Fungal Ecology 32 (2018) 72–79

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Rivers may constitute an overlooked avenue of dispersal for terrestrial fungi

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ARTICLE INFO

Article history:
Received 25 September 2017
Received in revised form
30 November 2017
Accepted 11 December 2017

Corresponding Editor: Felix Bärlocher

Keywords:
Dispersal
Phosphorus
Freshwater
Microbial communities
MiSeq
Fungi
Environmental gradients

ABSTRACT

While fungi are intimately associated with substrates in freshwater systems, the role of fungi in the open water column is less well defined. Using next generation sequencing of 0.2 μm–1 μm filtered water column samples, we detected abundant and diverse fungal sequences across 25 stream and river sites in the Ozark region of Oklahoma and Arkansas. Fungal communities were only weakly related to stream environmental metrics with the exception of total phosphorus (TP). We infer from our results that TP is acting as a proxy for unique catchment effects. We observed patterns of dominant community taxa at higher taxonomic groupings but lower taxonomic groupings were site specific. OTU functional assignment showed the majority of sequences to be related to plant and animal pathogens, and some saprotrophs. The likely allochthonous origin and strong site specificity of these fungi suggest overlooked dispersal via lotic waterways, which may have important biogeographic consequences for fungi.

1. Introduction

Fungi in aquatic ecosystems have been extensively studied, typically focusing on substrate surfaces such as allochthonous leaf litter in freshwater in the context of decomposition (Suberkropp and Klug, 1976; Nikolcheva and Bärlocher, 2005; Sridhar et al., 2008). One of the topics that has been largely neglected is fungi in the water column not associated with substrates or sediment. One study that assessed fungal biomass in the upper 1 m of the water column in 32 temperate streams in Poland found a significant correlation between fungal biomass and total nitrogen (N) and phosphorus (P) in water using regression and Pearson correlation analysis (Gorniak et al., 2013). Community structure and role were not directly investigated but direct fungal participation in water column nutrient cycling was hypothesized (Gorniak et al., 2013).

Another possible explanation for fungal presence in the water column is simply the deposition of hyphal fragments or other potential propagules from air, upstream water, detritus deposition, and root to stream contact. Water column dispersal is well studied for aquatic hyphomycetes (Ingold, 1942; Thomas et al., 1991; Suberkropp and Wallace, 1992; Sridhar and Bärlocher, 1994) but such inputs could provide an overlooked means of dispersal for terrestrial fungi as well. In fact, fungus-like oomycete plant pathogens of the genus *Phytophthora* are well known to disperse via river systems (Li, 2016). Recent studies clearly show that some true fungi are dispersal-limited (Peay et al., 2012; Cline and Zak, 2014; Peay and Bruns, 2014), leading to strong biogeographic distribution patterns (Taylor and Bruns, 1999; Peay et al., 2010).

Most work on dispersal in terrestrial fungi has focused on movement of aerial spores (Brown and Hovmøller, 2002; Pashley et al., 2012; Savage et al., 2012; Grinn-Gofroń and Bosiacka, 2015), including next generation sequencing studies of indoor air (Amend et al., 2010; Adams et al., 2013). While aquatic hyphomycetes have received attention with respect to diversity of decomposers and macroscopic life stages (Fabre, 1998a, 1998b, 1998c), there have been few studies of the roles of river systems in the dispersal of true fungi in general, particularly by very small propagules. Given the fact that streams and rivers serve as ecological aggregators of processes throughout their watershed catchments (Frissell et al., 1986; Allan, 2004; Bormann and Likens, 2012), and that riverine dispersal

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https://doi.org/10.1016/j.funeco.2017.12.003
1754-5048/

is important in numerous other taxa, e.g. fish, insects, reptiles, and plants (Maguire, 1963; Bermingham and Avise, 1986; Bunn and Hughes, 1997; Bernatchez and Wilson, 1998; Miller et al., 2002; Santamaría, 2002; Petersen et al., 2004; Pellegrino et al., 2005; Vanschoenwinkel et al., 2008), the lack of fungal studies represents a major gap.

