5-2022

Nitrogen availability in dune systems and Its effect on root fungal endophyte communities.

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Nitrogen Availability in Dune Systems and Its Effect on Root Fungal Endophyte Communities

By

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Submitted in Partial Fulfillment for Graduation *summa cum laude*

and

for Graduation with Honors from the Department of Biology

University of Louisville

May 2022

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Lay Summary

As global change persists, changes in resource availability have the potential to influence root fungal endophytes, which are fungal species inhabiting plant root systems. To better understand how resource availability can influence these interactions and species diversity, I focused this research on how varying nitrogen (N) levels affect root endophyte communities in the plant species *Ammophila breviligulata*. This plant species, known as an ecosystem engineer, is critical to the survival of the dune system. I analyzed the relationship between nitrogen addition and microbial community composition across 60 plots treated with three nitrogen addition levels (control, low, and high) in a long-term experimental field site in the Lake Michigan dunes. I identified *Ammophila breviligulata's* root endophyte community after creating a culture collection and performing DNA analysis on samples from each of the 60 plots. I clustered genetic sequences into sixteen taxonomic units using a statistical analysis medium before entering them into genomic databases for species identification. The data also resulted in identifying three species significantly associated with specific treatment levels. Our results indicate that any level of nitrogen addition had cascading effects on the endophyte community. This research contributes novel insight to the more significant discussion of global change and discusses the implications of increased atmospheric nitrogen on microbial communities. Our findings can further research focused on better equipping our environment for the imminent global change threat.

Abstract

As global change persists, changes in resource availability can influence plant-microbe interactions. To understand how resource availability can influence these interactions and species diversity, I focused this research on how varying nitrogen (N) levels affect root endophyte communities in the plant species *Ammophila breviligulata*, an ecosystem engineer in the dune system. I analyzed the relationship between nitrogen addition and microbial community composition across 60 plots treated with three nitrogen addition levels (control, low, and high) in a long-term experimental field site in the Lake Michigan dunes. I identified *Ammophila breviligulata's* root endophyte community after creating a culture collection and performing DNA analysis on samples from each of the 60 plots. Our results indicate that any level of nitrogen addition had cascading effects on the endophyte community. I identified sixteen operational taxonomic units (OTUs) and sixty-six morphospecies using genetic sequence clustering techniques. Indicator species analysis identified three species significantly associated with specific treatment levels. This research contributes novel insight to the more extensive discussion of global change and the implications of increased atmospheric nitrogen on microbial communities. Our findings will further research focused on better equipping our environment for the imminent global change threat.

Introduction

The global change crisis is rapidly advancing and causing cascading effects from microbiomes to entire ecosystems. Fungal endophytes are microorganisms that live within plant roots, stems, or leaves. They can impact the host species as either a mutualist or parasite depending on a multitude of both biotic and abiotic factors (Jumpponen et al., 1998). While some well-established research on leaf and stem endophytes is available, root fungal endophyte research is novel, and their significance is relatively unknown (Rodriguez et al., 2009). As the biosphere continues to change rapidly, it is even more imperative that plant symbionts are studied to find possible mutualistic relationships and evaluate their ability to support plant nutrient uptake and stress tolerance in ever-changing ecosystems (Kivlin et al., 2013).

Nitrogen deposition, nitrogen input from the atmosphere into the biosphere, is increasing dramatically and is particularly important in the larger conversation surrounding global change. Researchers have found that nitrogen deposition is primarily due to increased agricultural and industrial activity (Zhang et al., 2021). The effects of nitrogen deposition can be catastrophic to community composition and species diversity.

In plant communities, it is well known that nitrogen can be a limiting nutrient impacting plant species richness (Stevens et al., 2004). Previous research has found that whether this impact on plant species and their foliar fungal endophytes is negative or positive is both community- and species-dependent (Simkin et al., 2016). With added nitrogen in the biosphere, selection favors plants well-adapted to high nitrogen levels, leading to some species out-competing native species well-adapted to low nitrogen levels, ultimately decreasing species diversity and richness. (Stevens et al., 2004). There is currently very little research on the impact this may have on the microbial community, specifically root fungal endophytes. The cascading

effects nitrogen deposition may have on microbes could lead to a better understanding of how ecological communities will adapt to increased nitrogen in the biosphere.

