Title: Monitoring fungi in ecological restorations of coastal Indiana, USA

Running head: Monitoring macrofungi

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Abstract

Monitoring of ecological restorations has rarely focused on fungi. In this study, we conduct a first-ever monitoring of macrofungi in ecological restorations of coastal Indiana (USA) and present an approach and considerations that can be followed elsewhere. Forty-two sites were surveyed over a two-year period for the presence of saprotrophic, mycorrhizal and parasitic macrofungi. Sites included those considered to be restoration, pre-restoration or reference and were in wooded, semi-wooded or grassland habitats. With 1103 observations, 277 species of fungi were identified. Most fungi were found in wooded habitats though some were in grassland restorations. Invasive plant cover negatively impacted fungal species richness. Monitored sites were compared to a set of reference sites using two different similarity indices (overlap and Jaccard), as well as the ratios of different fungal functional guilds, revealing that choice of index can impact how restorations are perceived to match targets. Last, we present a novel, tractable and conservative way to assess and rank sites by the functional trait guilds of fungi. We show that such an approach can provide important additional information about the success of restorations such that functional guild ratios could be used as an indicator of restoration progress early-on while functional-values are better used in later phases.

Keywords: macrofungi, restoration, functional trait guild, ectomycorrhizal, soil exploration type

Implications

- Monitoring fungi in restoration is important because of their essential roles in ecosystem processes.
- Habitats with woody plants (e.g., live and dead trees, logs and other downed wood) had more macrofungi; thus practices that increase trees and woody debris can benefit fungal community development.
- Practices that target non-native plant removal may enhance macrofungi.
- Comparisons of macrofungi in restorations to other sites (e.g. references) should use multiple approaches to determine if restoration targets are met.
- Tools are provided to estimate amounts of fungal function and other valuable fungal data relevant to restoration success. One benefit of these tools is that their use does not require extensive mycological expertise.

Introduction

The goal of ecological restoration is to establish and sustain the components and processes of natural ecosystems. Often, the primary focus of such efforts is to ensure that autotrophs (i.e. plants) are introduced, maintained and productive. Heterotrophs (i.e. fungi and animals) are typically excluded from active restoration efforts, as they are expected to self-colonize once the plant community is established. Plants are also typically the focus when restorations are assessed for success, as the standard means by which success is gauged is by monitoring and measurement of the plants (Society for Ecological Restoration 2004).

Although monitoring plants is vital in assessment, other components of ecosystems can be measured to make assessments more comprehensive and thorough. For instance, fungi contribute major ecosystem roles in decomposition, nutrient cycling, especially carbon, and soil aggregation. Furthermore, fungi can be a large component of biodiversity in even the most species-poor plant communities (e.g. Taylor et al 2014). Given this, assessing the fungi of restorations would seem to be a critical part of determining the success of restorations (Keddy & Drummond 1996; Harris 2009).

However, fungi are hard to monitor. Only some fungi, like those that form mushrooms, have macroscopic structures that can be seen readily by eye, and even these are ephemeral and episodic. Hyphae, the metabolic structures of fungi, are cryptic, with the majority hidden from sight as they grow in their substrate or form mycorrhizas with plant roots. Molecular approaches of iden-

tification that uncover these fungi are common (Peay et al. 2008) but require substantial costs and expertise, and thus are usually beyond the means of restoration managers charged with monitoring. As a result, the methods and resources required to monitor fungi in restorations are seldom employed. Not surprisingly, few restorations have monitored fungi and used fungal data as part of the assessment of restoration progress and success (Harris 2003).

Despite the lack of fungal monitoring in ecological restorations, a large number of studies have been conducted to examine the role of fungi in the process of restoration (e.g. Shearer 1986; Smith et al. 1998; Korb et al. 2003; Allison et al. 2005; McKinley et al. 2005; Giai & Boerner 2007; White et al. 2008; Olsson & Jonsson 2010; Banning et al. 2011). Many of these studies focused on one or a small number of restoration sites or restricted their analyses to a general category of "soil fungi" rather than views of multiple trophic groups, or guilds, of fungi and few provided specific applications for restoration management. But, the overarching implication is that heterotrophs like fungi have important roles and should be monitored in restorations (Harris 2009, Olsson & Jonsson 2010).

The development of functional communities of fungi in a restoration likely includes the establishment of three major guilds: the decomposers, the mutualists and the parasites. It follows that determining the trajectory of a restoration should incorporate a level of measurement of these functional guilds. How to measure this is unclear, though, as fungi could be measured by numerous means of varying complexity that range from taxonomic identification of species to de-

scriptions of fungal-driven ecological process. For instance, a large, fleshy mushroom growing on a stump could be described simply as a "saprotroph" or more precisely as a "brown rot wood decomposer". Or a mutualistic fungus such as an ectomycorrhizal (EcM) fungus in symbiosis with tree roots could be described just as a "mycorrhizal" or as an "EcM fungus of a hydrophobic, long distance exploration type, with rhizomorphs." To make monitoring fungi in restorations tractable, useful and efficient, an appropriate level of monitoring complexity should be determined.