A potential hurdle to investigating dispersal via the water column is determining whether or not fungi detected are active aquatic community members or are transient and inactive. There is a large body of evidence linking fungi to the phosphorus (P) cycle in soils (Bolan, 1991; Schachtman et al., 1998; Van Der Heijden et al., 2008). High total phosphorus (TP) is also an indicator of excessive nutrients from catchments feeding into streams and rivers (Schindler, 1977; Carpenter et al., 1998) and has a large impact on aquatic systems (Bennett et al., 2001; Anderson et al., 2002; Hart et al., 2004). Exploring fungi across a gradient of TP allows identification of relationships of free living fungal communities to P in the water column.

The bulk of water-column particulate matter consists of eroded soils and particulate organic matter (Schlesinger and Melack, 1981; Waters, 1995; Bilotta and Brazier, 2008). Fungi are both ubiquitous in soils and are directly involved in colonizing and decomposing organic matter in streams (Christensen, 1989; Gessner, 1997). To focus on dispersal via small fungal cells, fragments, and spores in the water column, it is desirable to exclude fungi associated with particles using a method like size filtering (APHA, 1998). Here, we analyze total fungal diversity in a microscopic fraction (0.2–1.0 μm) across a river system spanning a range of TP. While many fungal cells are larger than 1 μm, we anticipated good detection of fungi through small cells, spores, and cell fragments. The ecological gradient is representative of differences in multiple catchment properties such as vegetation and nutrient cycling across the varied watersheds enhancing the exploration of relationships between fungal communities and the environment in the system.

2. Methods

2.1. Sampling

Extraction of genomic DNA (gDNA) from water-column filter samples was described in detail in LeBrun et al., (2017). The study area was a collection of mid-order (3^{rd}-5^{th}) streams and rivers along the Oklahoma-Arkansas border, an area with known P enrichment problems (Fig. S1) (Green and Haggard, 2001; Haggard and Soerens, 2006; Haggard, 2010). Sampling was performed in October of 2014. Additional site characteristics are also available through a study by Cook et al., 2017 (*in press*) where data on these sites was collected at regular intervals over 2 y. The sampling sites represented a gradient of TP levels from 7 to 173 μg/L. Sample processing involved a stacked filtration of 50 mL of water from ~10 cm below the surface in the water column through a 1 μm glass fiber filter and then a 0.2-μm filter. Only components collected on the 0.2-μm filters were used in extracting the gDNA for this study (i.e. the 0.2–1.0 μm size fraction).

2.2. Environmental data

Environmental data including dissolved organic carbon (DOC), total phosphorus (TP), total N (TN), C:N ratio, C:P ratio, sestonic chlorophyll-a, total suspended solids (TSS), turbidity, pH, dissolved oxygen (DO), temperature, and specific conductance in stream/river water along with catchment size and catchment and land use factors including percentage pasture land, percentage impervious cover, percentage developed land were collected as reported in LeBrun et al., (2017). In brief, water chemistry was measured using YSI EXO2 multiparameter data sonde (Yellow Springs, OH) and standardized water testing (APHA, 1998). Catchments were delineated using ArcGIS and land usage was estimated from the National Land Cover Data (NLCD) raster (ESRI, 2011. *ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.*, n. d.).

2.3. Library preparation

Library preparation for this study started with the gDNA collected in LeBrun et al. (2017). An initial PCR amplification of the ITS2 region was conducted using 5.8S_Fun and ITS4_Fun primers (Taylor et al., 2016) modified to include adapters for future indexing. PCR was performed using 2× Platinum™ Green Master Mix from Invitrogen. PCR specifications were 1 cycle for HotStart step at 94 °C for 2 min followed by 30 cycles of 94 °C denaturation step for 45 s, 50 °C annealing step for 1 min, and 72 °C elongation step for 1.5 min. Successful PCR was identified through 1% agarose gel electrophoresis run at 70 V for 40 min. PCR cleanup was conducted using an Agencourt AMPure XP kit (Beckman Coulter Life Sciences) following the manufacturer's protocol. Final PCR product quantification was conducted using a Qubit 3.0 system.

A second round of PCR amplification was run to add unique indices to each sample as well as Illumina sequencing adapters. PCR was again performed using 2× Platinum™ Green Master Mix. PCR specifications for the second round of PCR were 1 cycle for HotStart step at 94 °C for 2 min followed by 8 cycles of 94 °C denaturation step for 45 s, 59 °C annealing step for 1 min, and 72 °C elongation step for 1.5 min. Cleanup and quantification were performed in the same manner as the first round of PCR. Samples were then pooled so that 10 ng of DNA from each sample was present in final library.