The ecology of sand dune systems is particularly fascinating regarding resource availability, considering they are typically low nutrient systems. When investigating increased nitrogen deposition in the environment, looking towards systems like the dunes can provide clear insight into nitrogen addition's impact on low nutrient systems. This system is especially applicable because its natural lack of nutrients decreases confounding variables within the experiment. The results of this experiment allow insight that is globally relevant as resource availability continues to change drastically. Species historically present in sand dunes have evolved to be successful in this nutrient-deficient habitat and will be heavily impacted by increased nitrogen richness (Frosini et al., 2012).

Nitrogen addition has been found to have both positive and negative relationships with plant species richness and is often dependent on the level of nitrogen added and the ecosystem the experiment is taking place in (Simkin et al., 2016). One relevant hypothesis could be modeled off the findings that nitrogen deposition leads to a substantial decrease in plant diversity but a nominal decrease in foliar fungal endophyte diversity and only a slight decrease in endophyte species richness (Henning et al., 2020). This research would support a similar finding in the dune system and root fungal endophyte species diversity and richness. However, an alternative hypothesis could be shaped by another study that indicates nitrogen deposition's effect on foliar endophyte species richness is dependent on the ecosystem as a whole and the level of nitrogen added. This research found that with low nitrogen levels, species richness increased, and with high levels, it decreased (Simkin et al., 2016). It pinpointed nitrogen deposition as a significant threat to plant diversity but recognized that the extent of this threat is

determined by the ecosystem's ability to adapt to different resource conditions (Simkin et al., 2016). Although both studies have different findings and rationale, they both suggest that nitrogen addition's impact is dependent on both the species and the ecosystem in which it lives.

This research aimed to analyze how resource availability, with a particular focus on nitrogen, impacts the root fungal community. Our overarching question was: Does nitrogen addition in dune systems impact root fungal endophyte communities? We conducted this research using the plant species *Ammophila breviligulata*. *Ammophila breviligulata* is an ecosystem engineer in dune systems along the Great Lakes and other coastal dunes that colonizes dune blowouts and uses its root system to accumulate and stabilize sand (Emery et al., 2015). Ecosystem engineers, in general, are species that are vital to building and maintaining habitats (Emery et al., 2015). Our field site located at the Leelanau State Park dunes on Lake Michigan, is especially applicable because of its coastal location, the dune system's proximity to industrialized regions like Chicago, and the focus on an ecosystem engineering plant species. Since previous research has shown that nitrogen decreases diversity and decreases species richness in aboveground foliar microbe communities (Henning et al. 2020), I expected to find a decrease in both diversity and species richness within nitrogen addition plots. Because nitrogen deposition is a significant threat to plant diversity, it is imperative to find whether root fungal endophytes, important plant symbionts, are threatened.

Methods

Experimental Design

Ammophila breviligulata is an ecosystem engineer in the dune system, making them vital to building and maintaining the habitat. A long-term experiment conducted by Dr. Sarah Emery and collaborators at Leelanau State Park on the shore of Lake Michigan evaluates the species *Ammophila breviligulata* under different resource availability conditions (Emery et al., 2015). The field site is in a coastal region near industrialized cities with increasing nitrogen deposition levels. The site consists of ninety plots established in 2011 on a blown-out dune in Leelanau State Park, MI. Each of the ninety original plots, once bare, were initially planted with *Ammophila breviligulata.*

Nitrogen Treatment

Starting in 2016, the plots at the field site were manipulated with three different nitrogen treatments levels: high nitrogen addition, low nitrogen addition, and control levels of nitrogen, mimicking different severities of nitrogen deposition. Twenty plots were treated with the low nitrogen addition (0.5g NH^{4+} m^{-2}) fertilizer, 20 plots were treated with high nitrogen addition (10g NH^{4+} m^{-2}) fertilizer, 20 control plots were left at ambient conditions and received no additional fertilizer to serve as a control, and the remaining thirty plots have been let at ambient conditions since the field site was established. The nitrogen treatment levels were intentionally chosen to represent the levels of nitrogen deposition the dune system will endure with continued global change (Emery et al., 2015). The high-level treatment was chosen to release the plant from nutrient stress, while the low nitrogen addition treatment is comparable to current nitrogen

levels in the industrialized area of Chicago, Illinois (De Vries et al., 2018). Control levels were tested to compare the high and low nitrogen level outcomes with current ambient levels.