In this study, we examine this gap in how to monitor fungi in restorations. We take advantage of the overlap of a system of restoration monitoring (Northwest Indiana Restoration Monitoring Inventory, NIRMI, 2011-2016) and mycological expertise in a region where many restorations exist (Botts 2006; Calumet Stewardship Map 2015) and mycological studies have occurred (Leacock et al. 1999; Schmit & Mueller 1999; Avis et al. 2008). We used this system to initiate fungal monitoring in restoration sites across a region of coastal Indiana extending from northeastern Illinois to very southwestern Michigan — an area often referred to as the "Calumet Region."

This study had four objectives:

1) To monitor the macrofungi (i.e. mushrooms) of restorations systematically and examine ways of comparing fungi in different restorations as a means of assessment.

2) To use a set of monitoring data to test hypotheses about factors impacting macrofungal observations in restorations. Specifically, we hypothesize that favorable collecting conditions (e.g. rainy and warm weather for over a week in the summer) and the presence of wood (a prerequisite for the growth of certain functional groups, e.g. live trees for EcM fungi, or dead wood for saprotrophs), increase the numbers of macrofungal observations, since they are required for the development and production of sporocarps. In contrast, we hypothesize that the presence of non-native plants will have a negative impact on the number of macrofungi recorded since introduced plants can escape the parasites of their native regions or antagonize fungi encountered in the introduced habitats (Roberts & Anderson 2001; Mitchell & Power 2003; Callaway et al. 2008).

3) To compare restoration sites to reference sites. Specifically, we examine the performance of two community similarity indices (overlap and Jaccard) and the proportion of fungal species in different functional guilds providing comparisons between restoration and reference sites. This allowed us to test aspects of the hypothesis that greater similarity between a restoration and reference site implies that a restoration is "closer to target", at least in terms of fungal community development.

4) To rank restoration sites based on fungal species richness as well as a novel metric developed in this study called "functional-value". This metric characterizes fungal functional guilds and their prevalence within a site and provides a way to estimate the development of the ecological

function of the fungi in restorations. We hypothesize that ranks of restoration sites by functional-value differ from those based on fungal species richness as richness alone is very limited as a characterization of fungal ecological function. Furthermore, this metric implies that restorations with higher functional-values are more "restored" since they exhibit higher levels of documented function.

These objectives, hypotheses and comparisons each provide guidance for restoration practitioners and we show how these data can be used to assess and determine the success of restorations based not only on community comparisons but also on functional attributes of the fungi.

Methods

Study Sites

The 42 sites examined in this study (Table S1) are a subset of the 45 sites that the Northwest Indiana Restoration Monitoring Inventory (NIRMI) monitors primarily for plants and other variables (NIRMI 2011-2016). The sites include a range of habitats (woodlands to grasslands, upland to wetland), restoration types (from baseline/pre-restoration, to recent and/or long-term), restoration activities (intensive to minimal) and land owners (private and public, large and small, federal to local).

Reference sites

Seven locations served as reference sites, four within Indiana Dunes National Lakeshore (IN-DU): Headquarters (HQ) Woods aka Bailly Cemetery, Cowles Bog, Miller Woods, Tolleston Dunes; and three others: Indiana Dunes State Park; Gibson Woods Nature Preserve; and Green Lake Savanna. These sites have been studied and monitored by mycologists for the past 20 years and provide a regional pool of observations to which restoration sites were compared (Table S2).

Study plots

The study utilized NIRMI plots in which data are gathered in a spatially defined way. The plot design follows the Carolina Vegetation Survey (Peet et al. 1998) and the area of the plot surveyed is that recommended for fungi by Mueller et al. (2004) and Berglund et al. (2005). Plot locations were determined by coordination with site management and typically overlapped with or were adjacent to areas undergoing or expected to undergo restoration activities. In some sites, restorations occurred near target habitats in the reference sites. In nearly all cases, these plots are 0.1 ha in area (1000 m^2) with dimensions of 50 m by 20 m and divided into ten 10 m by 10 m (100 m^2) modules. Four of these modules contain intensively monitored nested sampling corners that span spatial scales from 0.01 m^2, 0.1 m^2, 1 m^2, to 10 m^2. In this particular study, though, we aggregate data for the entire plot area of 0.1 ha (1000 m^2). In these same plots, plant communities are monitored allowing for relationships between fungi and several vegetation variables, including tree cover and non-native plant cover (NIRMI 2011-2016), to be examined in a spatially explicit manner.

