Patterns of microbial colonization and succession in *Guadua weberbaueri* internodes

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Keywords:

Microbial, ecology, colonization, succession, community-assembly, bamboo

ABSTRACT

The integration of microbial perspectives in colonization and succession theory is an important horizon in community ecology. Currently, most colonization theory is focused at the macroscopic level. Studies that do consider microbial colonization typically do so in the context of animal-microbiomes which may not be the most reliable model system. We seek to determine the extent to which microbial colonization and succession of freshwater in bamboo internodes follows classical ecological theory. We sampled the water inside internodes of 8 different bamboo stalks near the Los Amigos Biological Station in Peru. Using 16S-V4 Illumina MiSeq sequencing we considered the taxonomic density, diversity, and metabolic function of these microcosmic communities over a period of 168 hours. We observed that all of the internodes had a shared primary colonizer, genus *Halomonas*. This primary colonizer was overtaken by *Comamonadaceae* and *Acinetobacter* over time, but was not completely lost from the community. We also found that our micro-communities displayed an increase in taxonomic density and diversity over time, measured through unique taxa counts, Bray-Curtis distance, and NMDS ordination. Finally, we used PICRUSt to identify two distinct patterns of metabolic functional acquisition over time. These data suggest that microbial colonization and succession in plant hosts adhere well to classical ecological theory and previous animal microbiome studies. We also suggest the potential for bamboo as a new model system for theoretical microbial colonization and community assembly studies.

INTRODUCTION

Host-associated microbial communities are an integral aspect of symbiotic ecology and may have crucial implications for host health, nutrient acquisition, and competitive exclusion of pathogens and parasites (Turner, 2013). These internodes serve as natural controlled microcosms, due to their small size and relatively closed boundaries, to interrogate theories of ecological processes (Jiang, 2008). Such processes include colonization and succession of newly available habitats. However, few studies focus on colonization of plant-associated microbiota. This study seeks to test hypotheses about plant microbiome structure for two essential reasons. First, plant microbiomes can serve as a test of classical ecological theory, but at the microscopic scale. Identifying incongruences between macroscopic and microscopic theory can better integrate microbial perspectives in community ecology. Second, comparison to better-studied plant systems and animal microbiomes may inform the generality of processes that drive the assembly of these important communities. We aim to address this knowledge gap with the following driving question: In what ways do the assembly patterns of host-associated microbial communities adhere to established theories about colonization and succession?

Classical ecological theory seeks to explain the processes by which new habitats are colonized. Current understandings of the development and structure of novel communities are informed by a step-wise process in macroscopic organisms. First, a disturbance occurs which makes new habitats or niches available (McCook, 1994). These newly empty habitats are characterized by their sparse distribution of resources and environmental constraints (Shea, 2002). A given species' physiological and ecological characteristics define its ability to be an effective colonizer (Shea, 2002). Species with high colonization rates establish themselves in these new habitats and eventually change the environmental composition through their collective metabolic processes (McCook, 1994). These primary colonizers are of special note because they are able to survive in nutrient-sparse environments and often exhibit striking tradeoffs between competitive growth rates and strong colonizing traits (Vellend, 2010). Next, secondary colonizing species recruit to the area and find it hospitable due to the manipulation of resources and nutrients by the primary colonizers (Vellend, 2010). There is a vast body of evidence to support this sequence, and ecological theory predicts changes in species distribution, abundance, and dominance at the macroscopic scale (Vellend, 2010).

On the other hand, at the microscopic level, ecology-focused efforts to determine colonization and succession patterns yield conflicting results (Nemergut, 2013). These studies are hindered by the difficulty of drawing boundaries around microbial communities given the prolific, perhaps global, dispersal of microbes throughout all habitats (Nemergut, 2013). Further, it can be difficult to ascertain which taxa contribute meaningfully to the manipulation of resources of a niche given the significant metabolic redundancy of microbial communities (Allison, 2008). However, microbes adhere to some classical ecological theories of colonization and succession. Most relevant is the observation that microbial taxa may act as ecosystem engineers in early colonization and alter the environment for future successive taxa (Nemergut, 2013). Further, microbial communities undergoing colonization tend to follow both classical abundance curves and "species-time relationship observations" (Nemergut, 2013).

Given these knowledge gaps, microbiomes are compelling models to study the colonization of new hosts. They give the potential to track the entire process of colonization and succession on a short time scale without waiting for natural disaster or inducing experimental disturbance. Plants are interesting host-models because of their interactions between various microbiota spheres. The plant microbiome is defined as any area with associated-microbiota above or below the soil, as well as around and inside the roots and seeds (Hirsch, 2012). Plant-associated microbiota play key roles in nutrient uptake from the soil, tolerance to environmental stress, hormone production, pathogen protection and more (Schlaeppi, 2015). Many plant microbiome studies focus heavily on the root-associated rhizosphere microbiota (Hirsch, 2012). This bias is due to the rhizosphere's rich taxonomic diversity and key role in nutrient acquisition and cycling (Hirsch, 2012).

This study focuses on the internal endosphere microbiota of the *Guadua weberbaueri* species of neo-tropical bamboo. *Guadua weberbaueri* is a significant component of the Amazonian flora and ecosystem (Griscom, 2007). Bamboo species are a unique study system because their robust endospheres are composed of closed hollow internodes filled with nearly-sterile freshwater (Loutin, 1996). The bamboo endosphere is often exposed to the external environment, typically by foraging spider monkeys (Izawa, 1997). Therefore, its microbiota should be reflective of two main sources. First, there will likely be some primary "endogenous" microbial colonization from the rhizosphere (Turner, 2013). Rhizosphere bacteria can enter root nodules passively, through cracks,

or actively, through non-pathogenic levels of cellulolytic enzymes, and migrate to the endosphere (Hirsch, 2012). Therefore, a non-random concentration of taxa is likely to be sourced from the rhizosphere. Second, the opened endosphere may be exposed to "exogenous" sources of colonization, such as air, rain, and animal-mediated dispersal (von May, 2012). *Guadua weberbaueri* internodes are closely associated with the life-cycles of various vertebrates and invertebrates, such as the rhinoceros beetle, *Enama pan,* and frog, *Ranitomeya sirensis*, (Jacobs, 2012; May 2008). The potential interplay between these two microbial sources may influence colonization and assembly of the bamboo endosphere microbiome.

