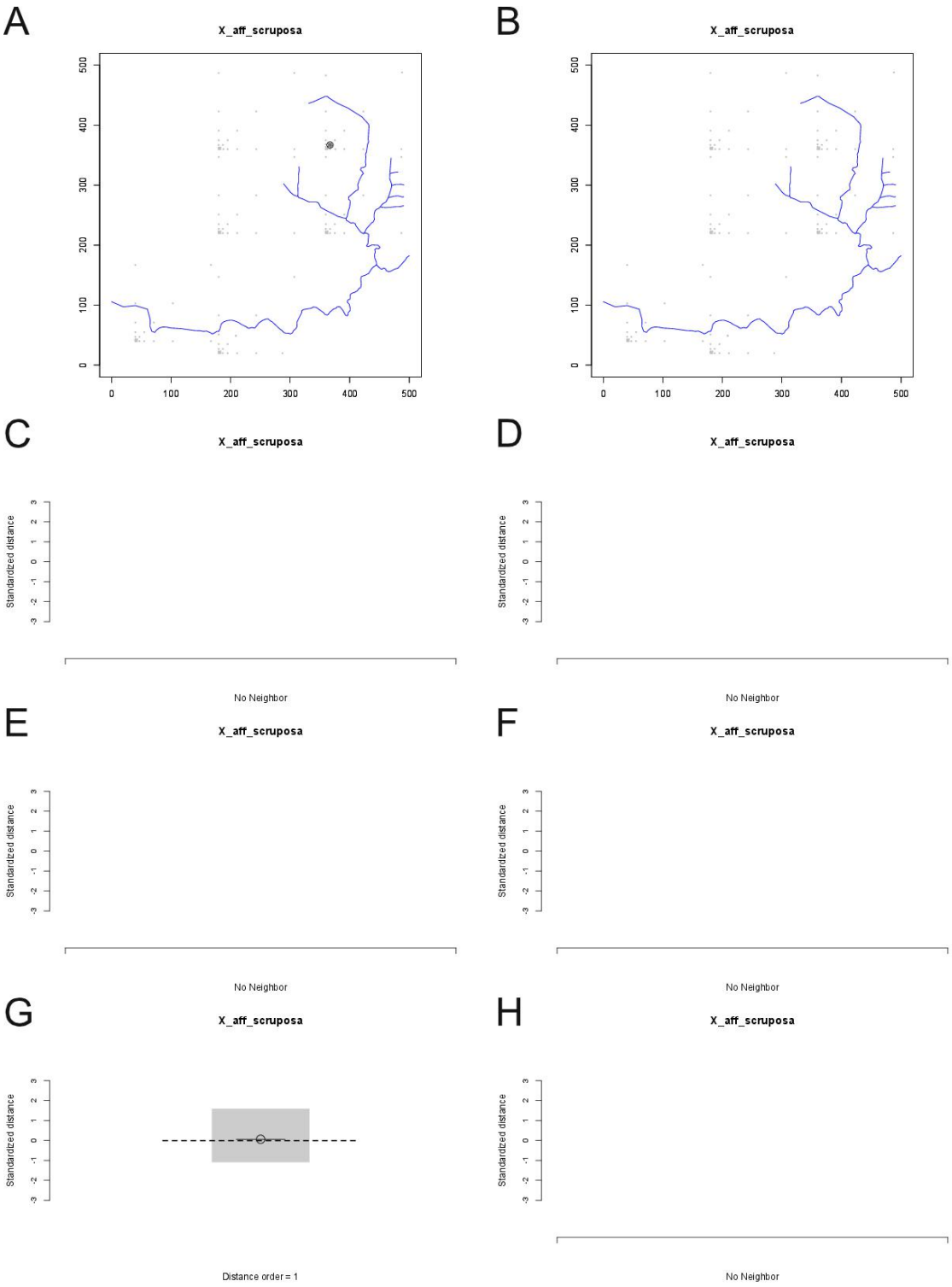


FIGURE S25: *Xylaria* aff. *scruposa* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

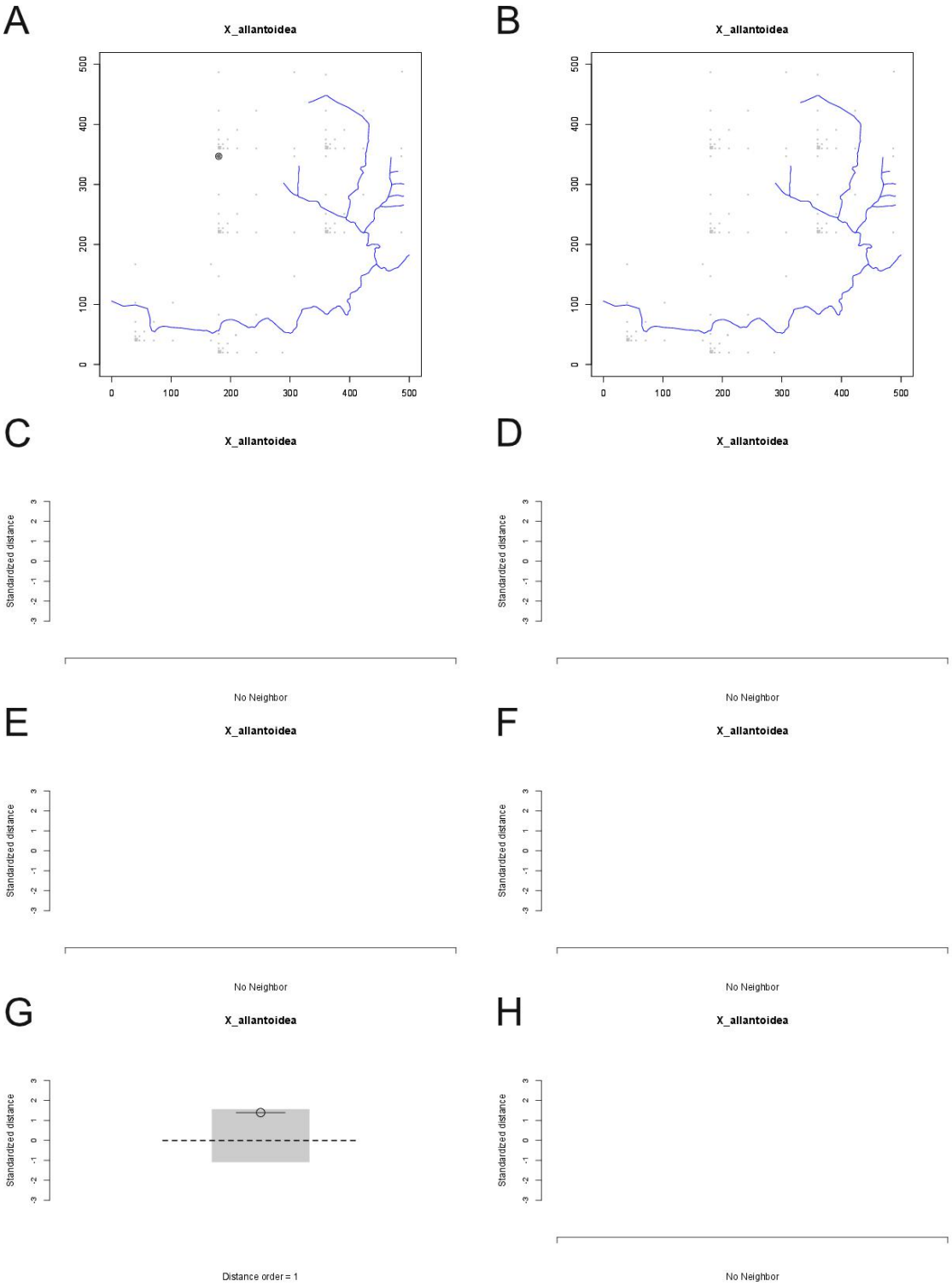


FIGURE S26: *Xylaria allantoidea* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