Sequencing was performed on an Illumina MiSeq system using a MiSeq Reagent Kit v3 2 × 300 with paired-end reads. Libraries were spiked with 20% PhiX control.

2.4. Sequence processing

Demultiplexing was conducted through Illumina BaseSpace. Paired-end read FASTQ files for each sample were extracted for downstream processing. Additional sequence processing was carried out using Quantitative Insights Into Microbial Ecology (QIIME) version 1.9.1 (Caporaso et al., 2010). Paired-end reads were combined using the fastq-join algorithm from ea-utils (Aronesty, 2013). Un-paired reads were discarded at this time. The resulting sequences were then filtered with a maximum unacceptable Phred quality score of 20. Chimeric sequences were identified and removed using the UCHIME algorithm within USEARCH (Edgar, 2010). Operational taxonomic unit (OTU) picking was performed via open reference with a 0.50 pre-filter using UCLUST against the dynamic UNITE database version 7 with a 0.94 similarity cutoff. Singleton sequences were removed during OTU picking and taxonomy was assigned with the UNITE database as reference. Reads identified as Plantae or Protista were then manually removed from the resulting OTU table via filtering.

Functional information in the form of guild assignment to OTUs was performed using the online version of FUNGuild (Nguyen et al., 2016). FUNGuild parses OTUs into “guilds” or “functional groupings” based on their taxonomic assignments and ecological data extracted from the literature (Nguyen et al., 2016). Guilds are representative of species, whether related or unrelated, that exploit the same class of environmental resources in a similar way.

2.5. Statistical analysis software

All analyses were performed in the R software package v.3.2.3 (R Core Team, 2015) using various packages and scripts as identified. OTU table BIOM files from QIIME were either exported to tab delimited format directly from QIIME or imported for use in R using

the phyloseq package version 1.14.0 in R (McMurdie and Holmes, 2013).

2.6. Diversity, ordinations, models, and comparisons

Diversity metrics for Shannon (H'), Simpson (1-D), and Inverse Simpson (1/D) indices were calculated using the vegan package version 2.4–0 (Oksanen et al., 2016). Sample overlap was calculated using the Morisita-Horn index and bootstrapping (n = 200) in the vegetarian package version 1.2 (Charney and Record, 2012). Heatmaps were built using the heatmap.2 function in the gplots package version 3.0.1 (Warnes et al., 2016). Heatmap dendrograms were built using Bray-Curtis distance. Taxa that do not represent more than 1 percent of relative abundance in any site were removed post clustering for visual clarity.

Non-metric multidimensional scaling (NMDS) ordinations were constructed to describe community dissimilarity in unconstrained space using the vegan package. NMDS using Bray-Curtis distance was performed for ITS2 and Guild datasets. NMDS plots were created using the ggplot2 package in R (Wickham, 2006). Environmental gradients were built on NMDS ordinations using ordisurf from the vegan package. Ordisurf uses Generalized Additive Modeling (GAM) model building to overlay environmental variables in the ordination space (Marra and Wood, 2011).

ITS2 and Guild data were compared with a Mantel test using the mantel function in the vegan package with the Pearson correlation method and 1000 permutations in order to test for similarity between the data sets (Smouse et al., 1986). PROcrustean randomization TEST of community environment concordance (PROTEST), a potentially more sensitive detection method than a Mantel test, was also used to compare NMDS ordinations in the vegan package (Jackson, 1995). PROTEST uses scaling and rotations to maximize alignment in ordinations as a multivariate measure of concordance in datasets along with permutation based significance testing (Jackson, 1995). All PROTEST analyses were performed with 1000 permutations. Mantel and PROTEST analyses were also used to investigate relationships between fungal community assemblages and the bacterial community assemblages from LeBrun et al. (2017) for the 23 sites shared between the two studies.

2.7. Environmental groupings and analysis

Due to the experimental design focusing on a TP gradient, sites were divided into groups of Low, Med, and High TP. Group cutoffs were determined at apparent breakpoints in the distribution of collected TP data. Low for TP was set for sites below 40 μg/L and High as above 70 μg/L. The designation of groupings for each site can be viewed in Table S1. Multivariate ANOVA (MANOVA) like non-parametric analyses including PERMANOVA and ANOSIM were then performed with 1000 permutations using the vegan package to test for significant TP group related dispersion and variation.