In this study, we had four goals to assess the processes driving colonization and succession of endospheric bamboo microbiomes. First, we tested whether each internode had the same initial colonizers. This determination was crucial to determining whether the *G. weberbaueri* endosphere selects for specific colonizer traits or if primary colonization is stochastic. We hypothesized that each internode would have the same primary colonizer because their rhizosphere microbiota are significantly influenced by shared soil composition and internal habitat (Griscom, 2007; Turner, 2013). Secondly, we tested for taxon-turnover with time and assessed whether there was an observed loss of initial colonizer species, as is consistent with macroscopic theory (Del Moral, 1993). We hypothesized that there would be distinct shifts in taxon-turnover over time and a loss of initial colonizer species (Nemergut, 2013; Del Moral, 1993). Next, we tested whether the endosphere microbiota maintained a classical species-time relationship. We hypothesized that the endosphere would display a positive species-time relationship with respect to taxonomic density and diversity (Chu, 2017). Finally, we tested the metabolic profiles of each community to observe changes in the dominant metabolic functions over time. We hypothesized that there would be differences in metabolic functional profiles over time due changes in taxon dominance (Nemergut, 2013). Overall, we hope to contribute to the unification of microscopic perspectives within theory regarding colonization and succession.

METHODS

Sample Collection:

We performed our experiment at Los Amigos Biological Station in southern Peru starting on November 30th of 2016. Los Amigos is in a lowland tropical rain forest near an Amazon river tributary. We collected water samples from one internode of eight discrete stalks of the bamboo species *G. weberbaueri*. The eight stalks were distributed across two distinct plots in terra firme forest that were within 200 meters of each other (Figure 1). We sampled four stalks from each plot. We determined the following eligibility for internode sampling: the internode must be 1 to 2 meters above the ground, contain water, and had not been previously opened. We opened bamboo internodes using a bleach-sterilized blade and cut a 20mm to 40mm notch horizontally near the top of the node. Then, we pulled back a section of the internode external skin using the edge of the sterilized blade to expose the surface of the internode water. We chose this method because it mimics the actions of foraging spider monkeys which open internodes in a similar fashion (Izawa, 1997).

At the time of opening, we collected 0.5mL of water from each bamboo internode using sterilized pipettes. We preserved these samples in 1.5mL of CTAB buffer. Samples were collected in a time series with the first collection taking place immediately after opening. We then subsequently sampled at 24 hours, 48 hours, and 168 hours after opening.

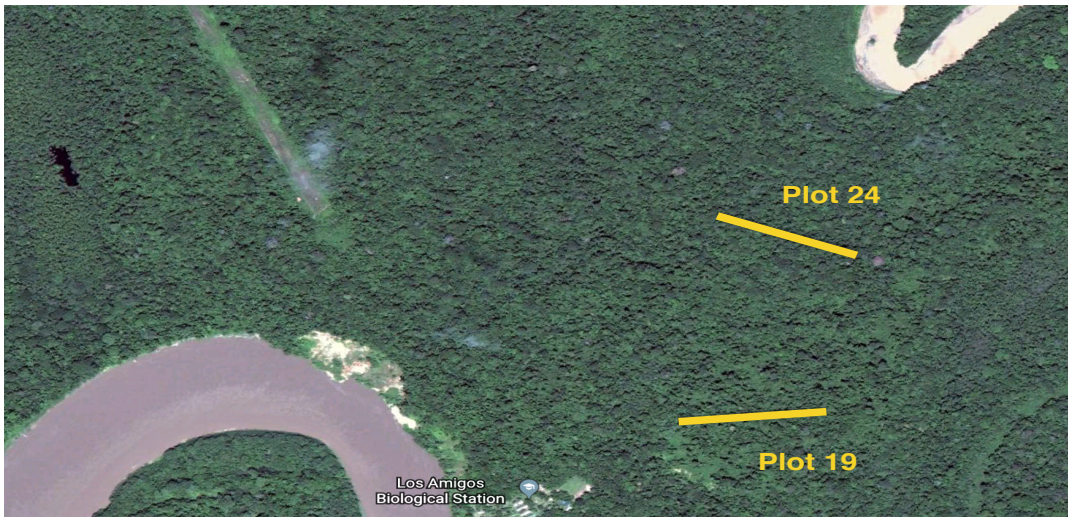


Figure 1: A map generated with Google Maps showing the location of the two plots where sampling was conducted near Los Amigos Biological Station, Peru. Four samples were collected from Plot 19 (samples 1-4) and four were collected from plot 24 (samples 5-8). Sample 8 was later omitted due to poor resolution after processing.

Nucleic Acid Extraction:

We extracted the total metagenomic DNA from the water samples by incubating 200 uL of CTAB and water mixture with 400 uL of buffer ATL from Qiagen's DNEasy Blood and Tissue kit with 10 uL of proteinase K. We incubated the samples at 65℃ for 24 hours. We then performed a phenol-chloroform-isoamyl alcohol extraction following Barker et al. 1998. We chose phenol-chloroform to minimize contamination by plant secondary metabolites that could inhibit PCR or sequencing reactions. We chose to remove an entire bamboo series, a set of four samples from a single bamboo, because its showed poor sequencing results and extremely low diversity relative to the other samples.

Purified and eluted DNA was stored at 4℃ before being sent for sequencing. Sequencing libraries were created with the NEBNext DNA Ultra prep kit 2 (cat# E7645L) with barcoded dual-index primers specifically developed to target the V4 hypervariable region of the bacterial 16S rRNA gene (Kozich, et al 2013). All sequencing samples included a negative control. We standardized all samples using a Qubit measurement and pooled equimolar quantities of samples for the final run. Libraries were sequenced at the University of Michigan's Microbiome Sequencing Core using the MiSeq platform (500 cycle, Illumina).

Statistical analyses:

Using demultiplexed reads, we used Mothur to prepare a curated set of reference OTU 16S bacteria sequences for later individual-specific analysis (Schloss, 2013). We used the program to make contigs from our paired-end sequences, select appropriately sized fragments, and remove low-quality sequences. We then aligned the sequences against the SILVA database, and removed the sequences that did not align well using a cutoff of greater than 20% sequence dissimilarity. Finally, we identified sequences to domain using the SILVA taxonomy, and discarded all non-bacterial sequences. We also removed duplicate sequences. We then used vsearch (Rognes, 2016) to cluster all sequences in the database to 97% similarity, retaining one sequence per cluster in the final database. Each sequence in our curated database was identified by the taxonomic identity of the SILVA reference sequence with which it most closely aligned. We used vsearch to make contigs for each individual, and then aligned the contigs against the

bacterial database. We used a custom script in R to parse the resulting file into a sample-by-OTU community matrix.