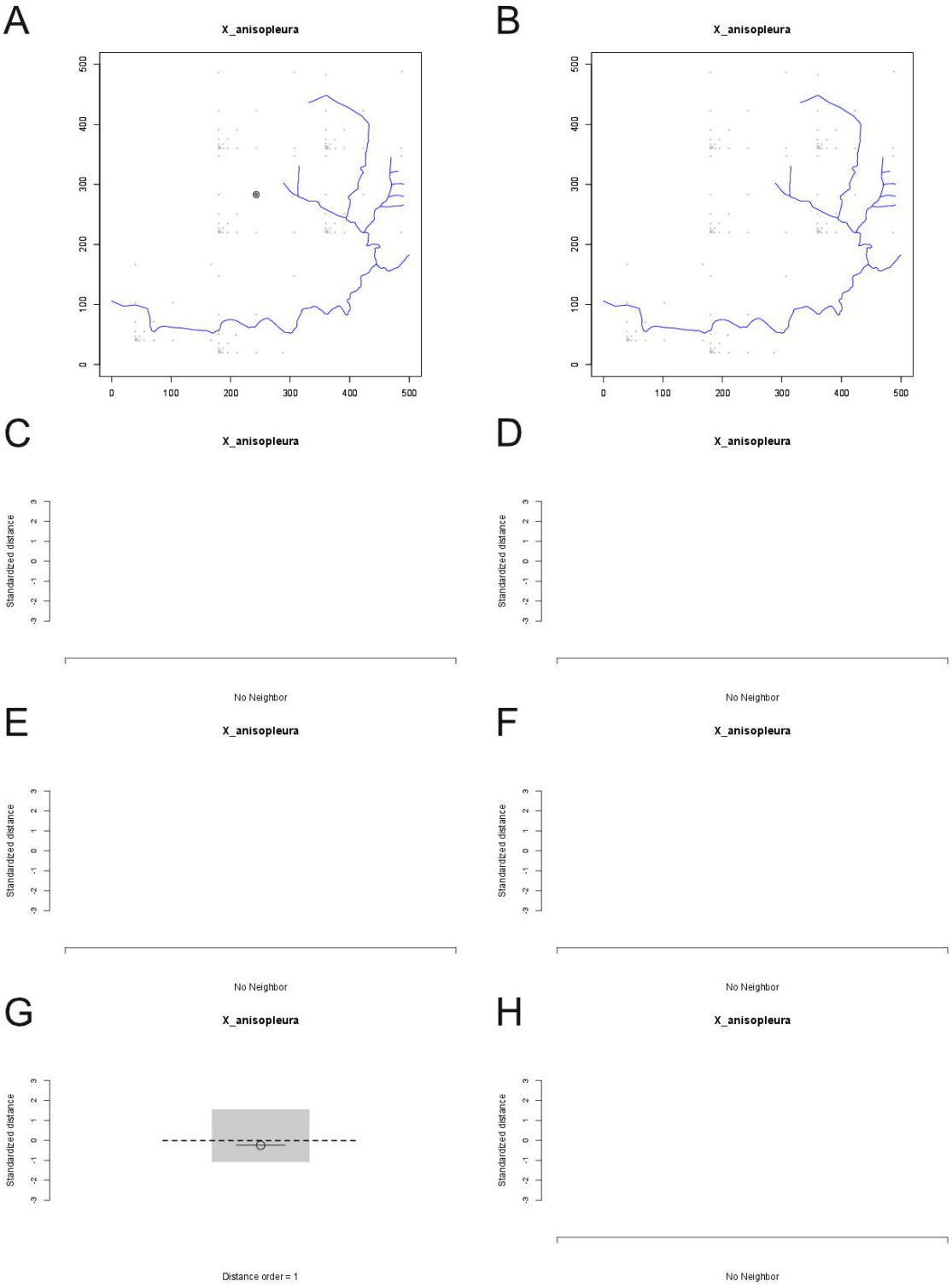


FIGURE S27: *Xylaria anisopleura* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

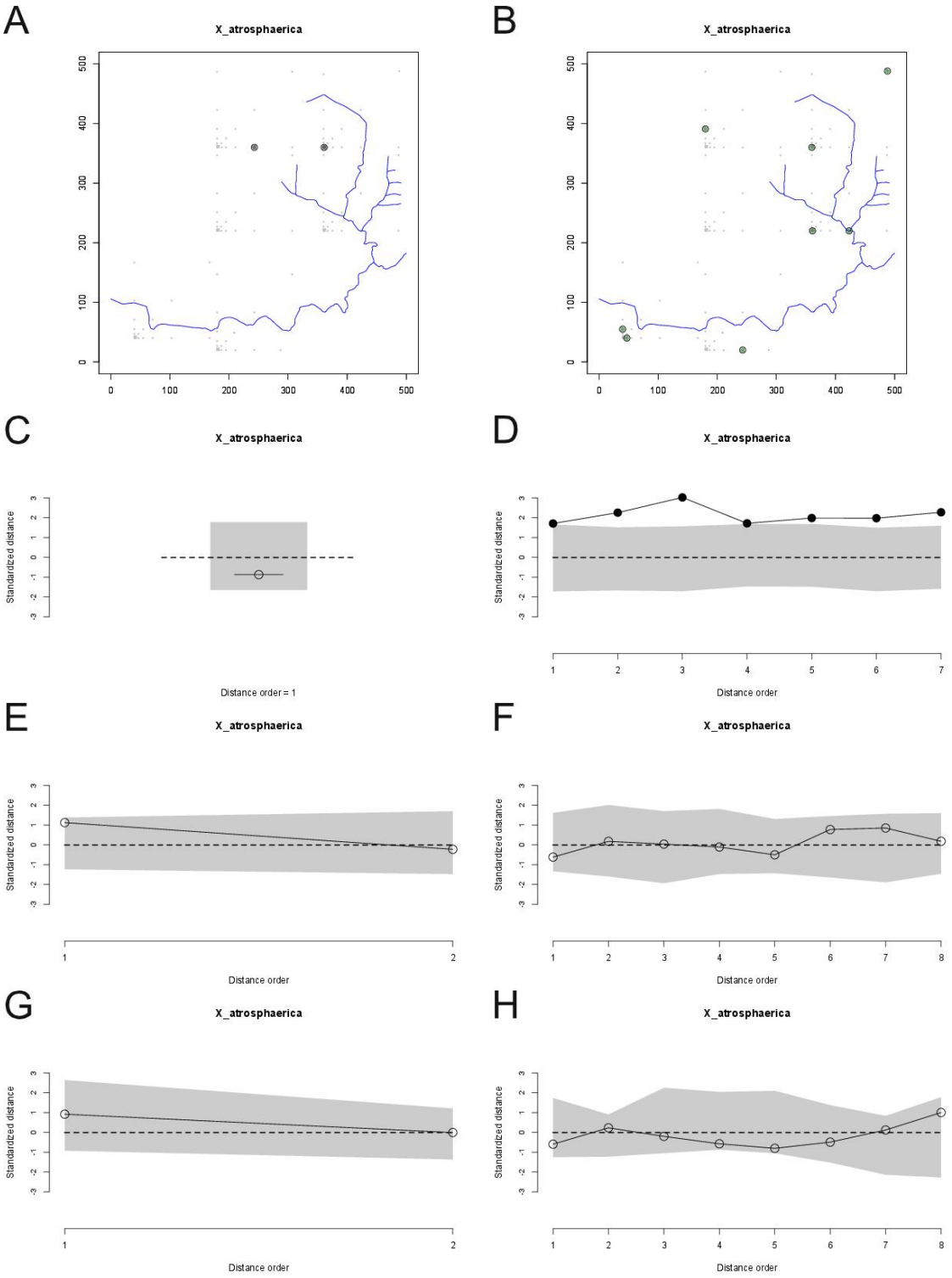


FIGURE S28: *Xylaria atrosphaerica* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