2.8. Network construction and analysis

To visualize taxa relatedness and clustering, networks were constructed from data imported through phyloseq using the network package version 1.13.0 and igraph package version 1.0.1 in R with Bray-Curtis distances (Csardi and Nepusz, 2006; Butts, 2008). Clustering for each network was performed using a spinglass model and simulated annealing via the cluster_spinglass function in the igraph package.

2.9. Additional modeling and testing

Redundancy Analysis (RDA) models were built in the vegan package (Oksanen et al., 2016) in order to describe the community structure in environmentally constrained space for both OTU and FUNGuild data. RDA model selection was performed by starting with an initial model including all collected variables and manually removing collinear variables in an effort to maximize adjusted R^2 and minimize the magnitude of difference between R^2 and adjusted R^2.

Generalized Additive Models (GAMs) were built individually for each collected environmental variable against Shannon, Simpson, and inverse Simpson diversity scores calculated using relative abundance data. GAMs were built using the mgcv package (Wood, 2001) for multiple environmental metrics.

Indicator species analysis was performed using the IndVal function in R from the labdsv package (Roberts, 2013) with 2:6 clusters and 1000 iterations. Indicator species analysis identifies important taxa for typologies created from any classification procedure independently from the classification method (Dufrêne and Legendre, 1997).

Threshold analysis for TP and turbidity was performed using the TITAN 2.1 package in R (Baker et al., 2015). Threshold Indicator Taxa ANalysis (TITAN) identifies environmental variable values maximizing taxa frequency and abundance using bootstrapping to identify reliable indicator taxa and the sum of indicator taxa value Z scores to identify the environmental values representing the peak of increase or decline of the taxa (Baker and King, 2010). The number of bootstraps performed in our TITAN analysis was 200. The genus taxonomic level was used and only taxa observed more than 3 times across all sites were used.

3. Results

3.1. OTU counts and site diversity

We identified from 48 to 168 fungal taxa at each site across the 25-site system (mean = 85, SD = 33.25) (Table S1). Shannon index values ranged from 1.59 to 3.36 (mean = 2.35, SD = 0.53), Simpson index values ranged from 0.66 to 0.94 (mean = 0.84, SD = 0.08), and inverse Simpson index values ranged from 2.98 to 17.25 (mean = 7.99, SD = 3.90) (Table S1). The Morisita-Horn overlap index was $C_D = 0.179$ (SE = 0.0003). The heatmap of taxa at the phylum level shows groupings of sites primarily dominated by one of the phyla Basidiomycota, Ascomycota, or Chytridiomycota (Fig. 1).

3.2. Network analysis

Network analysis showed small, distinct clusters of ecologically

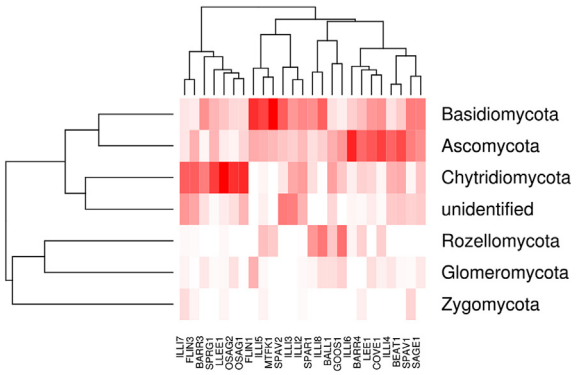

Fig. 1. Heatmap of community structure at the phylum level by site. Dendrograms are constructed with Bray-Curtis distance.