Initial colonizers and taxon turnover:

We aimed to determine the extent of taxon turnover within our sampled data set and compare the breakdown of taxa within each sample over a period of 168 hours. We then subset our sample-by-OTU matrix by internode and sampling time. Next, we selected the most represented taxa at one of three successional stages for each sample. The successional stages are defined as following: stage one is a composite of initial sampling time and time 24, stage two is time 48, and stage three is time 168. We plotted the mean abundances of these key taxa from all samples over time with standard deviation error bars (Figure 2).

Taxonomic density over time:

To evaluate the taxonomic density of each internode over time, we subset our sample-by-OTU matrix by the number of unique taxa in each sample. These values were plotted on a line plot, and colored by internode (Figure 3A). We also calculated the mean abundance of each unique taxa and plotted this value over time (Figure 3B).

Taxonomic diversity over time:

Beta-Diversity:

To distinguish the differences in diversity between groups within our dataset, we considered the beta-diversity between communities according to sampling time, plots and internodes. To do so, we created a Bray-Curtis distance matrix using vegan (Oksanen, 2013). This distance matrix was then ordinated using PCoA and plotted with unique coloring for sampling time, plot, and internode (Figure 4).

NMDS ordination:

To give a complete picture of the diversity within our dataset we created an NMDS distance matrix using vegan, with the number of reduced dimensions as 2. This distance matrix was then ordinated and plotted (Figure 5).

Community functional analysis over time:

To functionally characterize the communities in each internode and understand how community-metabolism may change over time, we applied PICRUSt, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (Langille, 2013). Manipulation of PICRUSt outputs in R allowed us to create a pathway-by-sample matrix. Euclidian distances between samples were then calculated and arranged into a distance matrix. These differences were ordinated and colored by sampling time (Figure 6).

To evaluate metabolic turnover over time and identify the gain or loss of metabolic processes, we plotted the metabolic profiles of each internode over time as a stacked bar plot (Figure 7).

RESULTS:

Initial colonizers and taxa turnover:

We found that *Halomonas* was the best represented taxon at time zero in all samples. Despite a *Halomonas* majority in the first successional stage, *Halomonas* abundance sharply decreases as time increases and the genus is still present in the final sampling time, but not well represented (Figure 2). *Acinetobacter* and *Comamonadaceae* emerge as the majority taxa in the final sampling for all internodes but we cannot distinguish between either as the dominant taxa given the overlap of error bars (Figure 2). Therefore, we can conclude that our hypothesis regarding shared primary colonizers was supported by the observation that *Halomonas* is the majority taxon in our initial sampling (Figure 2). We do not support our hypothesis of taxonomic turnover because, though there is a decrease in relative abundance of *Halomonas* and increase of *Acinetobacter* and *Comamonadaceae* over time, *Halomonas* presence is not lost as succession occurs (Figure 2).

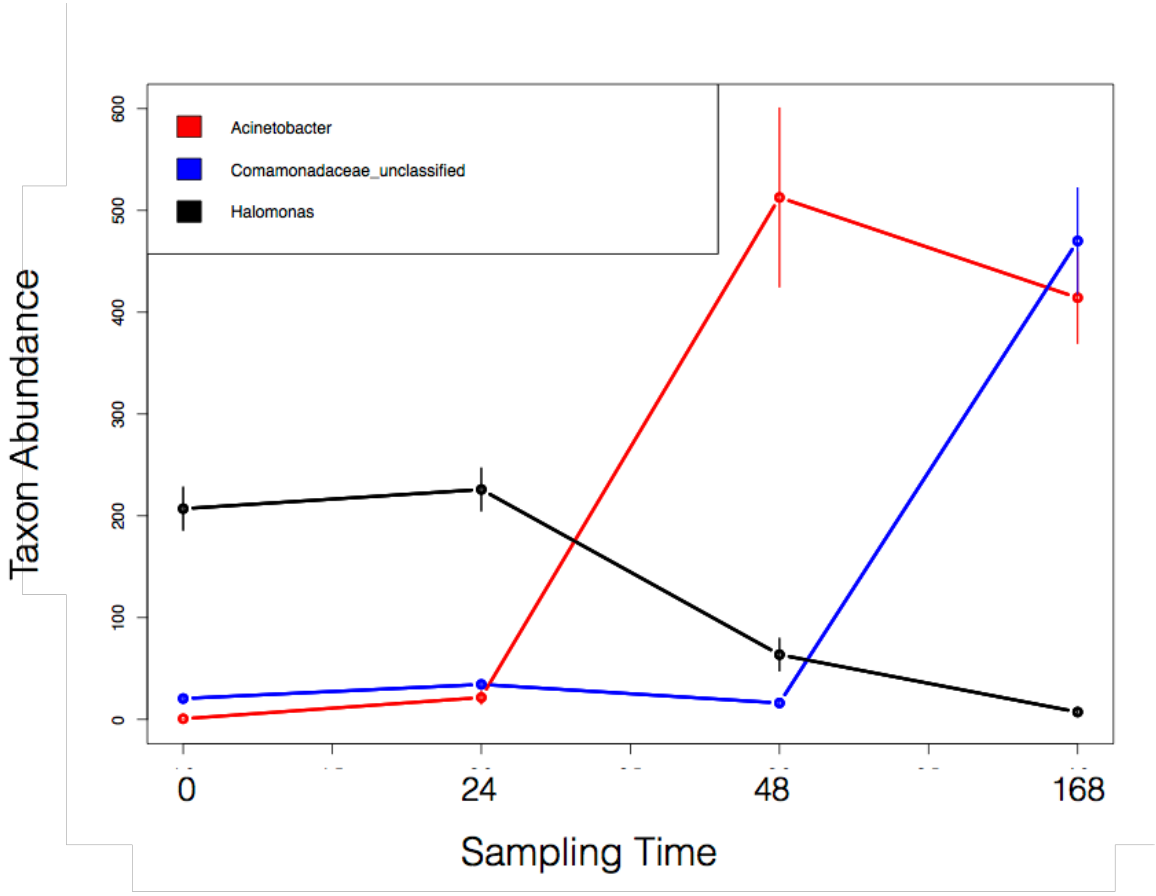
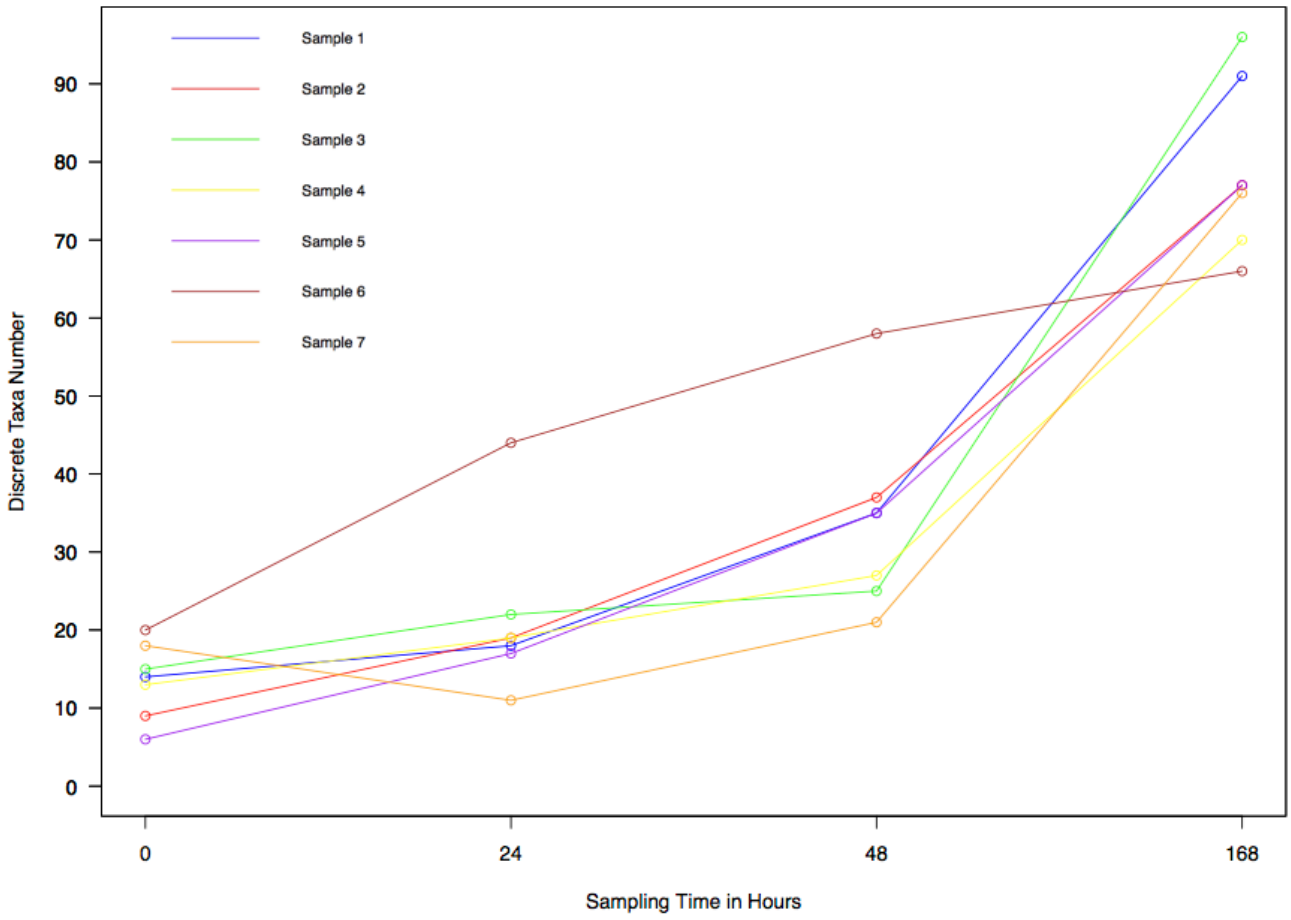