Macrofungal surveys

Each site was surveyed one to two times in 2012-2013. Surveys were conducted by walking the entire plot, observing and recording the presence and location of macrofungi (Mueller et al. 2004). We define "macrofungi" as any observable fungus with a cohesive structure over 5 mm in diameter. This includes primarily mushrooms but also obvious bracket (shelf), crust, cup, gasteroid (puffball), jelly and sclerotium forming fungi. We did not include observations of lichens or slime molds. Collecting conditions during surveys were categorized as "Good", "Fair" or "Poor" based on rainfall, temperature, and the presence of fungi at comparable sites in the area at the same time (within the same week) the survey was conducted. For this region, conditions typically considered "Good" for uplands include a seven to ten day span with four to five rain events of an average of at least 0.25 cm and high temperature >26 °C. Importantly, condition assessment depends on the site. A wetland often has "Good" conditions at the same date that a woodland site has "Poor" conditions.

Some specimens were collected for further identification. For these, microscopic observations were made and specimens preserved at Indiana University Northwest and the Field Museum of Natural History. Identifications relied on expertise of the authors as well as the use of keys by Arora 1986; Bessette et al. 2010; Beug et al. 2014; Kibby & Fatto 1990; Kuo 2000-2015; Kuo & Methven 2014; Rogers 1986. Nomenclature and taxonomy follow Index Fungorum (www.indexfungorum.org). In most cases, fungi were identified to species but in some cases

identifications were made to the genus or to a species complex. For simplicity, the latter situations were considered individual species.

Identification tools

For two abundant groups of EcM fungi found in the Calumet region, the boletes and russulas, novel on-line identification keys were developed. These are available at http://nirmi.org/keys/boletes/key.html and http://nirmi.org/keys/russula/key.html, respectively. Morphological information including features often easily identifiable by non-mycologists were compiled into species-character matrices which were then converted into an online system. The data for the keys are from Kibby & Fatto (1990) and Bessette et al (2014), edited and expanded based on author experience specific to the study region. Additionally, for the benefit of restoration practitioners interested in determining the general ecological role or functional guild of macrofungi collected in a site, a key was developed (see http://nirmi.org/identification_tools.php?pageNav=key_ecological&page=start).

Functional guild values, assignment and model

Each species observed was assigned a "functional-value" based on the number of services fungi have in Dighton (2003; see summary in Tables S3a, S3b). To do so, each species was first classified to categories as either "saprotrophic", "EcM" or "parasitic". Each nutritional mode was given a value based on the number of ecosystem services assigned to that guild (Dighton 2003).

The final functional value was the sum of all the services; but, for numeric simplicity, each sum was normalized by the category receiving the smallest (i.e. parasites). EcM fungi have 12 services, saprotrophs 9 and parasites 3, so the final functional values are EcM 12/3 = 4, saprotrophs 9/3= 3 and parasites 3/3 = 1 (see also Table S3b).

To rank restorations by functional guild characterization, fungi observed in each plot were assigned to functional guilds in two ways, and called "simple" and "complex". In the simple approach, fungi were only categorized as either "saprotrophic", "EcM" or "parasitic" based on the knowledge of fungal nutritional modes (as above). The, second, or complex characterization, was assigned by breaking the three simple categories into more complex functional guilds, again given the known aspects of the fungi (Tables S3a&b). Saprotrophs were broken into five subcategories based on type of rot and substrate they typically grow on. EcM fungi were broken into four categories based on the soil exploration type of the mycorrhizas (Agerer 2001; Agerer 2006; Peay et al. 2011; Tedersoo et al. 2012). Parasites were broken down depending on the type of host they infect.

Then, each of the 42 sites was scored and ranked using two formulas:

1) Simple Model

$$Site\ Simple = [F_{EcM} \times \ln(\#species_{EcM} + 1] + [F_{Sap} \times \ln(\#species_{Sap} + 1] + [F_{Par} \times \ln(\#species_{Par} + 1]$$

Where F is functional-value as defined in Table S3b, EcM is EcM fungi, Sap is saprotrophic fungi and Par is parasitic fungi.

2) Complex Model

$$
\begin{aligned}
Site\ Complex = {} & \left[F_{EcMContact} \times \ln\left(\#species_{EcMContact} + 1\right)\right] \\
& + \left[F_{EcMShort} \times \ln\left(\#species_{EcMShort} + 1\right)\right] \\
& + \left[F_{EcMMedium} \times \ln\left(\#species_{EcMMedium} + 1\right)\right] \\
& + \left[F_{EcMLong} \times \ln\left(\#species_{EcMLong} + 1\right)\right] \\
& + \left[F_{SapUnknown} \times \ln\left(\#species_{SapUnknown} + 1\right)\right] \\
& + \left[F_{SapBrown} \times \ln\left(\#species_{SapBrown} + 1\right)\right] \\
& + \left[F_{SapWhite} \times \ln(\#species_{SapWhite} + 1)\right] + \left[F_{SapSoil} \times \ln(\#species_{SapSoil} + 1)\right] \\
& + \left[F_{Par} \times \ln(\#species_{Par\ for\ each\ plant\ host} + 1)\right]
\end{aligned}
$$

Where F is functional-value as defined in Table S3b, EcM is EcM fungi, Contact is contact exploration type, Short is short exploration type, Medium is medium exploration type, Long is long exploration type, Sap is saprotrophic fungi, Unknown is the saprotrophic but of uncertain substrate, White is white rot, Brown is brown rot, Soil is soil saprotroph, and Par is parasitic fungi.