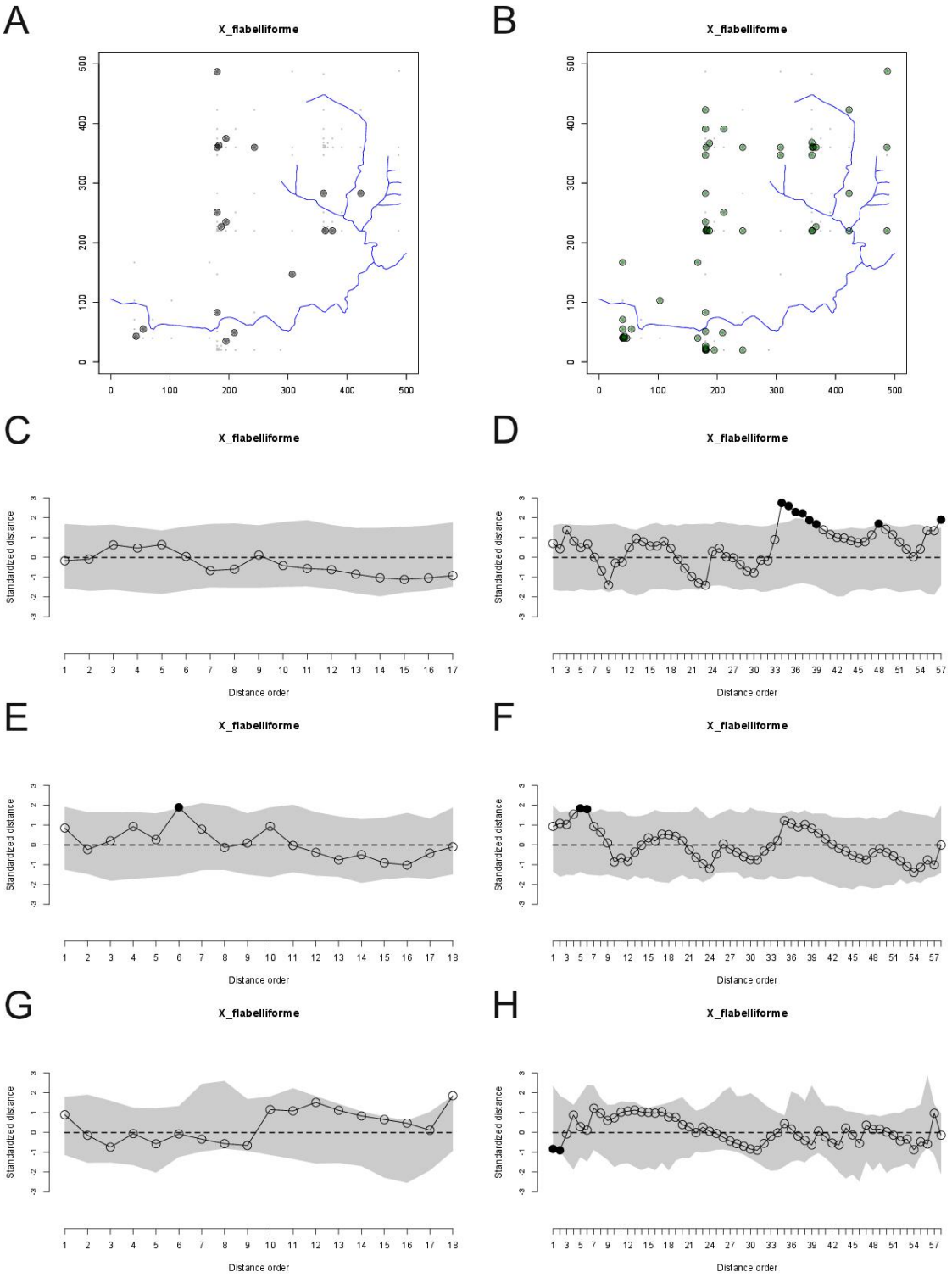


FIGURE S29: *Xylaria flabelliforme* (penzigioid) – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

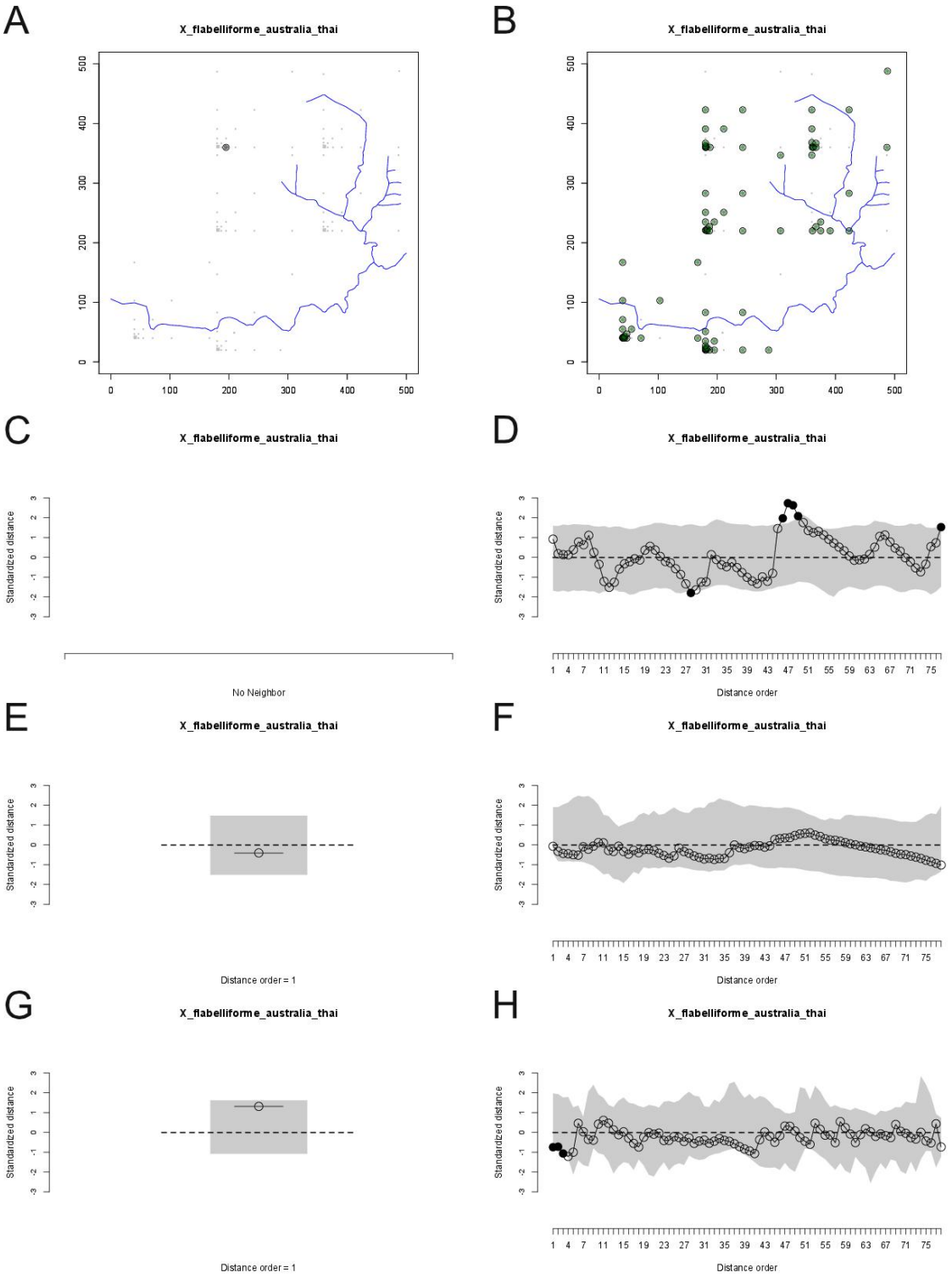


FIGURE S30: *Xylaria flabelliforme* (non-penzigioid) – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