Initial Morphospecies Identification

During the July 2020 field season, two root samples from each of the sixty treatment level plots (n=120) of *Ammophila breviligulata* were collected and transported to the University of Louisville. Using the Rudger's protocol (Jumpponen et al., 2017), surface sterilization was conducted to isolate only the fungus growing within the plant roots. First, I cut the roots into small sections before initially washing them with tap water. After this preliminary step, I continued with the protocol. The process was as follows: I placed the roots placed into 95% ethanol for four minutes, then into a 1% Clorox solution for one minute, next I washed the samples with 70% ethanol for two minutes, and finally, they were washed with autoclaved water using sterile techniques (Jumpponen et al., 2017). After surface sterilization of each plant root, I placed the samples into Petri dishes containing 2% malt extract agar. These plates also contained *Penicillin streptomycin*; an antibiotic used to decrease the risk of bacterial contamination.

Plates from individual *Ammophila breviligulata* plant roots each grew multiple fungal species. I then sub-cultured each of these plates until only one morphospecies (a putative species based on the physical appearance of the fungus) was present in each agar plate. This subculturing process yielded over a thousand plates and allowed us to catalog which fungal morphospecies was present in each plot. Once each morphospecies identified in the original dataset was isolated properly and without contamination, the culture collection consisted of 1500 malt extract agar plates. These plates, each with only one morphospecies growing, were representative of the

morphospecies present in each plot. I identified over 130 morphospecies by cataloging the physical appearance of each culture.

DNA Extraction

DNA was extracted from multiple samples of each morphospecies present in the culture collection. I used molecular techniques to refine the identity of each morphospecies. First, I extracted DNA from each sample and performed PCR using Sigma Extract n' Amp Plant PCR Kit (Weber et al.). First, I added 100 µL of extraction buffer to a tube containing metal beads, then a small sample of fungus up to only 0.5 square centimeters in size was also placed in the tube. I then inserted it into a homogenizer, also known as a bead-beater, to break down the tiny sample into a homogenous mixture. This mixture was then heated at 95˚C for ten minutes. One hundred µL of dilution buffer was added to the sample before freezing at -22˚C for storage until Polymerase Chain Reaction and Gel Electrophoresis were performed on each sample. I performed this entire extraction process in a laminar flow hood to decrease the likelihood of contamination. During this process, I saved a small sample of each fungus in a voucher to allow for long-term storage.

Polymerase Chain Reaction and Gel Electrophoresis

The polymerase chain reaction (PCR) technique was used to both target and amplify a specific fungal DNA region called the internal transcribed spacer (ITS) gene, which is present in all fungal species and widely varies among species. To allow for future phylogenetic analyses, I chose to amplify a portion of the gene slightly more significant than the ITS region; I did this using the forward primer ITS1F and the reverse primer LR3. PCR is a lab technique that

replicates and closely resembles the process of natural DNA replication. Instead of helicase breaking the bonds between nitrogenous base pairs, heat starts the denaturation phase. The forward and reverse primer were selected using previous research as a model to ensure that only the target region was flagged for amplification. The annealing phase temperature is primer dependent, so for the selected primers ITS1F (Gardes & Bruns, 1993) and LR3 (Vilgalys, 1992), 55˚C was the optimal temperature for the primers to bind to the target region of the genome. The third and final phase is the extension phase and refers to replicating the DNA strand that the primers have marked. These three phases are all heat-dependent and are done using a BioRad T100 Thermal Cycler. The thermocycler settings were conducted as follows: three minutes of denaturation at 95 ˚C, 30 seconds of annealing at 55 ˚C, and 45 seconds of elongation at 72 ˚C. Each of these settings was repeated for 35 cycles, with the final cycle adding a ten-minute final extension at 72 ˚C. The product of PCR is essentially millions of copies of the same strand of DNA.