Data analysis

A set of statistical tests were conducted to test hypotheses and make comparisons. Linear regression was conducted using proc lm in R (v3.2.2) to examine the impact of tree cover and non-native plant cover on the number of macrofungal species observed. Restoration sites were compared to reference plots using 1) pairwise Jaccard coefficients (= [# species appearing in both lists] / [total different # species in both lists combined]; for example, the Jaccard of [abcde] and [acxyz] = [#ac]/[#abcdexyz] = 2 / 8 = 0.25)) and, 2) a metric set up in this study called "overlap" (= percentage of species on the smaller list (e.g. restorations) that are found in the larger list (e.g. reference sites)). The ratios between the number of species of saprotrophic and EcM fungi were calculated and used as a basis for comparison, as were the ratios for saprotrophic, EcM and parasitic fungi (Table S1).

Results

A total 1103 observations were made and 277 fungal species identified across the 42 sites (Table S1). Sites had a mean and median of 16 and 7 macrofungal species, respectively, and ranged from having 0 to 95 species. Macrofungi observed included saprotrophs (167 species, 815 observations), EcM (92 species, 229 observations) and parasites (15 species, 54 observations), with a few (3 species, 5 observations) of uncertain nutritional lifestyles. Sites were sampled primarily when conditions were "good" (n=27) while on occasion sampled when "fair" or "poor" (n= 5, and n=4, respectively). Collecting under "good" conditions resulted in more macrofungal species and observations. Twenty-six sites contained plots that were considered woodland (>5% tree

cover) while 16 were grasslands. There was a significant positive relationship between macrofungal species richness and dominant tree % canopy cover (Figure 1; $R^2 = 0.3722$, $p<0.001$, $F=20.75$, $df=1, 35$). However, the relationships between fungal species richness in both woodlands and grasslands with percent cover of non-native plants were negative when the full range of coverage by non-natives was included in the analysis. For sites considered "woodlands" the relationship was significant (Figure S1; $R^2=0.478$, $p=0.013$, $F=9.146$, $df=1,10$; one outlier site, Meadowbrook, was removed from this analysis as it was >3 standard deviations from the mean richness; note that removing the two sites with the highest cover resulted in a non-significant positive trend for sites with less than 10% cover, not shown; $R^2=0.072$, $p>0.05$). For grassland sites, the relationship trended negative but was not significant (not shown; $R^2=0.126$, $p>0.05$, $F=1.1446$, $df=1,10$).

The similarity indices used to compare restoration sites to reference sites gave contrasting views (Table S4). With the overlap measure, most sites matched the two reference sites with highest species richness and highest number of visits and observations (HQ Woods and Cowles Bog in Table S4). However, using the Jaccard measure, a different set of reference sites (e.g. Green Lake) tended to be the top matches (Table S4). Only six of 28 sites included for this analysis had the same top match with overlap and Jaccard and, of these, only three were exclusive matches (i.e. Ivanhoe Dune and Swale, INDU Lake Plain Prairie and Marquette Pannes). Few sites had both restoration and reference sites (as noted by * in Table S4) and in these cases overlap only matched the top similarity in one of three (HQ woods to HQ woods, but Miller matched Cowles

and Gibson matched HQ woods) and Jaccard only matched one of three (Miller to Miller, but HQ woods to Green Lake, and Gibson to Miller).

The ratio of saprotrophic and EcM species in the reference sites had an average of 1.3 with a range of 0.8-2.7 (Table S2). For the restoration sites, this ratio averaged 7.8, and ranged from 0.7 to 37.0 (Table S1). Seven restoration sites including Meadowbrook, Ambler, Ivanhoe South, Buckeye and Ivanhoe Dune and Swale overlapped with reference sites in terms of the S:E ratio. The number of parasitic/pathogen species observed was typically low in comparison to saprotroph and EcM species (Tables S1 and S2).

The two functional-value ranking approaches resulted in similar but not equal ranks of sites as that provided by fungal species richness alone (Table S5). The rank order using the simple and complex approaches was different for more than half of the sites than when using species richness alone (see totals at bottom of Table S5). When ranks were different, the functional-value approaches usually increased the rank position of a site, rather than decreased it. Nonetheless, the relationship between fungal species richness and site functional-values was significant (Figure 2, R^2=0.95, $p<0.001$, F=274.2, df=2, 32 for the simple model). The relationship between simple vs complex approach was strong as well (Figure 3, R^2=0.97, $p<0.001$, F=1101, df=1, 32).

The functional-values (simple model) in the reference sites had an average of 32.3 with a range of 24.1 – 39.1 (Table S2). For the restoration sites, the average site functional-value (simple

model) was 11.5, and ranged from 0.7 to 27.9 (Table S1) with Meadowbrook the only restoration site overlapping with reference sites.