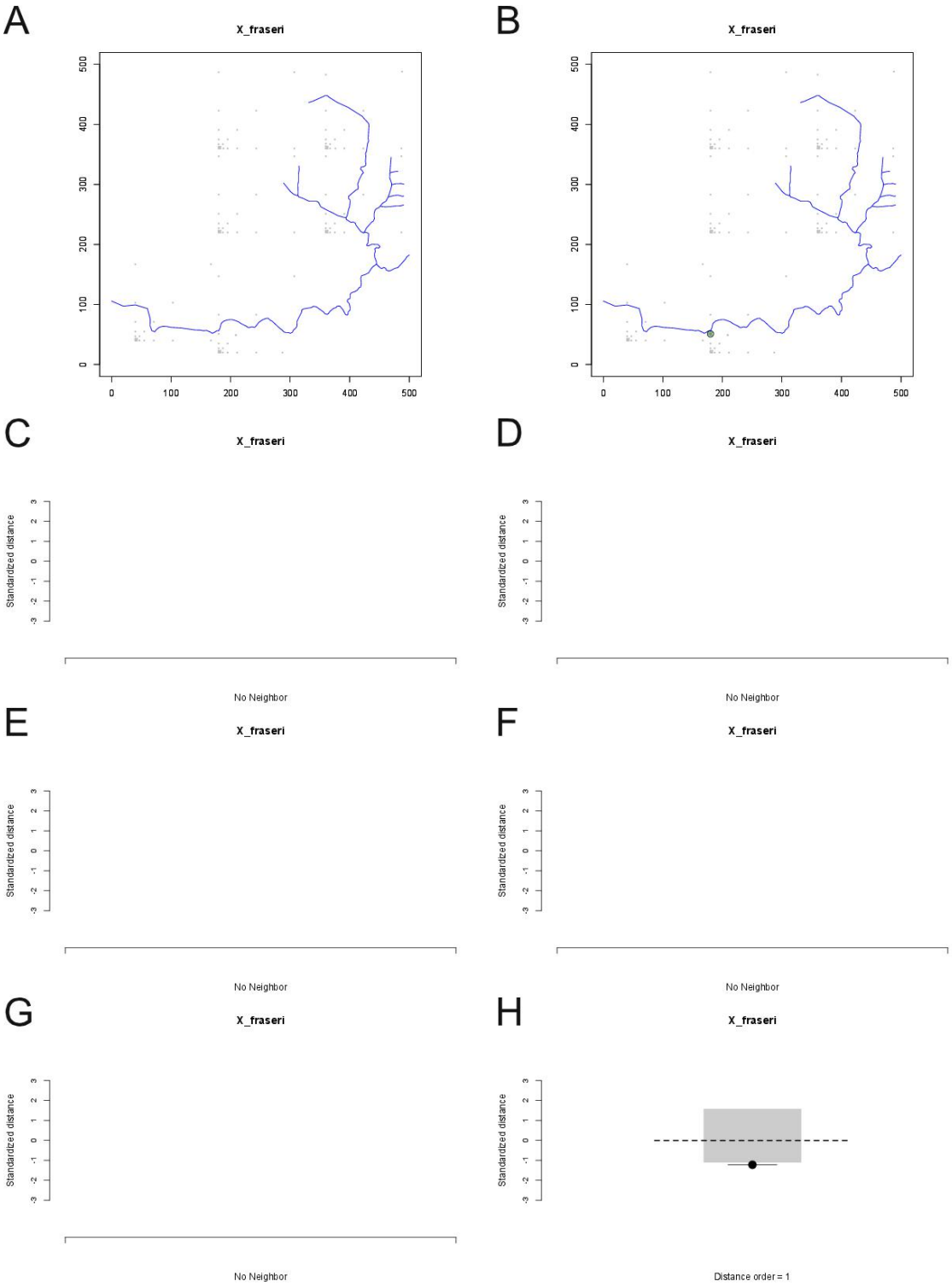


FIGURE S31: *Xylaria fraseri* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

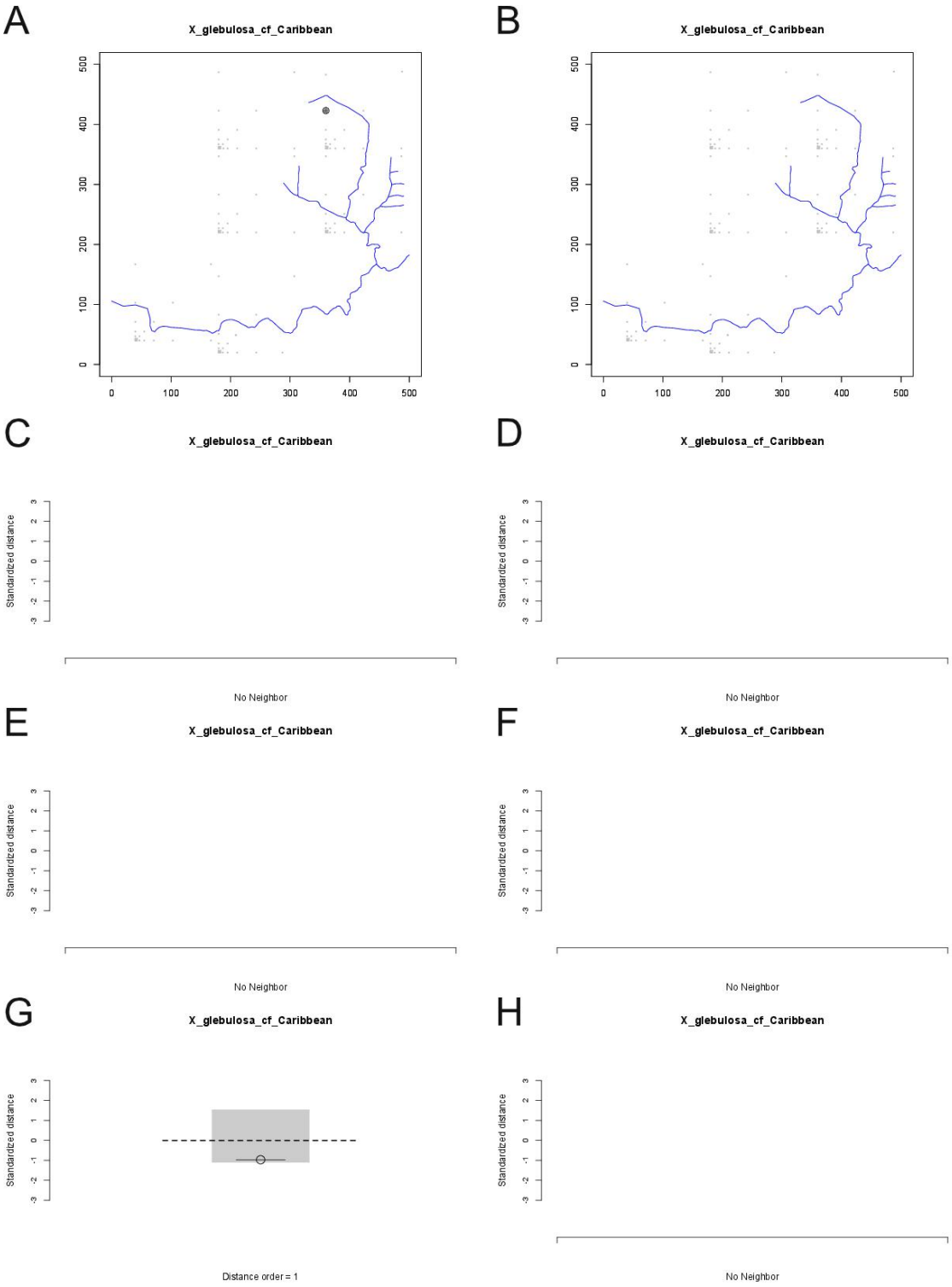


FIGURE S32: *Xylaria glebulosa* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