Figure 2: Line plot showing taxon turnover and change in taxon abundance over time of three key taxa: *Halomonas*, *Acinetobacter,* and *Comamonadaceae*. Taxa were chosen based on abundance at three key successional stages, time zero and 24, time 48, and time 168. Errors bars indicate standard deviation.

Taxonomic density over time:

The relationship between taxa density and time appears to follow a classical positive correlation (Figure 3A, 3B). The mean taxa density and time plot appears to support this cumulative trend and is indicative of an overall increase in taxonomic density over time (Figure 3B). This observation supports our hypothesis that there will be an increase in taxonomic density over time.

A



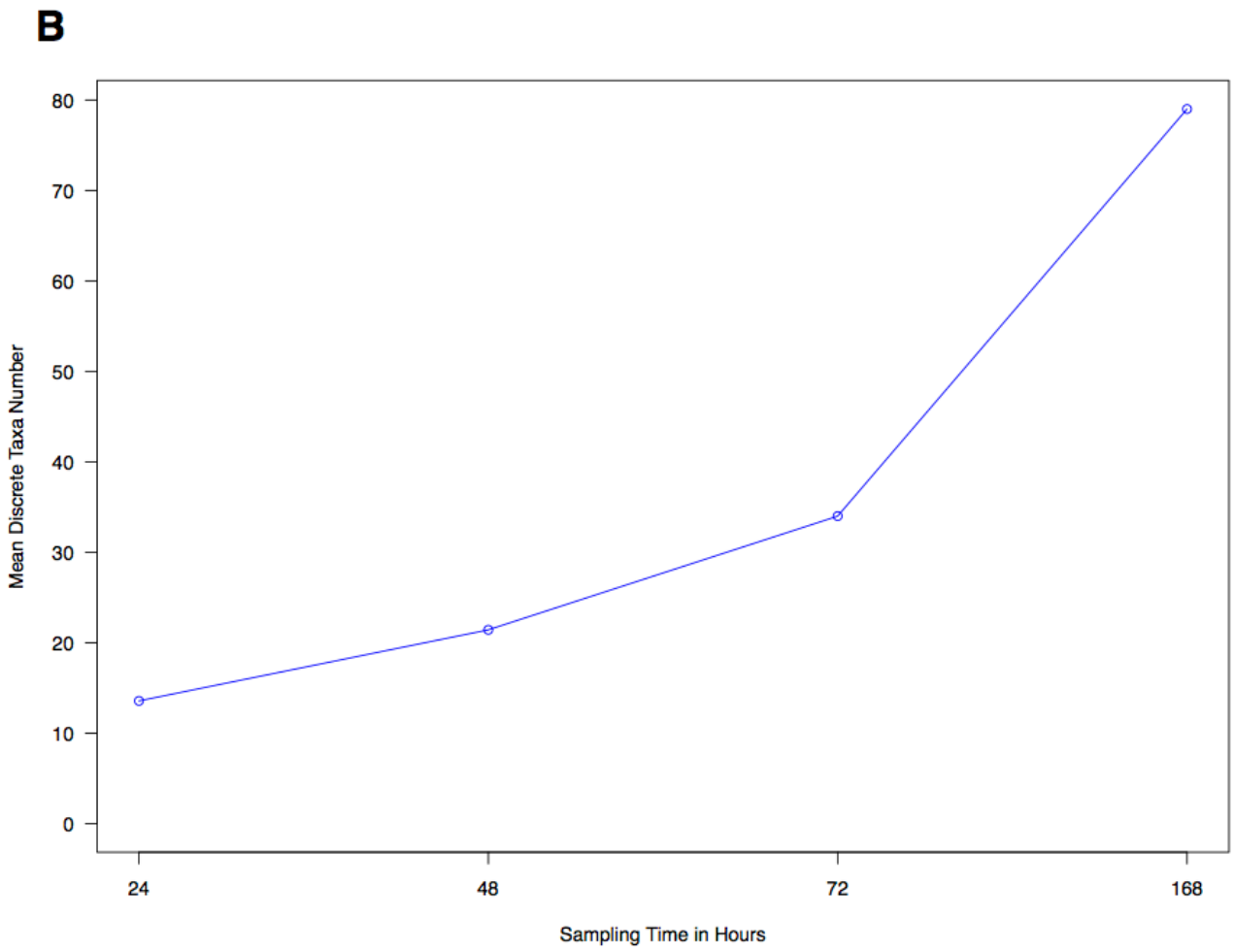


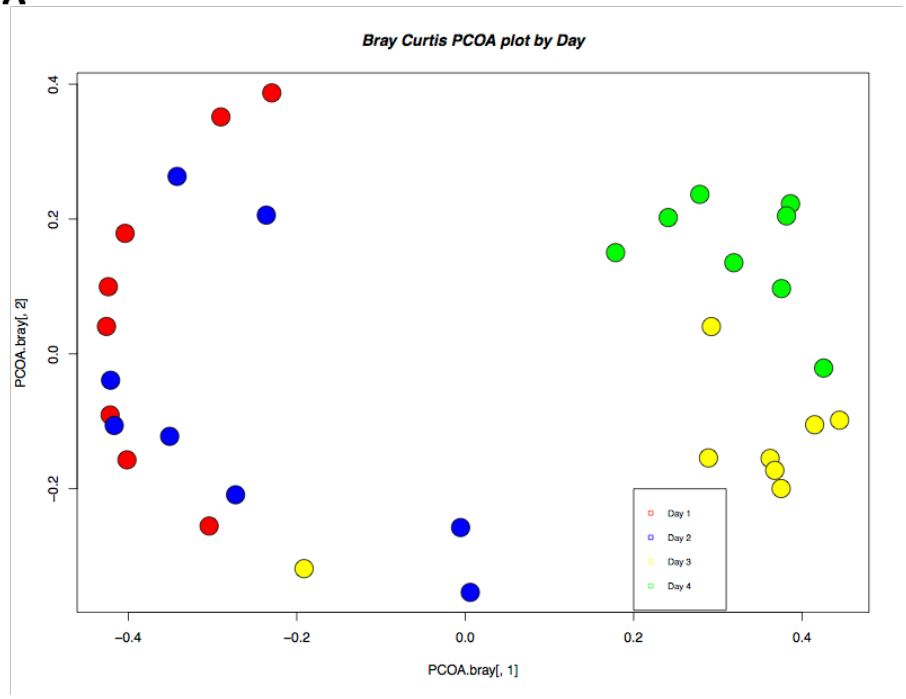
Figure 3: Taxonomic density over time for internodes over a period of 168 hours. A: The discrete number of taxa for all internodes across all time points were calculated and plotted in a line plot. **B:** The mean discrete number of taxa across all times were calculated and plotted in a line plot. There appears to be a clear increase in taxa density over time.

Taxonomic diversity over time:

Beta-Diversity:

We can see two distinct clusters; one cluster of time zero and time 24 samples and a second cluster of time 48 and time 168 samples (Figure 4A). This suggests a clear separation of taxonomic diversity between our initial and final sampling that is indicative of taxonomic succession. We do not see any meaningful separation of samples when considering separation by plot (Figure 4B). Finally, we also do not see any meaningful clustering of samples when considering separation by specific internode (Figure 4C). This suggests that plot location and bamboo stalk are not driving factors for the observed difference in taxonomic diversity, but time is.

A


Bray Curtis PCOA plot by Day
PCOA.bray[, 2]
PCOA.bray[, 1]
0.4
0.2
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Day 1
Day 2
Day 3
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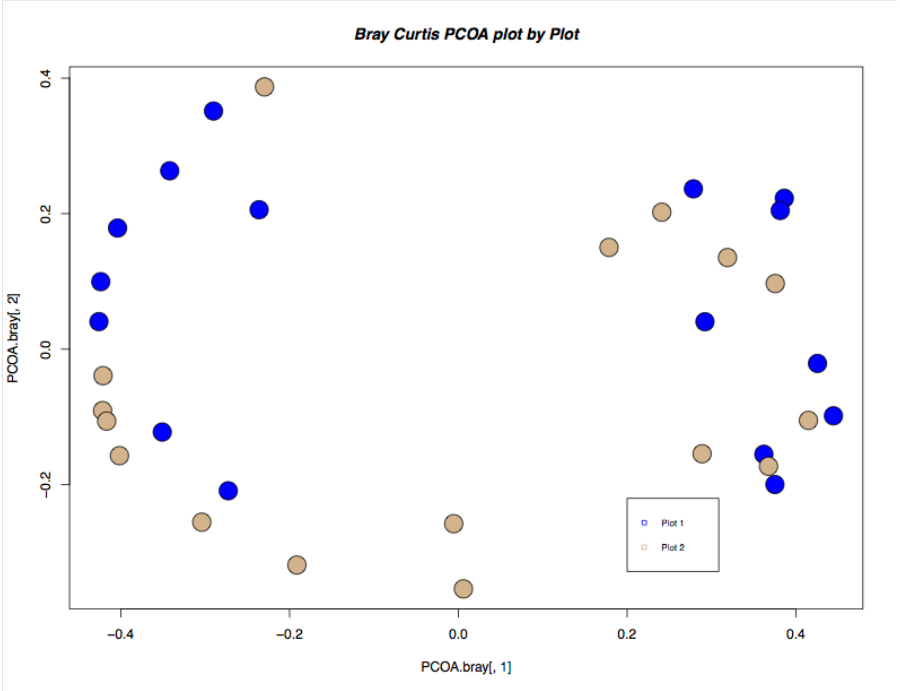
B