Discussion

This study established a baseline for the ecological restorations in coastal Indiana and is an important step in monitoring the fungi of these sites for the long term. In this regard, fungi-focused monitoring adds great value to the already impressive effort to monitor the region's restorations (NIRMI 2011-2016). Furthermore, this study provides a model that others can adopt in order to include fungal monitoring in the assessment of restoration success.

Collecting conditions and macrofungal observations

The results support our hypothesis about the factors impacting macrofungal observations in restorations. First, the collecting conditions (especially moisture and temperature) during which surveys occurred played a major role. When precipitation was substantial and temperatures were high during the summer, macrofungi were abundant, especially in woodland sites, in comparison to when conditions were poor (i.e. dry). This is expected given the cues that trigger macrofungal development of fungal structures like mushrooms are dependent upon optimal moisture and temperature (Duggar 1905). This is consistent with the research conducted in this region over 20 years that indicates many of the fungi identified are noticeable during warm and wet parts of the

growing season. This is an important aspect to consider for restoration practitioners and monitoring programs as it will greatly impact any assessment of macrofungi. Restoration managers in much of North America, especially in the Midwest and east of the Rocky Mountains, who want to incorporate fungal monitoring can expect summer and early fall as periods when optimal monitoring conditions exist (e.g. Pinna et al 2010). However, fungal phenology is affected by shifts in temperature and precipitation caused by climate change (Kauserud et al 2012). Therefore, approaches to monitoring fungi should take local predictions of climate change into consideration.

A parallel concern is the issue of how many surveys should be conducted in order to record an accurate picture of the fungi in a restoration. Based on our experience in the Calumet Region which is consistent with research elsewhere (Mueller et al 2004), an ideal amount of monitoring to establish a baseline is to visit a plot once per month from June through October for three to five years. If possible, a visit in May would be good as well as one in November especially as climate changes. Once a protocol is established, one to two individuals could survey two plots per day depending on the level of fruiting. Even a minimal, infrequent effort (one to two surveys per year) with an eye for the long-term (over five to ten years) would be valuable.

Presence of wood and macrofungal observations

The second hypothesis, that the presence of wood would have a positive impact on macrofungal observations, was also supported. Our proxy for the presence of wood, was tree cover and the major pattern observed was that the number of macrofungal species observed was a matter of

habitat type – woodland sites with greater tree cover had more wood and thus greater macrofungal richness. Woodlands have many substrate types for macrofungi including host tree roots for EcM fungi and organic matter for saprotrophs. Even in the cases where macrofungi were found in grassland restorations, residual woody organic matter or a lone tree influenced the presence of macrofungi. Interestingly, this is consistent with the practice of adding wood mulch into harsh restoration sites (e.g. mine spills) to improve restoration (Blanco-Garcia & Lending-Cisneros 2005).

For restoration managers interested in promoting macrofungi, this result suggests that the amount of woody matter (live or dead) is important to consider. Our results indicate that restoration activities that result in increased tree canopy likely result in increased macrofungi. Similarly, techniques to increase coarse woody debris (e.g. Shoo et al 2014) and introduction of tree seedlings with soil containing intact root and mycelial systems from comparable and local woodlands (e.g. Avis & Charvat 2005; Hankin et al 2015) is expected to promote macrofungi as well.

The impact of non-native plants

Our hypothesis that non-native plants would have a negative impact on macrofungal observations was supported, as the trend was negative in both woodland and grassland restorations. Such a trend is consistent with the enemy release hypothesis (Mitchell & Power 2003), which

posits that invasive plants leave behind their fungal pathogens. It further supports studies showing negative impacts of non-native plants on mycorrhizal fungi (Roberts & Anderson 2001; Callaway et al. 2008). Given our results, restoration managers could consider ~10% cover by non-natives a critical threshold, where above this level, non-natives have an effect on macrofungi and warrant invasion control.

It is important to note that the negative relationship between non-native plants and macrofungi was only significant when sites with relatively high cover values were included. Although sites with high levels of invasion are common in restorations, the removal of these highly invaded sites in the analysis produced non-negative relationships. Although contrary to our hypothesis, this suggests consideration of additional relationships between invading plants and macrofungi. For instance, introduced plants could act as vectors for fungi (piggybacking on hosts or in the growth media in which plants are moved). The movement of plant-growth substrates may be more of a concern than most realize. Soil, woodchips, mulch, or peat are often moved independent of the plants that would grow in them and this raises the question of invasive fungi, a growing concern (e.g. Schwartz et al 2006; Vellinga et al 2009), but beyond the scope of our study.