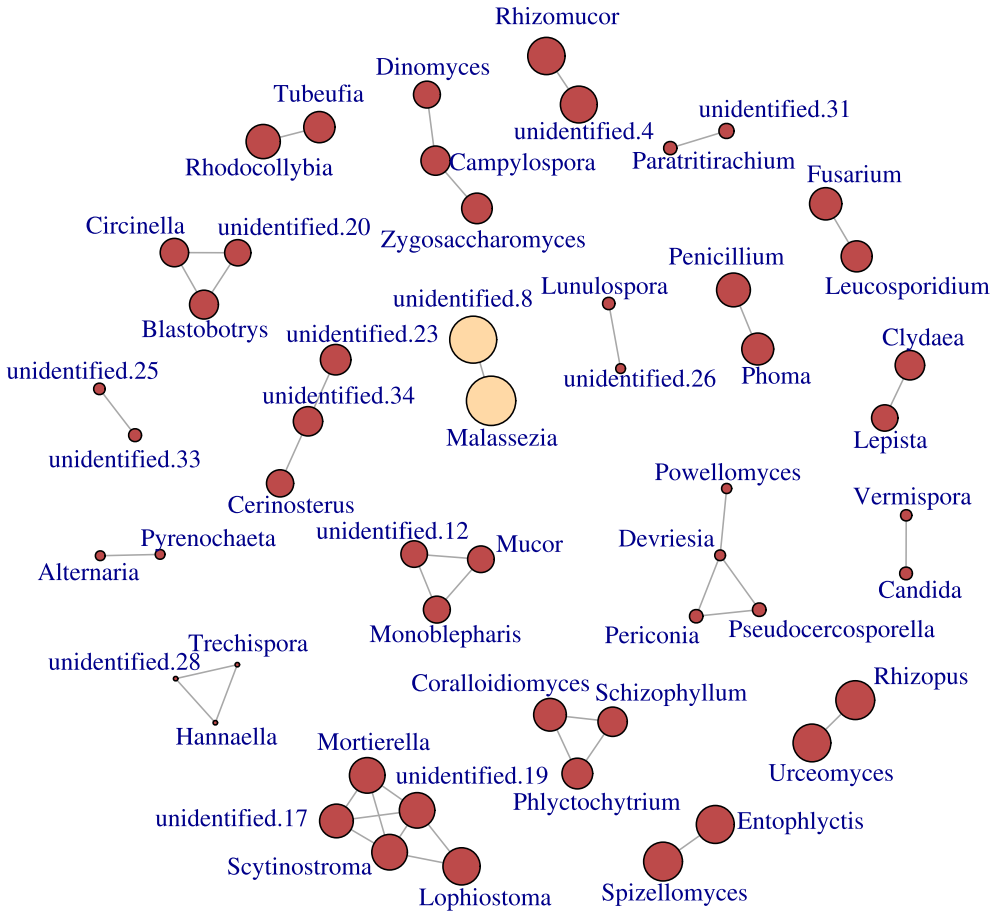

Fig. 2. Taxonomic network generated using Bray-Curtis distances. Isolated taxa have been removed leaving only taxa with at least one connecting edge. Node size represents total abundance of that taxon on a log scale. Taxa with red nodes represent less than 1% of total abundance while yellow nodes indicate taxa not considered "rare". Taxa labeled "unidentified.xy" were unable to be classified at the genus level.

related taxa at the genus level (Fig. 2). The majority of taxa represented OTUs that made up less than 1% of total abundance and so will be referred to as "rare" taxa for this study. The relationships shown are between taxa throughout the sites.

3.3. Guild designations

42% of ITS2 OTUs were classified into guilds by FUNGuild. The majority of taxa fell into animal and plant pathogen guilds as well as unidentified saprotrophs (Fig. 3).

3.4. NMDS ordinations and RDA models

For NMDS ordination with taxonomic data, sites were dispersed fairly evenly through ordination space with no apparent clusters of sites. However, a pattern of sites positioning in a related manner emerges when considering TP groupings (Fig. 4). The TP gradient fit to the ordination using GAM explained 32.6% of deviance with $p = 0.02$. NMDS ordination with the FUNGuild data showed slightly more separation between potential groups of sites (Fig. S2). The TP gradient fit to the ordination using GAM explained 48.2% of deviance with $p < 0.01$. Results from PERMANOVA and ANOSIM were both significant for TP groupings with $F = 1.48$ ($p = 0.02$) and $R = 0.131$ ($p = 0.03$).

The Mantel test between the fungal ITS2 data and the bacterial 16S NMDS data from LeBrun et al. (2017) for the 23 overlapping sites showed no significant correlation ($r_m = -0.033$, $p = 0.94$). Procrustean PROTEST comparison between fungal and bacterial NMDS ordinations had a correlation statistic of 0.48 with $p = 0.013$. The Procrustes error plot is shown in Fig. S3.

Attempts to model taxonomic community structure using Redundancy Analysis (RDA) and GAMs with collected environmental and land use variables were unproductive as none of the environmental variables resulted in a significant model. The final RDA model for FUNGuild data included TP, carbon to P ratio (C:P), DOC, DO, temperature, and pH with an adjusted R^2 of 0.345 and $p = 0.014$ (Fig. 5). The direction of C:P was the antithesis of to DOC and TP, indicating a relationship differing from TP or DOC, and so it was kept in the model.