Bray Curtis PCOA plot by Plot
PCOA.bray[, 2]
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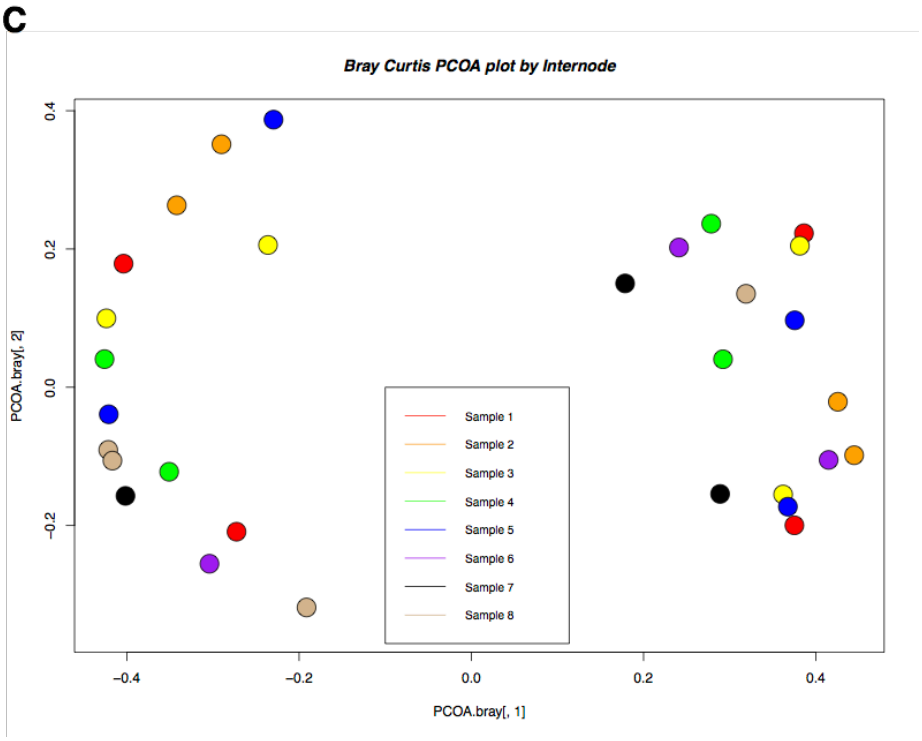


Figure 4: PCoA plot of Bray-Curtis distance between samples. A: PCoA colored by sampling time. Red points correspond to hour zero, blue points correspond to hour 24, yellow points correspond to hour 48, and green points correspond to hour 168. There appears to be distinct clustering of hour zero and hour 24 and of hour 48 and hour 168, with clear separation between both groups. **B:** PCoA colored by plot. Blue points correspond to plot 1, tan points correspond to plot 2. There does not appear to be a meaningful difference in clustering pattern. **C:** PCoA colored by internode. Red points correspond to internode 1, orange points correspond to internode 2, yellow points correspond to internode 3, green points correspond to internode 4, blue points correspond to internode 5, purple points correspond to internode 6, black points correspond to internode 7. There does not appear to be a meaningful difference in clustering pattern.

NMDS ordination:

The stress of our NMDS plot was 0.01, indicating that this metric provides a good representation of the data in reduced dimensions. We observe three distinct clusters of samples: one clustering of time zero and time 24 samples colored in purple, one cluster of time 48 samples colored in green, and one cluster of time 168 samples colored in red (Figure 5).

This metric further refines our understanding of the separation of samples from our Bray-Curtis ordination plots, but is able to give a higher resolution of the differences and separation between samples from hour 48 and hour 168 (Figure 4A, Figure 5).

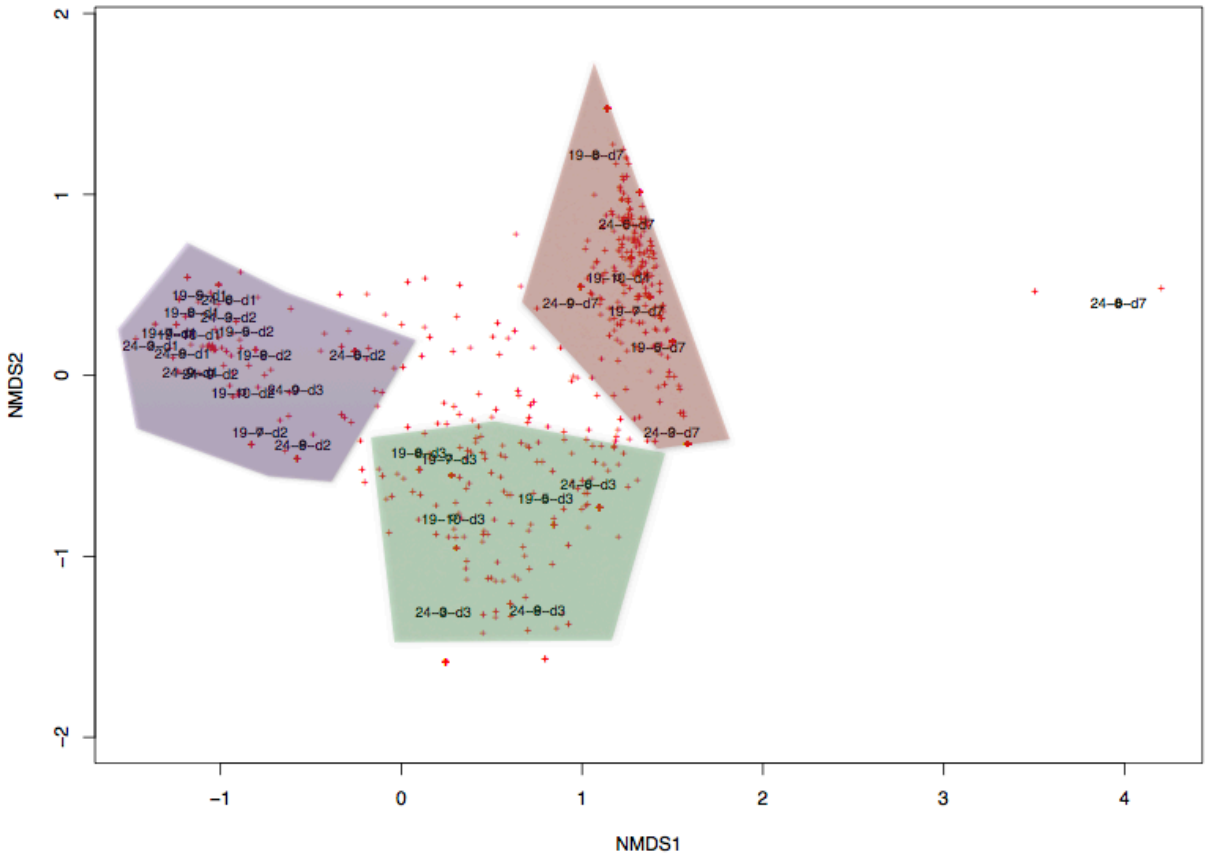


Figure 5: NMDS Euclidian distance ordination between samples. There appears to be no meaningful separation between samples from hour zero and hour 48. However, there is stronger, and distinct, clustering of samples from hour 48, as well as distinct clustering from samples from hour 168. Clusters are colored by time: time zero and time 24 are colored purple, time 48 is colored green, and time 168 is colored red.