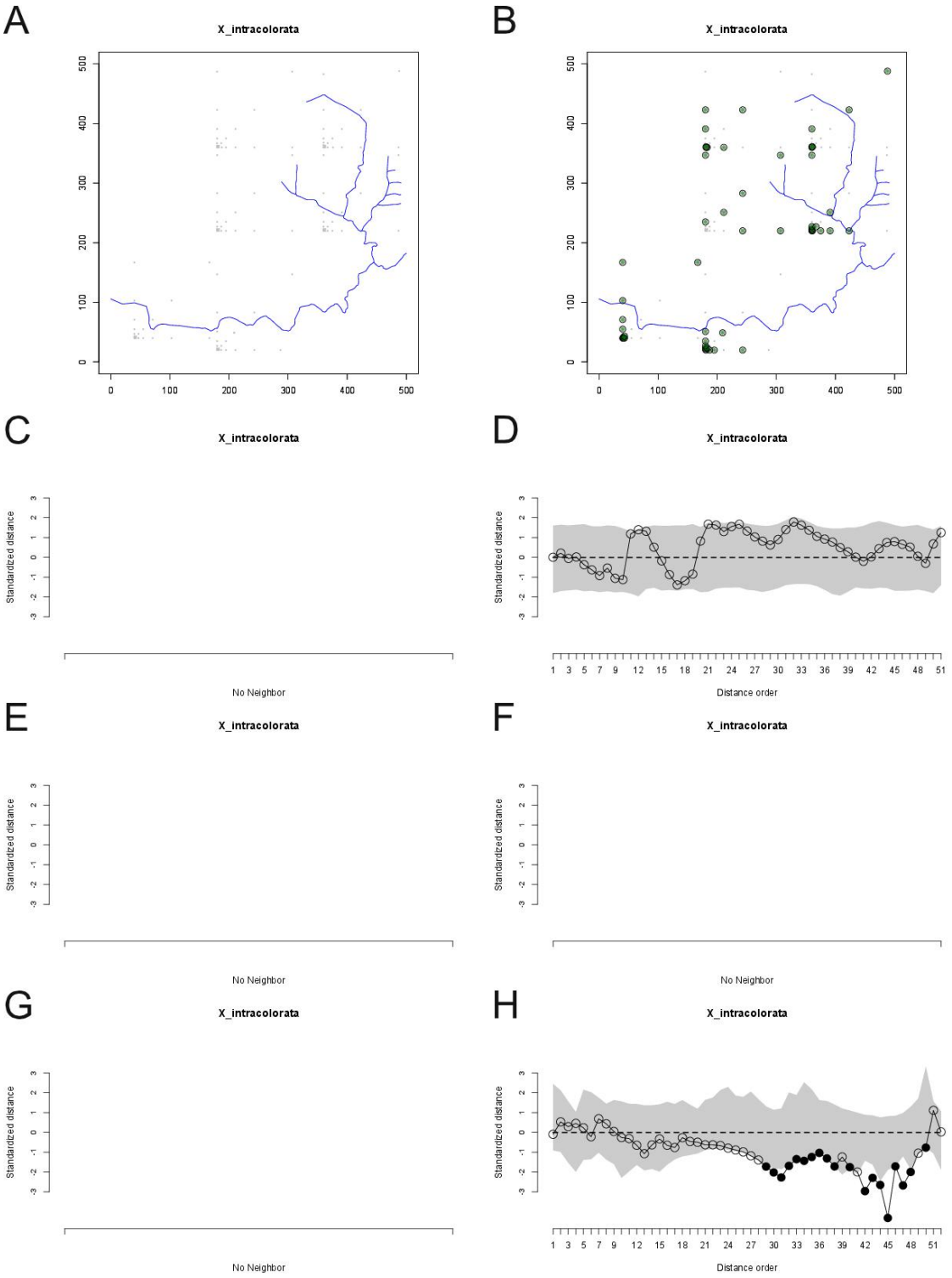


FIGURE S33: *Xylaria introcolorata* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

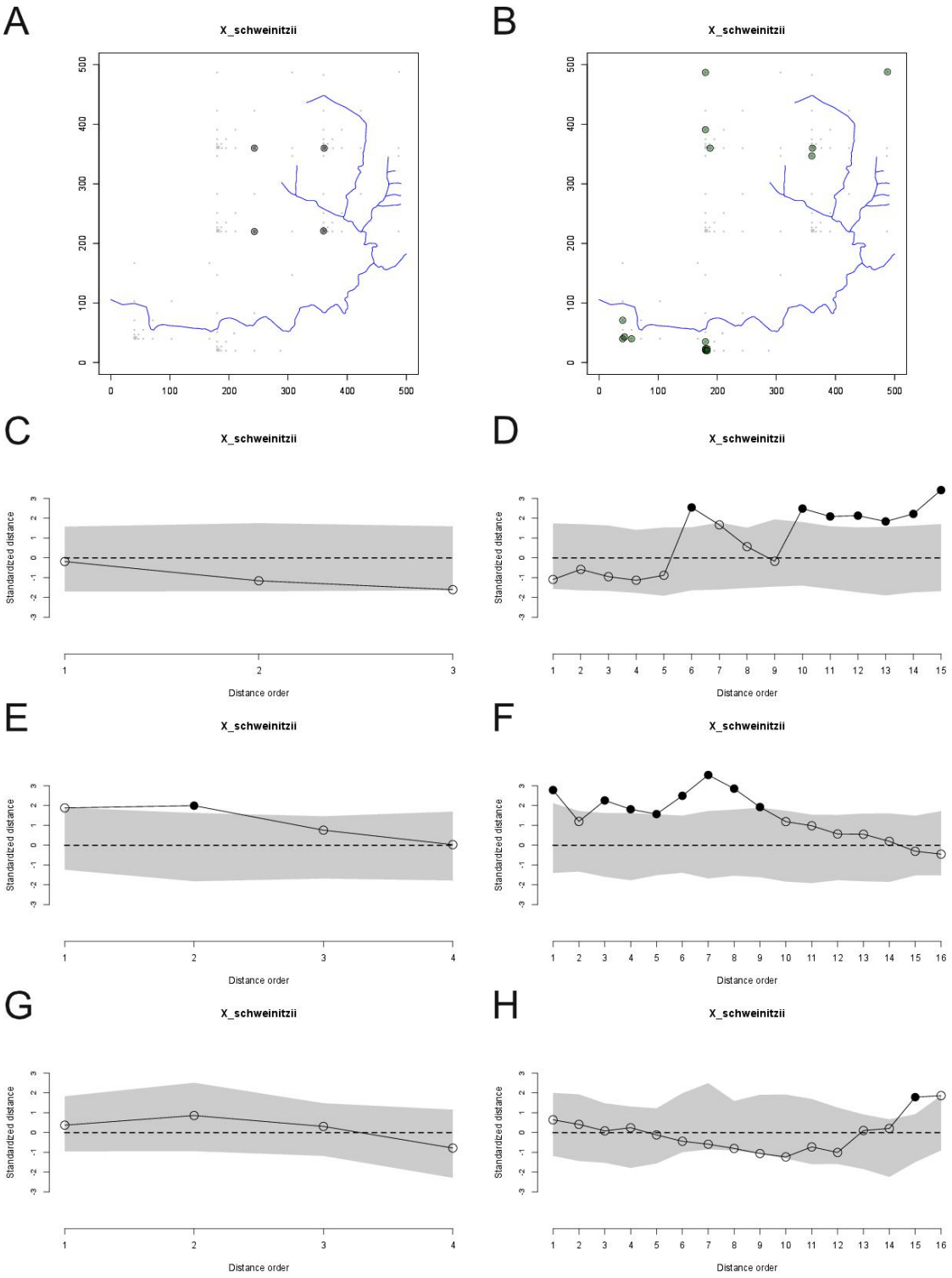


FIGURE S34: *Xylaria schweinitzii* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