3.5. Indicator species and total phosphorous gradient relationship

Indicator species analysis was able to identify a few indicator taxa in our system; however, the results were not consistent across multiple runs and clustering levels, and identified taxa were few and most only weakly significant. Two taxa that were consistently identified were the genus *Hygrocybe* ($d = 0.9804$, $p = 0.014$) at lower clustering levels (2–6 k-means clusters) and the genus *Entophlyctis* ($d = 0.948$, $p = 0.039$) at higher clustering levels (6–10 k-means clusters). Unfortunately, the reason for these species as indicators for the relevant sites remains elusive, although the log abundance of *Entophlyctis* was weakly related to TP by GAM (Deviance explained = 9.33%, $p < 0.001$). TITAN was unable to identify any reliable indicator taxa or change points in system

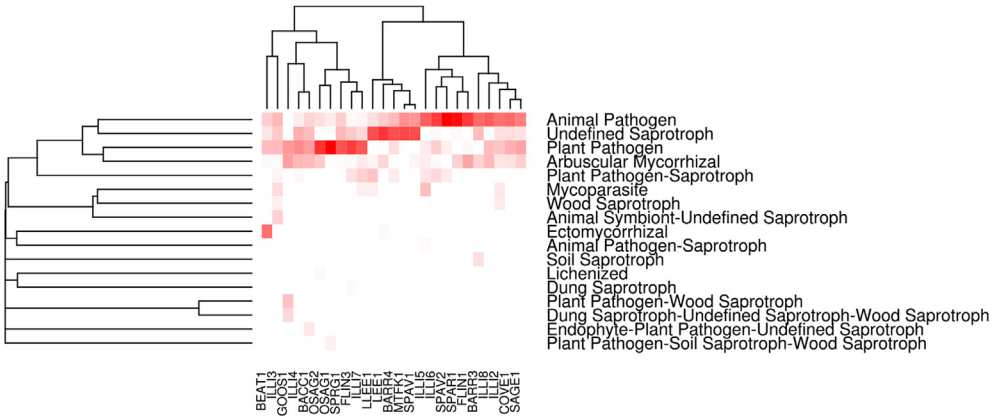

Fig. 3. Heatmap of FUNGuild identified guild abundance by site. Dendrograms are constructed with Bray-Curtis distance.

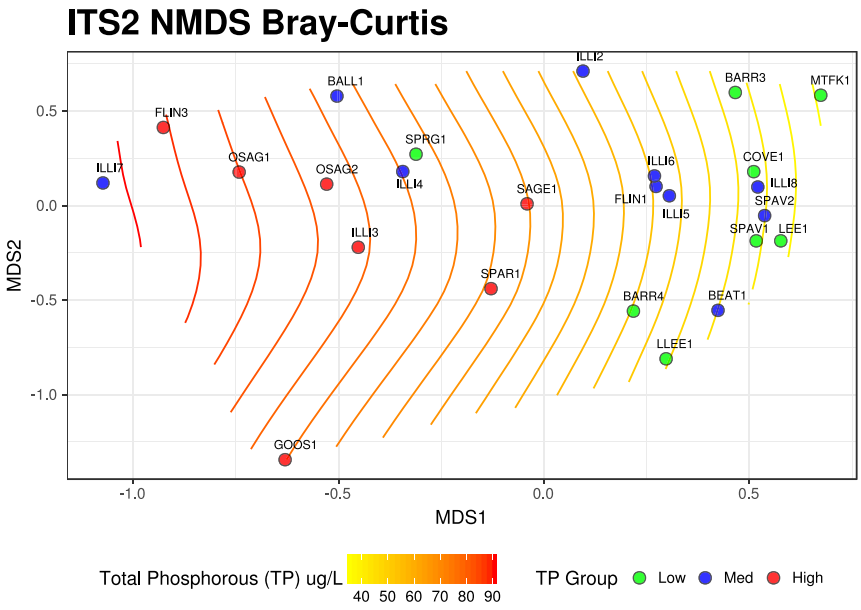

Fig. 4. NMDS ordination of ITS2 community with Bray-Curtis distance (Stress 0.155). Gradient represents environmental TP fit to ordination using GAM.

related to the TP gradient.