Community functional analysis over time:

In this ordination plot of Euclidian distances between metabolic functional profiles, we can see a moderately strong clustering of time zero and time 24 samples, as there was using our diversity metrics (Figure, 6, Figure 5, Figure 4A). This observation suggests shared metabolic function between time zero and time 24 samples. There appears to be much looser clustering of time 48 and time 168 samples, which suggests more varied and diverse metabolic profiles of these communities as time goes on (Figure 6).

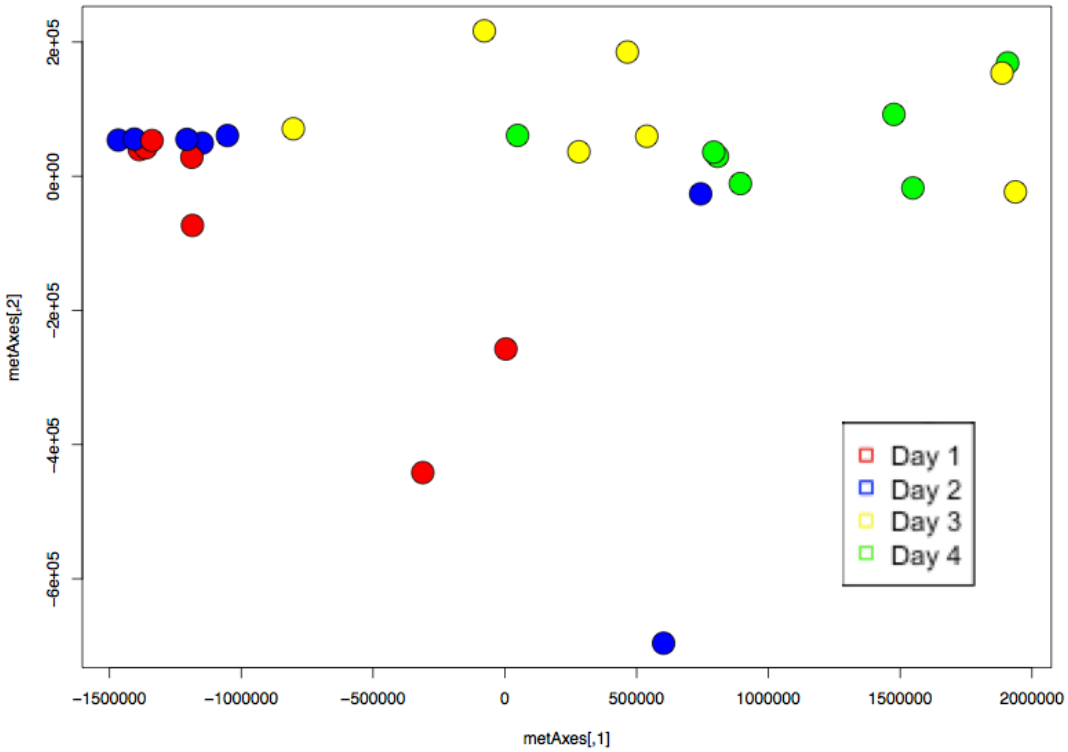
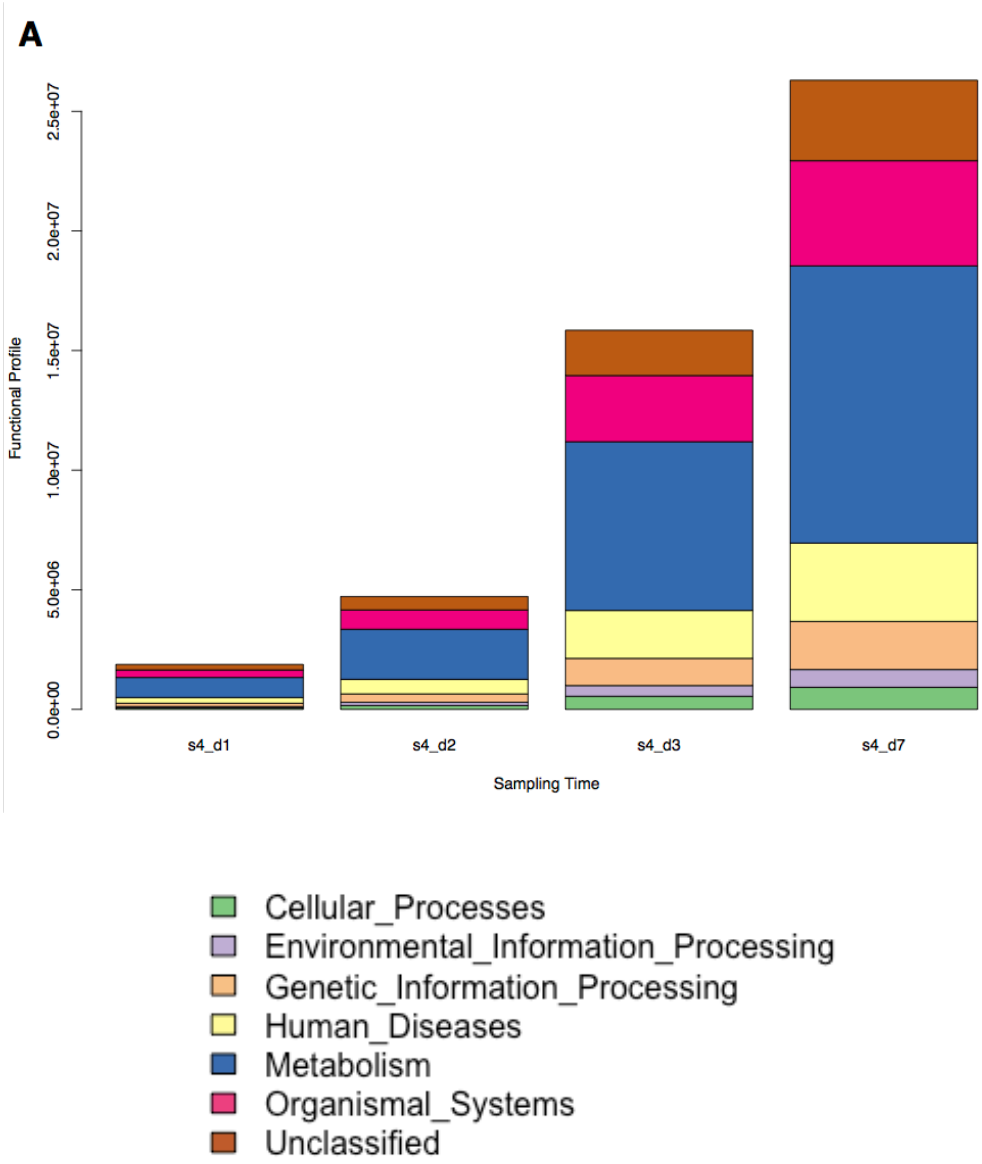