Comparisons and Caveats

The comparisons between restoration sites and the well-studied reference sites provided an opportunity to gauge how well these sites could be considered "restored" at least in terms of fungi in reference sites. As a result, we can provide insight about the effect of restoration on the fungi in these sites. Several caveats should be considered before elaborating on the latter. First, we assume that reference sites serve as optimal restoration targets. However, the reference sites used here are well-studied for fungi but were selected for those studies not necessarily because they serve as restoration targets (i.e. the overall habitat might be the same as what managers target, but other aspects of the sites might differ from what the restoration goal is). Although the reference sites are considered high-quality habitats, they vary in many ways (e.g., size, amount of habitat type, etc.) from the restoration sites. Therefore, differences between references and restorations may not simply be due to restoration activity or time since restoration started. However, we feel the reference studies offer a unique opportunity for comparison.

A second caveat relates to the mechanics and assumptions of how we conduct comparisons between reference and restoration sites – and this has practical implications for how restoration managers and their supporters (i.e. funding agencies) set and evaluate restoration benchmarks. If restoration is successful, it might be expected that any approach in comparison would give the same answer. For example, a restoration manager would pick a desired target level of diversity and species composition and simply compare observed to expected results. However, similarity indices challenge this assumption and the results of our study confirm this as the different indices

we used gave different answers (i.e. for most sites, the Jaccard metric and the overlap index ranked different sites as "most similar"). These inconsistencies are driven by the nature of the metrics: the overlap index screens if fungi found in a restoration site are also found in a reference site; but not what fungi are missing in the restoration site that are known in reference sites. As a result, when using the overlap metric for poorly sampled sites, the reference sites most "similar" are those with highest recorded species richness (i.e. the HQ Woods reference was the most species rich thus was the most similar match to most sites). The Jaccard metric, on the other hand, is more sensitive to the overall composition of sites as it accounts for not only the overlap of the restoration to reference site, but also what is not matched. As a result, the Jaccard shows low numerical scores when there is a great difference between species richness in reference and restoration sites. Given this, we encourage restoration practitioners apply multiple metrics rather than relying exclusively on one.

With caveats aside, this study provides insight about the effect of restoration on fungi, and the hypothesis that greater similarity between a restoration and reference site implies a restoration is "closer to target" at least in terms of fungal community and functional development. The ratio between saprotrophic and EcM fungal species (S:E ratio) appears to be useful in this regard. In a continent-wide study using next-generation DNA sequencing of soil fungi, the S:E ratio was correlated to ecological functions including the activities of major enzymes involved in carbon cycling (Talbot et al 2014). In that study, S:E was positively related to greater activity of the faster

cycling forms of carbon (e.g. simpler carbohydrates) while negatively related to the activity of enzymes cycling of more recalcitrant forms (e.g. nutrients complexed in soil organic matter). We can apply these relationships to our study where the reference sites had an average S:E ratio of 1.3 suggesting that a target fungal functionality (based on the survey data from reference sites used here) is a balance between saprotrophs and EcM fungi; effectively, a balance between those involved in the fast and slow cycling of carbon. In contrast, the restoration sites had an average S:E ratio five times larger suggesting that restorations were functionally more involved in the cycling of labile carbon. However, a few restoration sites had ratios near 1.0, in line with the average for the reference sites. This suggests that there are better performing restoration sites, at least in terms of the kinds of carbon cycling occurring. Presumably, with continued use of the particular restoration approach applied in those sites, the sites will continue to approximate reference targets and exhibit optimal levels of functionality.

Better-performing restorations exhibit particular qualities. Some sites, such as Ambler Expansion, have high levels of tree diversity which is one factor considered to drive levels of EcM fungal diversity (Dickie 2007, Spake et al 2016). This could explain the relatively high levels of observed EcM fungi (and thus lower S:E ratios) at sites like Ambler. Other sites, even though species-rich (e.g. Barker Woods and Munster), had higher ratios (i.e. fewer EcM fungal species). These specific sites had experienced limited to no restoration activity at the time surveyed. Thus, with possible additional activities such as planting of EcM host trees, a balance between fungal guilds may be attained.

Functional-value models

The functional-value we have generated is a novel metric that describes fungal attributes and we have used it to rank restoration sites. A functional-based approach is supported by research into the relationships between types of fungi and enzymes important in the decomposition process. Talbot et al. (2015) showed that a strong predictor of functional enzymes in decomposition microcosms was to divide the fungi into functional guilds. Given this, restoration practitioners could take what is known about the fungi observed in a restoration site via a monitoring survey as a minimum and conservative estimate of the fungal related ecosystem processes.

As we hypothesized, ranking sites by functional-value provided similar but not equal views of the restoration sites as the rank based on species richness alone. This suggests that the functional-value approach provides additional information to restoration practitioners. A moderately species rich site like Gibson Woods, which is a state-dedicated nature preserve with a relatively long history of restoration, may have more functional attributes than species richness alone might suggest. Similarly, some sites may appear species rich (such as Barker Woods, under more recent restoration), but be less functional than expected. In both cases, this information is important. For functionally over-performing sites, this can be highlighted and promoted as a special site quality and would support continued use of the management that was employed at that site. For under-performing sites, attention can be brought to the site and guide management to enhance the fungal communities.