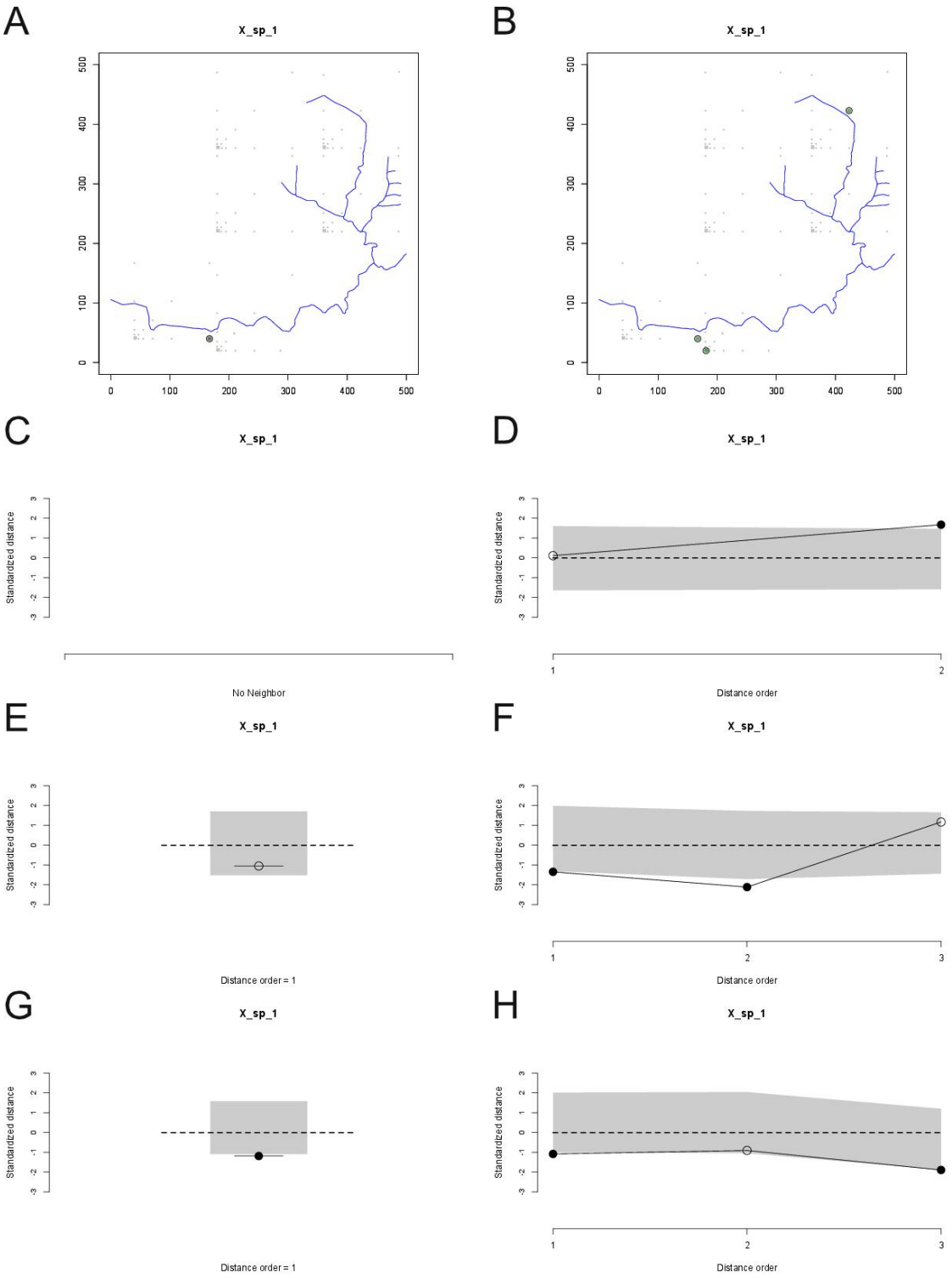


FIGURE S35: *Xylaria* sp. 1 – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

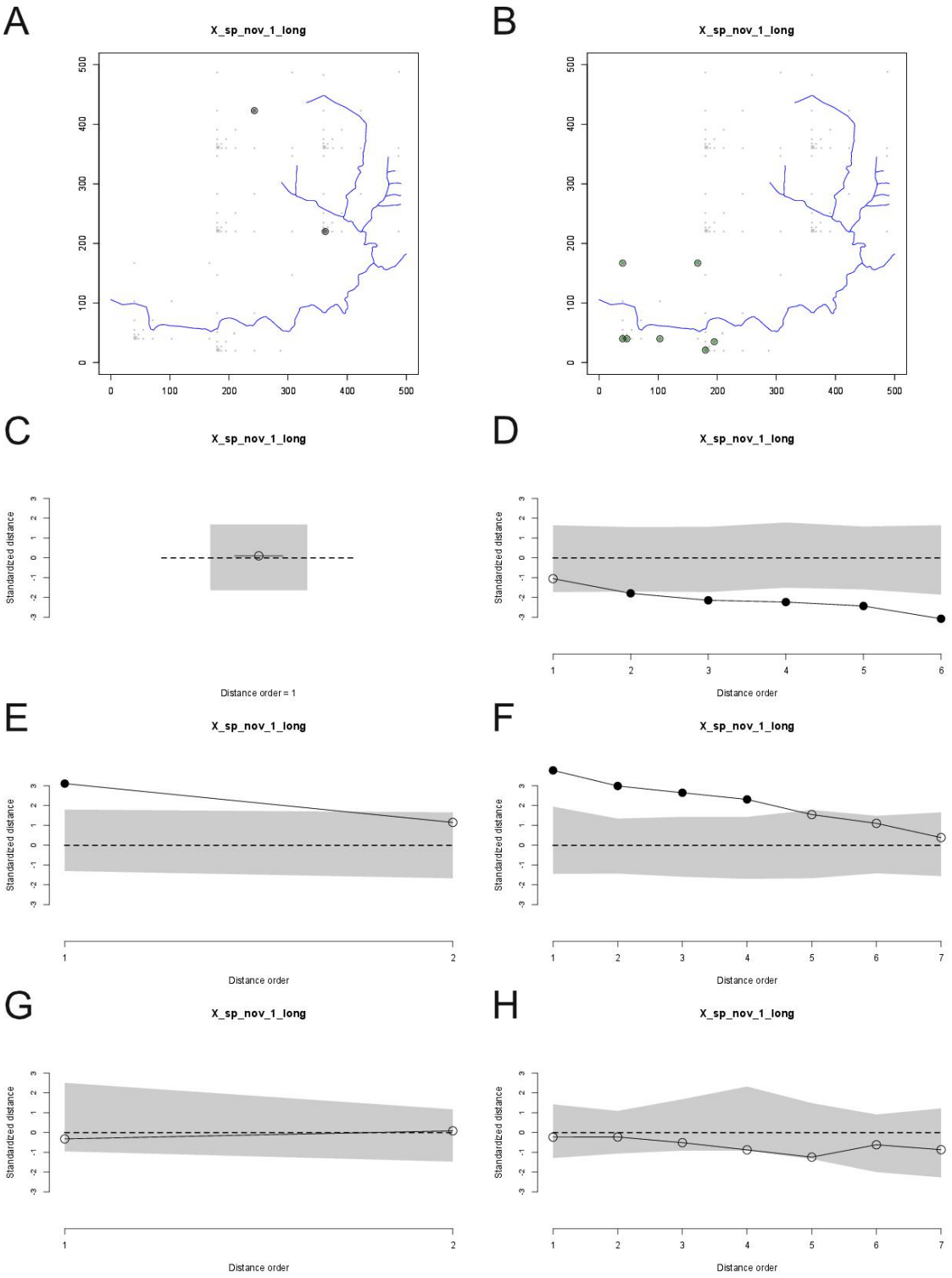


FIGURE S36: *Xylaria* sp. nov. 1– Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