4. Discussion

4.1. Site specificity of fungal assembly

Taxa in the system showed a high site specificity. Visually, site distributions in ordination space were widely dispersed and taxonomic heatmaps (Fig. S4 – Fig. S8) illustrate the increasing site specificity at lower taxonomic levels. This increase is to be expected; however, even at the class level, sites are distinct. We also found quantitative support for this site specificity. For Morisita-Horn, $C_D = 0$ represents a system of samples with no overlap, while $C_D = 1$ represents a system of samples with complete overlap (Jost, 2007). Thus, the observed value of $C_D = 0.179$ indicates very little taxonomic overlap. Little community overlap in the system likely affected network analysis with the majority of taxa being removed from the final network due to nodes having no edges along with making it difficult for indicator species and TITAN analyses to identify indicator taxa. In a previous study on bacteria and archaea within the same system, we were able to establish that there were no overwhelming effects of stream connectivity or downstream flow in the system (LeBrun et al., 2017). The site specificity in the current study also illustrates a lack of effects caused by any site flow connectivity (Fig. S8).

4.2. Taxa relationships identified by network analysis

The majority of identified relationships involve rare taxa (Fig. 2); taxa sharing a relationship tended to be found in the same abundances within the system. This finding could be indicative of taxa replacing each other in roles within the different catchments or may be an artifact of the network construction due to site specificity. Either way, these relationships warrant additional investigation. These rare taxa showed no relationship to environmental variables in the stream when separated from the overall community.

4.3. Nutrient cycling and environmental interactions

Analyses investigating fungal relationships to TP provided mixed results. The GAMs built with the ordinations for both taxonomic and FUNGuild data show a significant relationship between

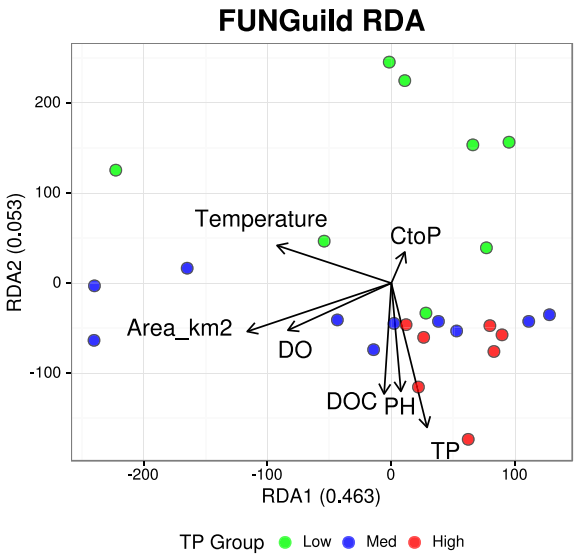

Fig. 5. RDA model of environmental variables and FUNGuild designations at each site (adj. $R^2 = 0.345$, $p = 0.014$).

TP and placement in ordination space. It must be taken into account that both ordinations had stress values bordering on the "suspect" range (0.155 & 0.104). However, a relationship to TP is supported by the significant PREMANOVA and ANOSIM tests. The taxonomic composition of the detected fungi did not correlate with any variables in RDA. Diversity was also unrelated to environmental metrics as shown by the lack of significant GAM models.

Functional composition of the fungal assemblage via FUNGuild designations appears to be more closely tied to collected environmental variables than was taxonomic composition. The TP gradient explains a higher percentage of deviance in the ordinations and the RDA model ties the functional designations to catchment and stream variables. The decoupling between taxa assemblage and function makes sense in light of the specificity of taxa at each site. However, the primary functional designations in the system, animal/plant pathogens and saprotrophs, do not intuitively apply to non-substrate (i.e. not on detritus, other organic material, or sediment) water column processes, meaning these fungi are likely transient.

Bacterial/archaeal communities in this system have previously been connected to wider ecological data (LeBrun et al., 2017) and the detected fungal communities are only weakly related to the bacterial communities. The small but surprisingly significant PROTEST correlation score is probably indicative of catchment effects, spatial autocorrelation, or a relationship to an allochthonous, terrestrial subset within the bacterial community. Ordinal TP GAMs explain significant site organization in ordination space for both fungi and bacteria. PROTEST is known to be more sensitive than a Mantel test (Jackson, 1995) and so is capable of capturing these types of minor effects; however, the Mantel test was insignificant and the Procrustes error plot shows no cohesive trends or patterns (Fig. S3).