I performed gel electrophoresis on each sample after PCR was complete to confirm successful gene amplification. Gel electrophoresis uses the high polarity of DNA molecules to classify the length of DNA fragments. The bands on the gel allowed us to visually confirm the successful amplification of the 1200 base pair gene. I also included a negative control for each PCR and used gel electrophoresis to confirm that each PCR reaction was clean of any contamination. If there was no band in the negative control, the sample was likely clean.

Sample Purification

After the PCR process was complete, each of the hundreds of completed PCR samples was purified before sequencing the DNA. The purification process used was one of two popular

methods that clean up access primer and base pairs leftover in the PCR product. The clean-up process included the addition of two different enzymes, exonuclease one and shrimp alkaline phosphatase (Nucleics 2021). Exonuclease 1 is an enzyme with nucleic acid cleaving capabilities and was added to break down excess primer in the sample. Phosphatase enzymes can remove phosphate groups from proteins, and in this reaction, Shrimp Alkaline phosphatase was used to break down excess deoxyribonucleotide triphosphates. Both excess primer and deoxyribonucleotide triphosphates are waste created during the PCR technique. After the samples were successfully purified, I sent the samples to Eurofins Genomics (Louisville, Kentucky) for DNA Sanger sequencing.

Species Identification

In total, I sequenced 150 purified PCR reactions. Raw sequence data were obtained for each of the samples and used to identify the species sampled. Using the sangeranalyseR package in R, I combined the forward and reverse sequences (Chao et al., 2021). These consensus sequences were used later to cluster sequences at a 95% sequence similarity threshold.

I used a blended approach to cluster the data for further analysis. First, the R package Kmer (Vinga & Almeida 2003) was used to identify the Operational Taxonomic Units (OTU) within the dataset. Essentially this technique clusters like genomic sequences, with a level of ninety-five percent similarity between the sequences. These OTUs were then entered into the NCBI Basic Local Alignment Search Tool Database (BLAST) (Altschul et al., 1990). This database returned species identification matches for each. The sequences were then entered in the Warcup (Wang et al., 2007) and UNITE (Wang et al., 2007) databases which also returned

species identifications. The results of each sample were compared to account for discrepancies between databases.

Statistical Analysis

All statistical analyses were completed using the R project for statistical computing (R Core Team, 2020). This programming language is widely used in the biology research community. Based on the currently available sequence data, many of the fungal isolates were unable to be clustered into OTUs at this time. Instead, I classified those fungi based on their morphospecies identification. This blended approach (molecular and morphological) provides the most complete but conservative description of the fungal community that is currently possible. I analyzed the composition of the fungal community using multidimensional analysis to account for the lack of normal distribution and multiple variables impacting the data analyses; this was done using the R vegan package (Dixon, 2003). A permutational multivariate analysis of variance (PERMANOVA) test was run using the R Vegan package (function 'adonis') to test how nitrogen affected fungal community composition. PERMANOVA tests compare the dispersion of multiple groups. ANOVA tests were also utilized to evaluate the impact of nitrogen treatment on species diversity and richness. ANOVA analyzes variance by comparing the mean of a response variable to multiple groups.

I used an indicator species analysis using the indicspecies package in R (De Cáceres et al., 2011) to determine which OTUs were significantly associated with specific nitrogen treatments. Indicator species analysis can provide helpful insight into species that potentially play critical functional roles within a system.

Results

OTU Species Identification

Sixteen OTUs were identified within the dataset, with an additional sixty-six morphospecies that were unable to be clustered into OTUs. The two most abundant OTUs in the ninety test plots were identified to be *Microdochium bolleyi* and *Kohlmeyeriopsis medullaris* (Table 1). *Microdochium bolleyi* was identified 108 times in the dataset, while *Kohlmeyeriopsis medullaris,* the second most abundant species was, only identified 16 times in the dataset.