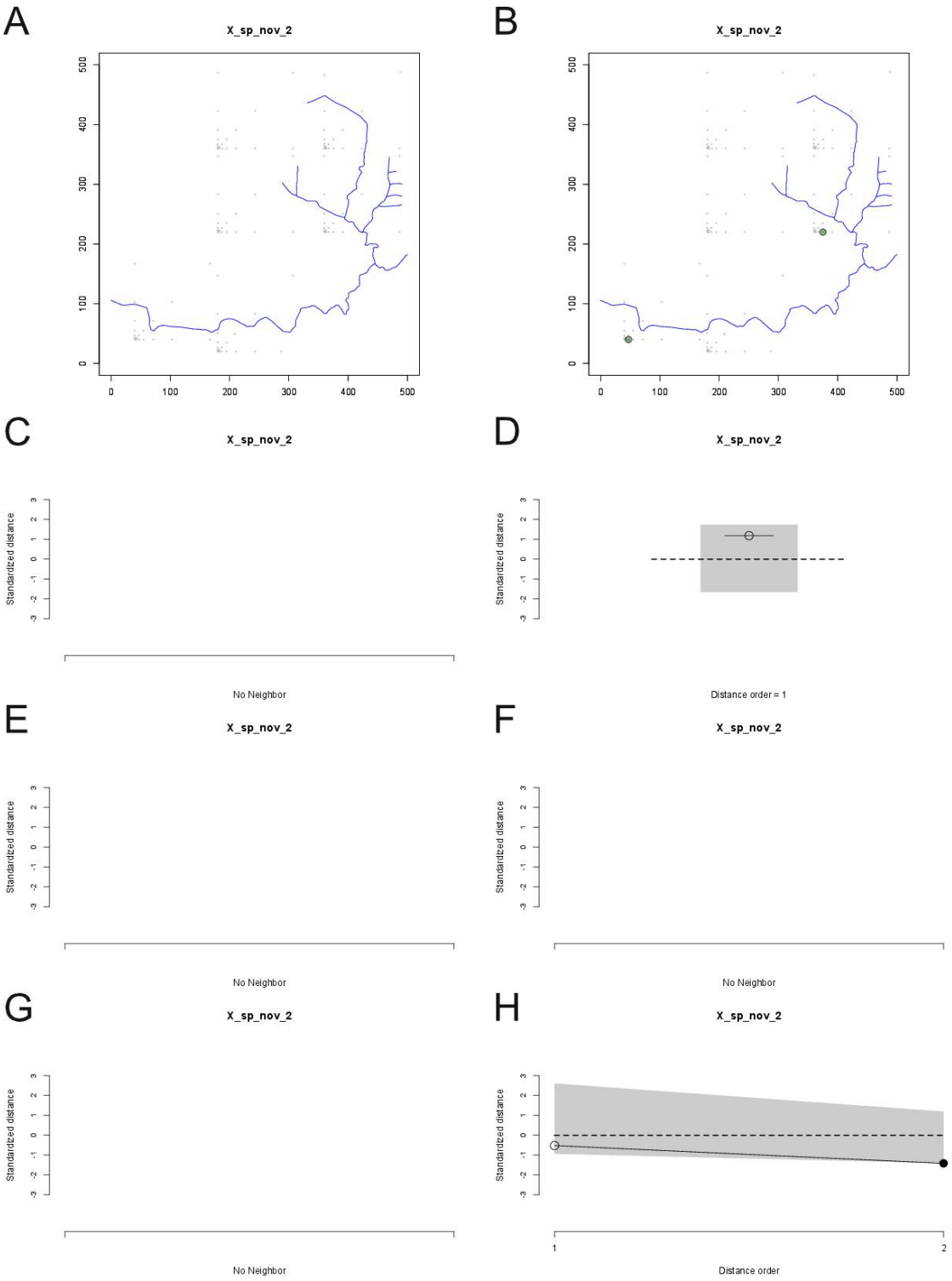


FIGURE S37: *Xylaria* sp. nov. 2 – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

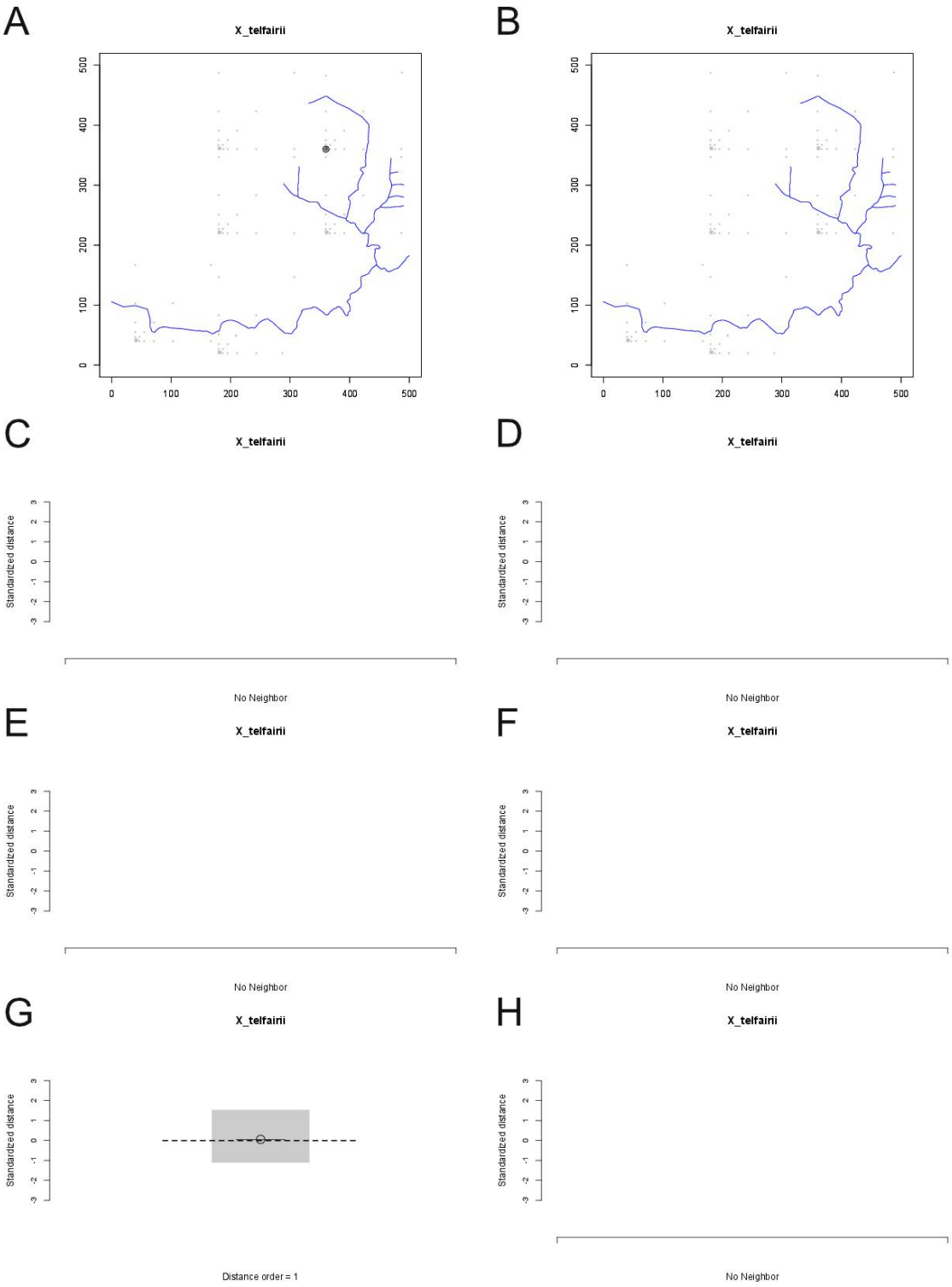


FIGURE S38: *Xylaria telfairii* – Maps of occurrences for: **A**, stromata; and **B**, endophytes. Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for this taxon showing clustering of: **C**, stromata, **D**, endophytes, **E**, stromata around endophytes, **F**, endophytes around stromata, **D**, stromata to the stream, and **H**, endophytes to the stream.

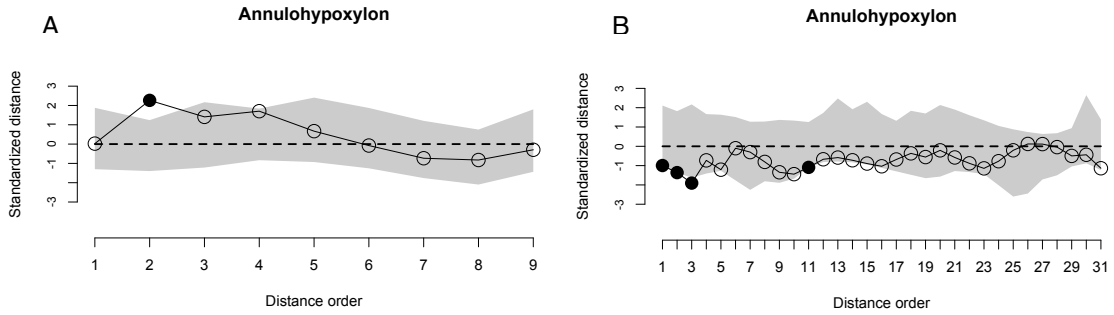


FIGURE S39: ***Annulohypoxylon*** – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for distance to the stream for all observations, treated collectively, for: **A**, stromata; and **B**, endophytes.