We infer from the collective results that a correlation does exist between the fungi detected at each site and TP but that the fungi are likely transient and allochthonous rather than being active water column community members. TP likely represents unique catchment features such as vegetation, that are difficult to identify due to a large number of environmental factors that could not be included in this study such as vegetation. Stream TP is a product of numerous catchment factors, so covariation of these factors with TP is very possible (King et al., 2005). This allochthonous origin is further supported by the identification of several taxa within the system such as the *Hygrocybe* identified in indicator species analysis. *Hygrocybe* is a terrestrial agaric with spore sizes larger than expected to pass through our 1-μM filter. Hence, it is unclear why it was so readily detected or whether we were capturing DNA from spores or cell fragments. While our findings corroborate prior findings of a TP correlation with fungi in the water column (Gorniak et al., 2013), our best guess based on our analyses of community structure and predicted function is that these fungi are not major participants in nutrient cycling within the water column itself.

4.4. Relationship of P to pathogens

In both constrained and unconstrained ordinations of FUNGuild data, there is a clear distinction between placement of Low and High TP grouped sites with Med grouped sites somewhat mixed in with one or the other. An increase in the abundance of some bacterial pathogens coincided with increased TP in a previous study of the same system (LeBrun et al., 2017). There appears to be a high potential for factors that result in high P (e.g. agricultural pollution or waste water inputs) to have a relationship to the presence and abundance of both bacterial and fungal pathogens. Pathogens in soil can cause negative density dependence in communities of vegetation (Laliberté et al., 2015). Distribution of vegetation has a strong relationship to both P levels and soil microbial communities (Langille et al., 2013). All of these inputs affect waterways (Bormann and Likens, 2012). While we have not established causation in the relationship between P and pathogens, there exists a potential feedback loop of pathogens affecting P and P affecting pathogens within catchments and waterways. This is likely a complex relationship that would require further study to fully understand as our data only hint at such a possibility.

4.5. The water column as a dispersal medium

There are multiple lines of evidence suggesting an allochthonous origin for fungal taxa detected in this study. The identified taxa are not likely active in stream nutrient cycles or processes and they are only loosely related to bacterial communities in the system, if at all. Our focus on a smaller size fraction favors detection of small cells and fragments. In guild analysis, most taxa were identified as pathogens and saprotrophs. Organisms in these categories have potential benefits from aquatic dispersal as streams are rife with the detritus for saprotroph colonization and have access to vegetation and animals for pathogen colonization.

Phytophthora, although not a true fungus, offers an excellent example of pathogen delivery to terrestrial plants via streams. Although *Phytophthora* is known for infecting agricultural and wild plants on land, its primary method of dispersal is through the water column and monitoring and detection is carried out directly in streams (Sutton et al., 2009; Hulvey et al., 2010). A fungal pathogen dispersing through an aquatic system is less dependent on chance than it is through aerial dispersal. The water column might act as a vehicle of pathogen delivery directly to a host, be it plant or animal. In addition, aquatic dispersal offers the advantages of protection from drought and UV stress that apply strongly to aerial dispersal. Further investigation of fungal pathogen dispersal patterns through natural streams and rivers seems warranted, as anthropogenic inputs to streams continue to grow (Søndergaard and Jeppesen, 2007).

5. Conclusions

Here we have documented a surprisingly large number of fungal taxa occurring in the 0.2 μm - 1 μm fraction of the water column. These taxa mostly consist of pathogens and saprotrophs from the

Basidiomycota, Ascomycota, and Chytridiomycota but their species composition varied greatly by site. We infer that these taxa are likely present due to deposition from allochthonous sources. The site specificity, diversity, and abundance of terrestrial fungi suggest an overlooked means of dispersal that could promote or reinforce biogeographic patterns in terrestrial fungal communities.

Conflicts of interest

The authors declare no conflict of interest.

Statement of data availability

Sequence data that support the findings of this study have been deposited in GenBank with the BioProject accession code PRJNA350288. The environmental data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

The authors thank Morgan Bettcher, Stephen Cook, Stephen Elser, Katherine Hooker, Lauren Housley and Caleb Robbins for their help in collecting field samples. We acknowledge the research support by Baylor University Office of Research and Baylor University Center for Reservoir and Aquatic Systems Research (CRASR).

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.funeco.2017.12.003.

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