OTU Number	*BLAST Database*	*Warcup Database*	*Unite Database*
*1**	Microdochium bolleyi [100%]	Microdochium bolleyi [100%]	Microdochium bolleyi [100%]
2	Myrmecridium schulzeri [100%]	Fusarium [73%]	Sodariomycetes [13%]
3	Sarocladium kiliense [97.70%]	Sarocladium [89%]	Acremonium [100%]
6	Talaromyces amestolkiae [99.79%]	Penicillium minioluteum [99%]	Talaromyces marneffei [100%]
7	Penicillium bilaiae [100%]	Penicillium bilaiae [100%]	Penicillium bilaiae [100%]
8	Alternaria [100%]	Alternaria [100%]	Dothiomycetes [92%]
9	Pleosporales [98.69%]	Massarina [86%]	Dothideomycetes unidentified [100%]
11	Phlebiopsis crassa [99.51%]	Phlebiopsis gigantea [93%]	Phanerochaete [100%]
13	uncultured fungal clone [98.38%]	Helotiales [75%]	Helotiales [79%]
14	Cadophora malorum [99.16%]	Pezizomycotina [100%]	Ascomycota unclassified [92%]
15	uncultured Exophiala [98.76%]	Pezizomycotina [100%]	Exophiala equina [100%]
*16**	Kohlmeyeriopsis medullaris [99.85%]	Hypocreales [96%]	Hypocreales [89%]
46	Sarocladium strictum [98%]	Microdochium bolleyi [99%]	unclassified fungi [100%]
47	fungal sp. [98.88%]	Microdochium bolleyi [100%]	Microdochium bolleyi [69%]
48	Microdochium bolleyi [92.22%]	Microdochium bolleyi [100%]	Microdochium bolleyi [100%]

Table 1. DNA sequences of the fungal OTUs were entered into three databases: BLAST (Altschul et al. 1990), Warcup, and UNITE (Wang et al. 2007). These databases each identified

either the species or higher taxonomic group based on genetic sequence similarity and identified a percent match rate. The two most abundant fungal OTUS are identified in the table with an asterisk.

Species Richness and Species Diversity

There was no relationship between increased nitrogen addition and species richness (p=0.232). No relationship was found between Shannon diversity and nitrogen addition (Figure 1; p=0.271).