We used two different functional-value models, simple and complex, to rank sites so that we could see if including different levels of monitoring complexity provided different answers. The reason for using two models is that the simple model is easily used by restoration practitioners; but, unless the simple model were compared to a more complex model, we would not know the extent of its utility and limitations. We therefore also chose a complex model that incorporated as much current knowledge on functional aspects of fungi as we could ascertain. The level of complexity to add for the saprotrophs and parasites was clear as the functional distinctions between brown and white rot for saprotrophs and plant host for parasites are largely accepted (Ingold & Hudson 1993). It follows that we should expect a functional way to distinguish EcM fungi from each other and we based our characterization on a set of studies that have shown that EcM exploration types can be considered "functional traits" (Koide et al 2014; Pena et al 2013; Tedersoo et al 2012; Peay et al 2011) and readily available (Agerer 2001; Agerer 2006; see also *DEEMY, Information System for Characterization and Determination of Ectomycorrhizae;* www.deemy.de).

Despite the increased amount of information put into the complex model, we found that the it yielded results very similar to and highly correlated with those of the simple model. This suggests that the important ecological qualities of fungi differ at the level of functional guild type (e.g. saprotroph vs. mycorrhizal) and depend less on the subgroupings of each guild (e.g. brown vs. white rot saprotroph). This finding is consistent with evidence that predicting fungal function

across systems appears to be more strongly related to functional guilds and less related to community composition (Talbot et al 2014). Furthermore, this indicates that the simple functional guild approach is enough to add to the monitoring analysis to gain functional information about a site. Such a simple approach is attainable for even the most modest restoration monitoring projects that aim to include macrofungal observations.

A special functional guild identification tool provided by NIRMI (http://nirmi.org/identification_tools.php?pageNav=key_ecological&page=start) and valuable global checklists (Rinaldi et al 2008, Tedersoo et al 2010) can assist the non-mycologist in guiding these determinations and including these observations in monitoring. This could be used alone or in tandem with mycologists who can assist in species identification.

Functional-values may provide opportunity to distill the temporal effects of restoration as we contend that sites with higher levels of functional-value are "closer to target". The comparison of Gibson Woods and Barker Woods illustrates a general model of how the sites included in this study compare in a very broad sense. Although the sites are from a range of types and have inherent site differences, they could be considered to fall into one of two categories, those with long- or short-term maintenance. Sites like Gibson that have been under restoration-minded management for longer often had higher functional-values. This suggests that focus on restoration over the long term is important.

The S:E ratio and functional-value approaches may be important to use at different stages of restoration in forested sites. When we compare restoration sites to reference sites based on functional-values, all but one restoration site had a functional-value below that range of values for reference sites. Given this, and also the knowledge that seven restoration sites had overlapping S:E ratios with the reference sites, S:E ratios might be better used as an indicator of restoration progress early in the restoration and monitoring process while functional-values might be better used in later phases as monitoring data accumulate. In grassland sites, where few large fungi are observed, other methods are needed, such as molecular diagnostic approaches. In particular, quantitative polymerase chain reaction (qPCR) can target different lineages of AMF (e.g. Gigasporaceae vs Glomeraceae) and has been examined recently to determine the abundance of these crucial fungi in grassland restorations (C. Palmer personal communication).

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Figure 1. Number of species of macrofungi as a function of percentage (%) of canopy cover of dominant tree.