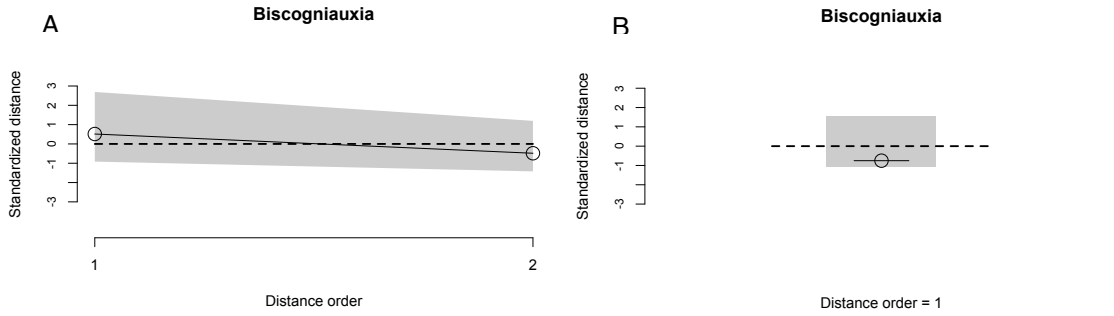


FIGURE S40: ***Biscogniauxia*** – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for distance to the stream for all observations, treated collectively, for: **A**, stromata; and **B**, endophytes.

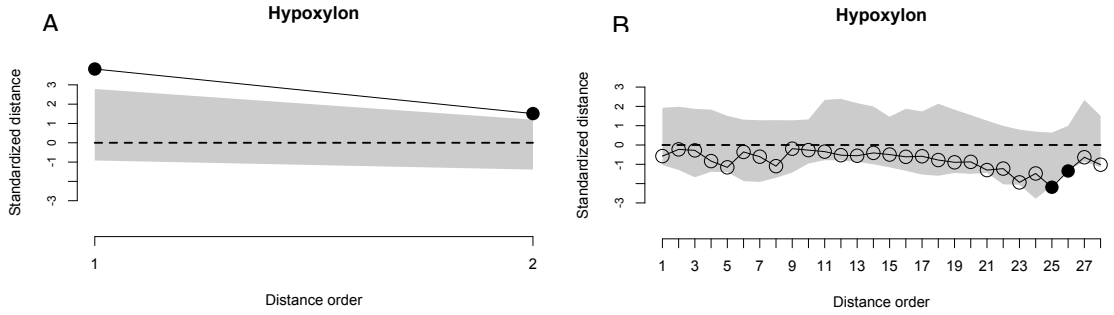


FIGURE S41: ***Hypoxylon*** – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for distance to the stream for all observations, treated collectively, for: **A**, stromata; and **B**, endophytes.

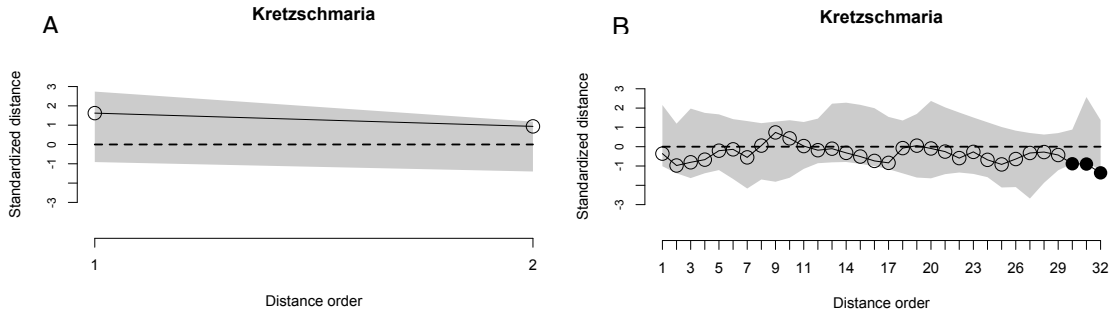


FIGURE S42: ***Kretzschmaria*** – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for distance to the stream for all observations, treated collectively, for: **A**, stromata; and **B**, endophytes.

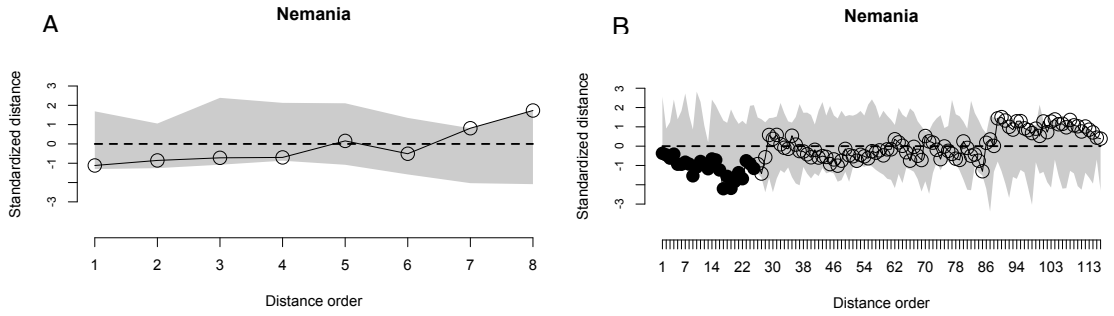


FIGURE S43: ***Nemania*** – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for distance to the stream for all observations, treated collectively, for: **A**, stromata; and **B**, endophytes.

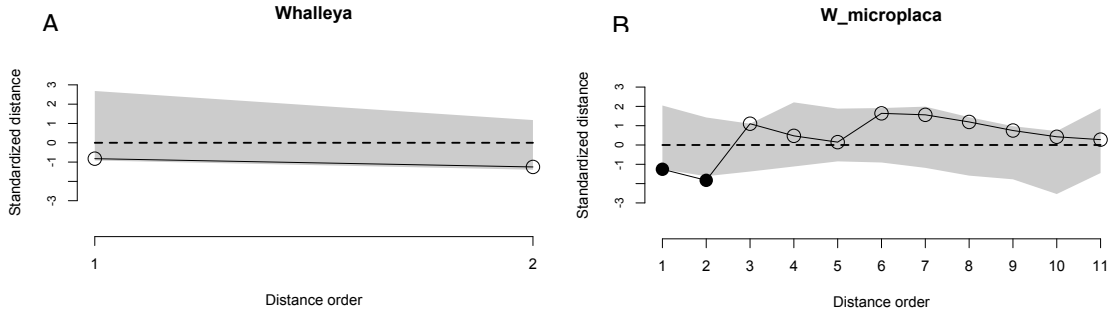


FIGURE S44: ***Whalleya*** – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for distance to the stream for all observations, treated collectively, for: **A**, stromata; and **B**, endophytes.

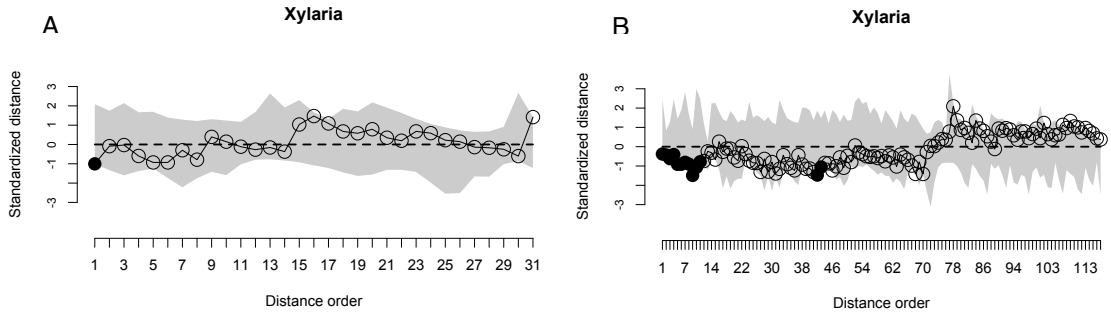


FIGURE S45: ***Xylaria*** – Graphical representation of the results of nearest neighbor Monte Carlo-type simulations for distance to the stream for all observations, treated collectively, for: **A**, stromata; and **B**, endophytes.

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CHAPTER IV

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