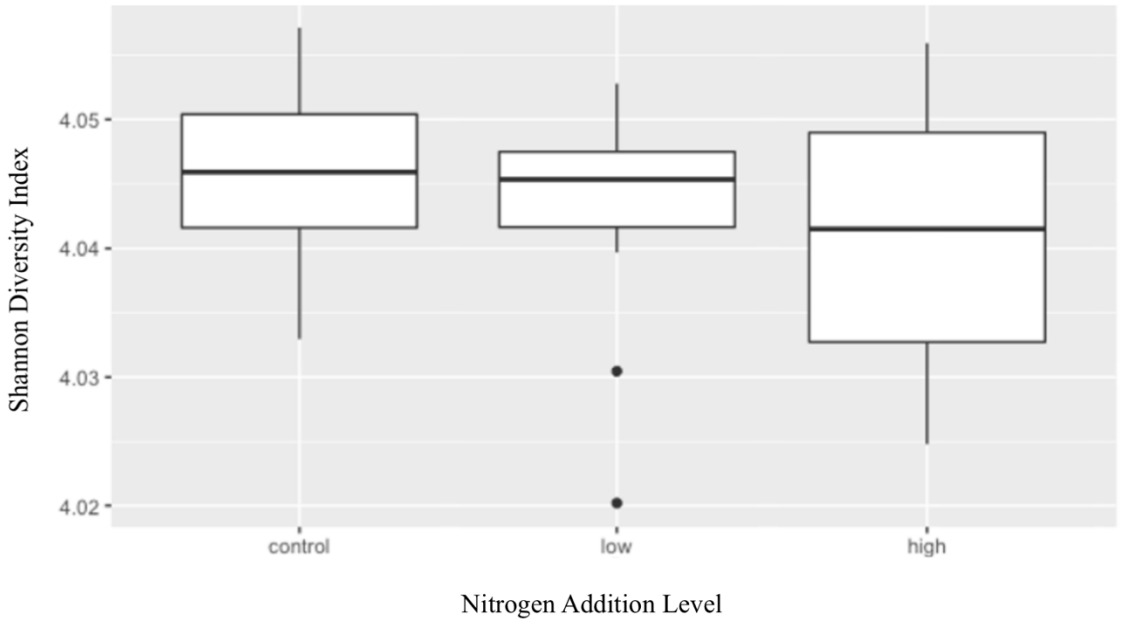

Figure 1. Changes in fungal species diversity (Shannon Diversity, H') in response to nitrogen addition.

Fungal Endophyte Community Composition Under Differing Nitrogen Conditions

Results from our PERMANOVA show Nitrogen levels alters the community composition of root endophyte species (p=0.073). The p-value from these analyses is greater than the typical

range of 0.05 used to identify statistical significance in datasets. When analyzing this data, I am taking a more liberal approach to the p-value because this data is trending towards statistical significance. Endophyte communities in high nitrogen plots show very minimal overlap with those in control nitrogen plots. Endophyte communities in low nitrogen plots overlap with both high and control plots but are more similar to high nitrogen plot community composition.

Following those results, I used an indicator species analysis to show that OTU 7 and 14 are abundant in low nitrogen plots, and OTU 13 is abundant in high nitrogen plots (Table 2).

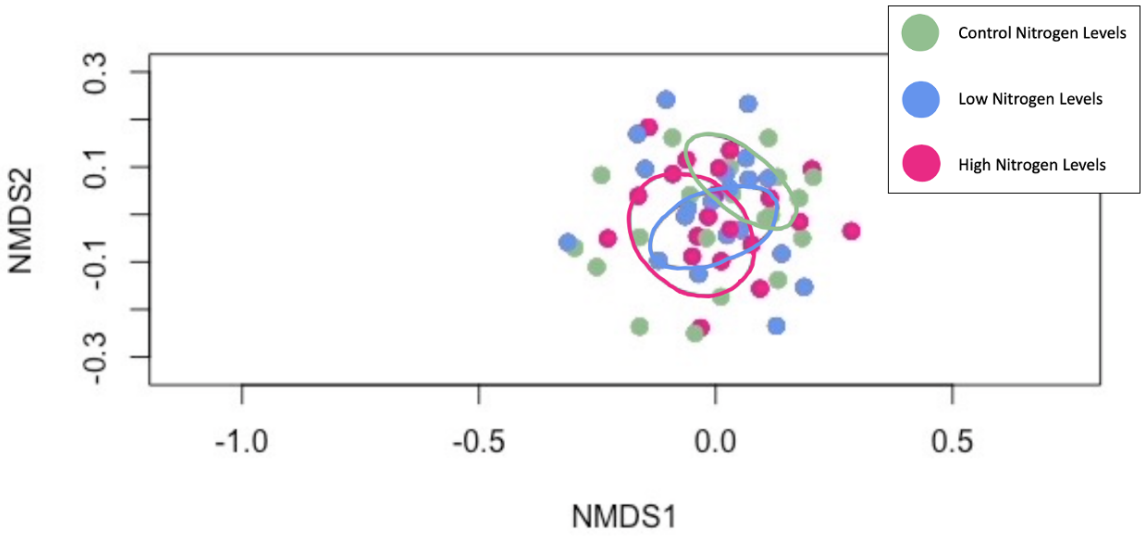

Figure 2. Non-metric multidimensional scaling (NMDS) of root endophyte community composition with a permutation of 999 (p=0.073). Each ellipse represents the community composition of different treatment levels based on species within the plots. The ellipses are drawn based on standard deviation point scores.

Fungal OTU	Database consensus species	Significance	P-VALUE
7	*Penicillium bilaiae*	Correlated with low nitrogen plots	0.044
13	*Helotiales*	Correlated with high nitrogen plots	0.039
14	*Cadophora Malorum*	Correlated with low nitrogen plots	0.044

Table 2. Indicator species analysis on fungal OTUs and their relationship to nitrogen addition plots.