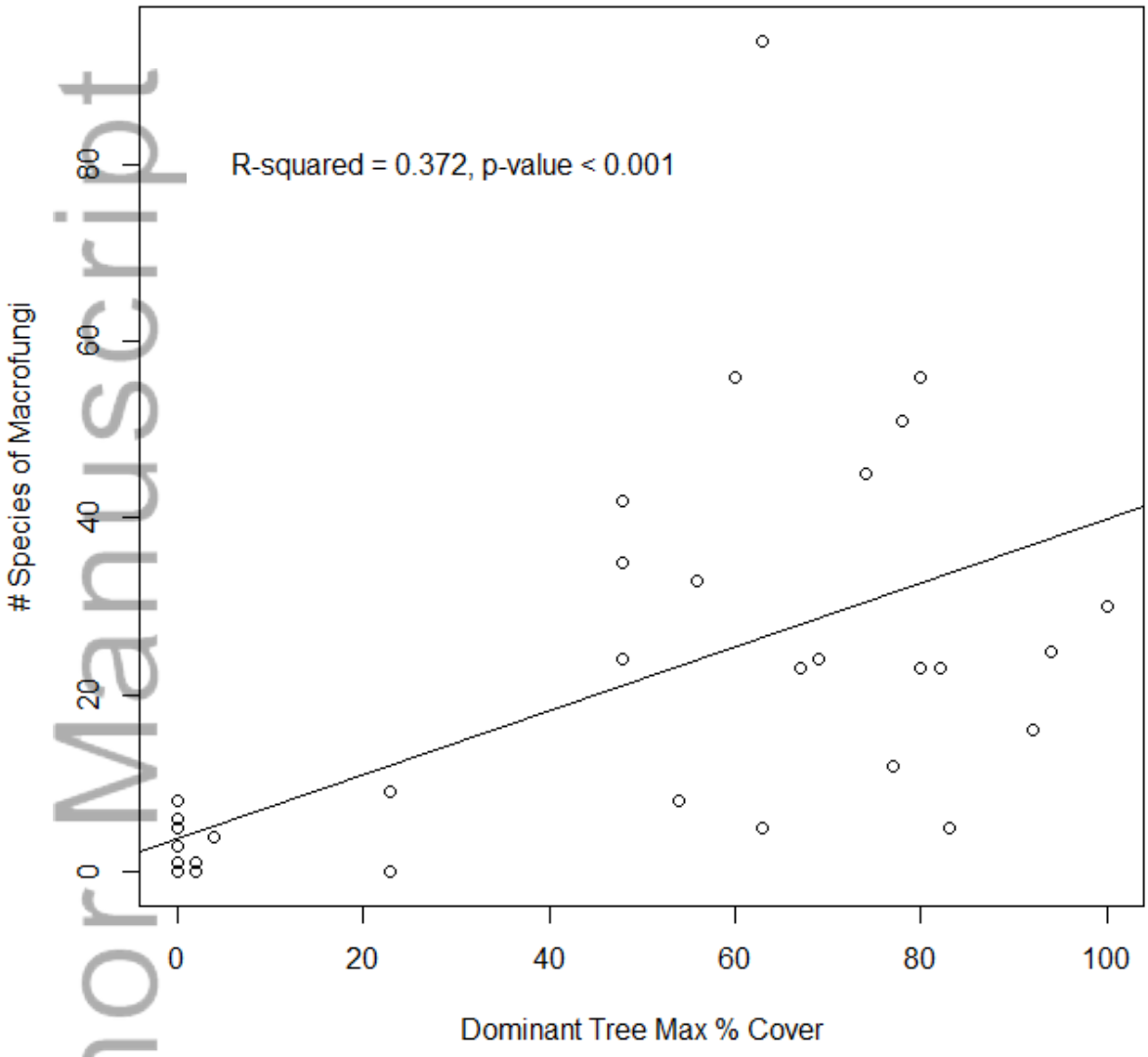

Figure 2. The relationship between log number of species of macrofungi and restoration site functional-value using the simple model.

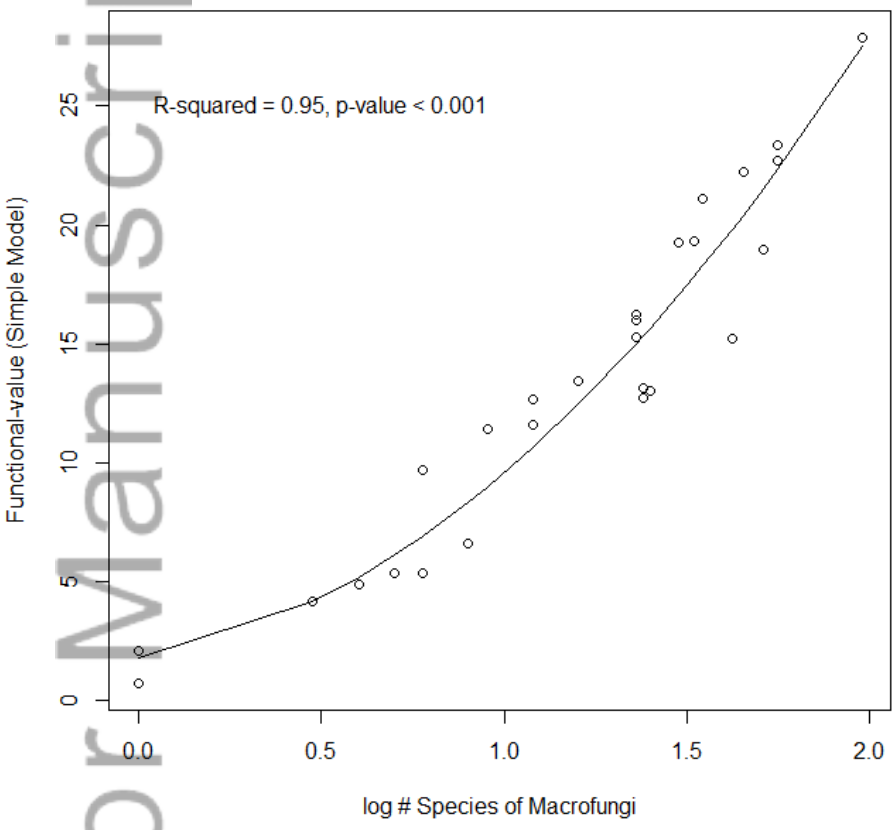

Figure 3. The relationship between site functional-value as determined using the simple model and site functional-value as determined using the complex model.