Discussion

OTU Species Identification

One of the Operational Taxonomic Units identified was more common than the rest in the dataset. Using the database results with the highest percent match rate, the species *Microdochium bolleyi* (n=108), corresponding with OTU 1, was identified as the most abundant species in the plots. This species is common in all three nitrogen levels, which indicates their resilience under different resource availability conditions.

Due to the high abundance of *Microdochium bolleyi*, it is critical to understand this species' function and natural history. This species is a very common endophyte among dune beachgrass species (David et al., 2016). Its function within its host species has not been determined, but it is typically considered a weak pathogen or commensal species (David et al., 2016). Further research on the function of this species would lead to a better understanding of its ability to survive in nutrient deficient systems and its relationship with host plants..

Nitrogen addition effects on richness and diversity

Leaf endophytes have shown decreased species diversity and decreased species richness with increased nitrogen levels (Henning et al., 2020). This previous work led me to originally hypothesize that a similar result would be found in root endophyte response to nitrogen addition. However, the current data showed no statistical significance between nitrogen levels and species diversity or richness ($p= 0.232$ and $p=0.271$, respectively). This unexpected result encourages further research into root endophyte resilience under different resource conditions.

Shannon diversity is a measurement that considers the number of species and their relative abundance, also referred to as richness and evenness. In Table 1, it can be recognized

that OTU 1, 47, and 48 were identified based on percent match rates to be the same species, *Microdochium bolleyi.* This is one example of an instance in the data where further clustering is necessary. Further clustering of these species, and others likely present within the data, would lead to a decrease in richness and evenness. This shifting in Shannon diversity may yield more insight into changes in species richness and species diversity under nitrogen addition. I expect this overlap between morphotyped and similar OTUs to be more significant as the remaining fungi within our culture collection are identified. Figure 1 also shows a much greater species spread in high nitrogen plots. This data further indicates that more precise grouping and continued examination may lead to more concrete conclusions about nitrogen addition's effect on species diversity and richness.

Nitrogen addition effects on community composition

By performing multivariate analysis, I was able to find the relationship between nitrogen addition and community composition. An NMDS plot was created to visualize the relationship between community composition and nitrogen treatment levels. Figure 2 shows three ellipses, each representing plots of different treatment levels, drawn based on the standard deviation of point scores. Plots with high and low nitrogen levels share more similarities with each other than they do with control plot community composition. Any level of additional resources, specifically in the form of nitrogen, is shifting communities to look more similar to one another than ambient conditions. This raises many questions about the cause of this shift, most notably: Are plant species taking advantage of increased resource availability in ways that plants under ambient conditions cannot? Research in the Emery lab is finding similar patterns of nitrogen effects with aboveground plant communities (K. Garces, unpublished data).

Three species with significance to nitrogen addition plots were identified by performing indicator species analyses, encouraging further exploration into their function within the system. *Penicillium bilaiae* and *Cadophora malorum* were found to be strongly correlated with low nitrogen plots. *Helotiales*, a diverse order of fungal species, were highly correlated with high nitrogen addition plots.

Identifying microbial species that demonstrate stress resilience will be imperative as global change drastically affects the environment. *Penicillium bilaiae* is a fungal species that has been shown to colonize under different phosphorus conditions and increase acidification (Raymond et al., 2018). This research identified their novel ability to solubilize sewage sludge under extreme conditions (Raymond et al., 2018). *P. bilaiae* was also able to colonize with nitrogen addition, which indicates this species' ability to withstand changing resource conditions. *Cadophora malorum* is a fungal species known to colonize marine plant systems (Almeida et al., 2010). This species was found to be heavily associated with and abundant in plots with low nitrogen addition. Nitrogen eutrophication continues to be a global problem in marine systems; this indicates that *C. malorum* may tolerate low nitrogen addition in marine systems.

Helotiales is a taxonomic order comprised of diverse fungal families and species. Recent research has indicated that this order of fungus has increased abundance under increased phosphorus conditions (Fabiańska et al., 2019). Given our indicator species analysis, this order of fungus may be well equipped to withstand habitats with high resource availability more generally.

Implications

The location of this experiment is important because of the dunes' low resource availability under ambient conditions. Addition of resources, particularly nitrogen, will disrupt species well-adapted to this resource-deficient habitat. Here I found that any addition of nitrogen leads to a cascading effect in the microbial community, especially with regards to community composition. *Ammophila breviligulata* is particularly important to dune systems; their resilience under different resource conditions is uniquely imperative to colonize dune blowouts. Although the symbiotic function of root endophytes has not yet been identified, it is vital that research continues to evaluate these relationships, as the microbial community may encourage the resilience of *A. breviligulata* in the face of global change. This research indicates that their microbial community will be impacted as global change continues to affect ambient atmospheric nitrogen levels. Continued research on the secondary successional species in the system, *Schizachyrium scoparium*, will likely yield more information on how nitrogen addition will impact less adapted plant species in the dune system.

The blended approach I took using both Operational Taxonomic Units and morphospecies as a species clustering method was a technique necessary due to the status of this research. This research allowed for a thorough exploration of my guiding research questions and hypotheses but left room for continued research. As this research continues, there are fungal samples that still need to be sequenced to move beyond morphological categorization and finalize the dataset of OTUs. Replicating the process used to identify the sixteen OTUs found will likely yield the identification of more OTU classifications, which would lead to a better understanding of the mycobiome. However, the current dataset still yields essential conclusions that have tremendous implications for global change.

Conclusion

Any level of nitrogen addition impacts the composition of root endophyte communities associated with *Ammophila breviligulata*. Specifically, communities in plots with nitrogen addition, both low and high levels, were more similar in composition than those in ambient plots. *Ammophila breviligulata* is a critical plant species to the coastal dune system, and fungal symbionts identified through this research may play an essential role in their survival as biosphere nitrogen levels continue to increase. This work illustrates that nitrogen deposition at any severity will have cascading effects on the environment, further emphasizing the importance of prioritizing environmental conservation efforts, even at the microbial level. This research is the first of its kind to study root endophyte community composition in relation to resource availability and therefore is an incredibly unique contribution to the field of ecology.

Acknowledgements

During my time at the University of Louisville, I have gained many skills and life lessons that I will carry with me for the rest of my life. Arguably some of the most important, both in my academic and personal life, can be attributed to the self-confidence I gained working in the Christian Lab. Being a biology major surrounded by pre-professional students is a recipe for a competitive weed-out culture. After my first year, although I managed to maintain good grades, my love for science was destroyed by this culture. The autonomy over my research project and my responsibilities while working in the Christian Lab allowed me to rediscover this passion. I am now more confident than ever in my abilities as a scientist.

Kylea Garces, both my research mentor and friend, is the ingenious mastermind behind this novel and groundbreaking research. Kylea has supported me as a scientist, friend, and woman for the past three years as I navigated college. Without her support and motivation, I would not have completed this project. Kylea is not only an incredible scientist, but she is also an eclectic fashion icon. She has shown me to be genuine and authentic to myself, no matter the audience. Because of her, I will never change my outfit to avoid judgment. I will never void my personality in trade for professionalism. She taught me that I can take up as much space in science and in the professional world as I want. On my first day in the Christian Lab working with Kylea, I wore long pants, a long-sleeved blouse, my hair tied up, and close-toed shoes. Little did I know, the only required dress code was to be myself. Although I am certain this is only the beginning of a lifelong friendship, I would redo my entire undergraduate degree to keep doing research with her every day!

My research advisor and mentor, Dr. Natalie Christian, has cultivated an inclusive, supportive, and nurturing environment to learn in. I am eternally thankful for her support and invaluable mentorship throughout my academic career.

I am also thankful to Dr. Sarah Emery and Dr. Lee Thompson for being on my committee and spending their valuable time reviewing my thesis. Dr. Emery has been supportive and encouraging throughout my time as an undergraduate researcher.

I am obliged to acknowledge both the GRFP grant awarded to Kylea Garces and the Student Research Opportunity Program I was awarded in May 2021 for funding this research.

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