Figure 6: PICRUSt Euclidian distance matrix ordination: Euclidian distance metrics were applied and ordinated. Red points correspond to time zero, blue points correspond to time 24, yellow points correspond to time 48, and green points correspond to time 168.

There appears to be a clear increase in the density of metabolic functions acquired by the community over time (Figure 7). However, there are two dominating patterns of metabolic acquisition within our sampled data. Two representative samples, sample 4 and sample 5 have been chosen to demonstrate these trends. The sample 4 trend shows a distinct, almost exponential, increase in metabolic function acquisition (Figure 7A). This trend applies to the overall community metabolic composition of samples 2, 3, 4, and 7 (Supplementary Figure 1). The sample 5 trend also shows an increase in metabolic functional acquisition over time but the overall metabolic activity drops between time 48 and time 168 (Figure 7B). This drop in metabolic functional community composition is distinct to samples 1, 5, and 6 (Supplementary Figure 1). This pattern is interesting because, despite an overall increase in taxonomic density and species-level diversity, these patterns are not always associated with a similar increase in metabolic function acquisition (Figure 4A, Figure 5, Figure 7B). This may have implications regarding the activity levels and

dormancy of these communities as well as evidence for metabolic redundancy within these communities.

Though the intermediate proportions appear to vary across internode, for almost all internodes the relative distribution of metabolic function appears to be comparable. The greatest proportion of the functional profile for all internodes is metabolism, followed by organismal structures and unclassified or human-pathogenic functions (Figure 7).



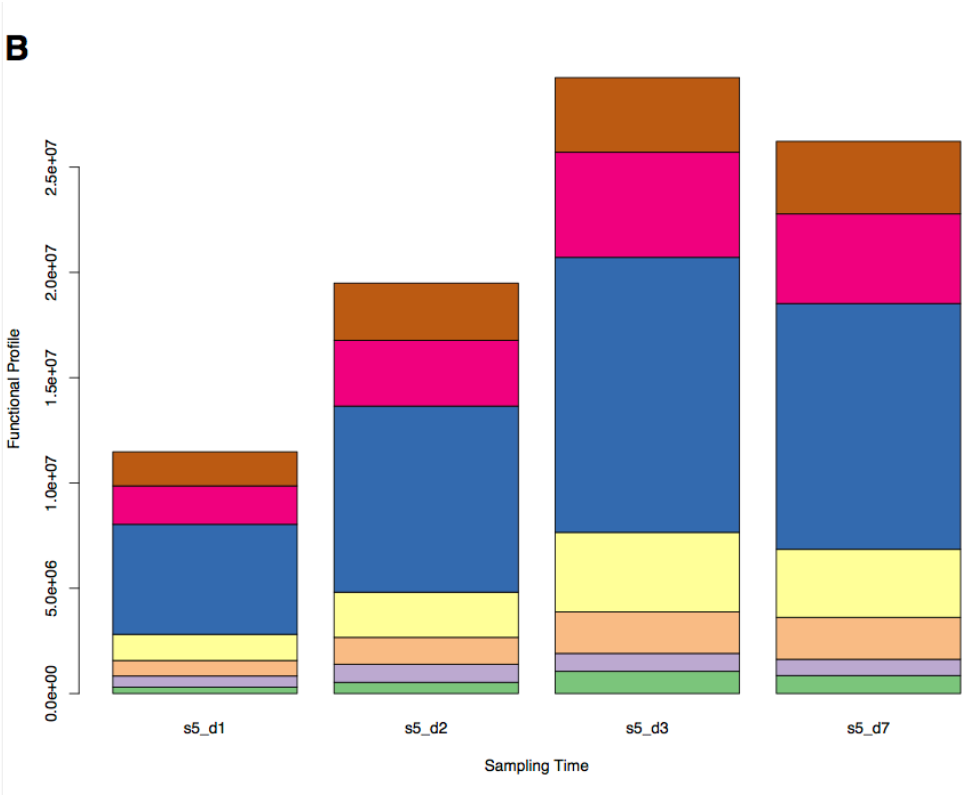


Figure 7: Comparative progression of functional profiles of internodes over a period of 168 hours. The functional pathway profile of each internode was determined using PICRUSt and plotted as a stacked bar plot over time. **A:** Internode 4 metabolic function profile over time. **B:** Internode 5 metabolic function profile over time.

These data suggest that our hypothesis of identifying clear patterns of metabolic acquisition is supported. We see two distinct patterns of metabolic acquisition over time, and also see a similar increase in the types of metabolic functions acquired over time (Figure 7). However, given the nature of this remote environmental sampling, it is possible that the PICRUSt metabolic database cannot accommodate a complete representation of the functions taking place in these communities.

DISCUSSION:

This study has been motivated by the intention of testing how patterns of microbial colonization and succession compare against theoretical expectations. Our hypothesis regarding shared primary colonizers was supported by the consistent primary colonization of *Halomonas* (Figure 2). Our hypothesis regarding taxon turnover was not supported. Based on successional theory, we expected to see a complete loss of primary colonizer *Halomonas* (Del Moral, 1993). However, we observed that *Halomonas* remained in the internode communities but its relative abundance dropped, and its role as the dominant taxon was overtaken by *Acinetobacter* and *Comamonadaceae* (Figure 2). This was the only instance in which our communities did not act in accordance with traditional theory. Our hypotheses regarding the positive relationship between species diversity and density over time were well supported by our unique taxa line plots and diversity metrics. We found that taxon density increased significantly over time and there was a significant difference in taxonomic diversity between our initial and final sampling time (Figure 3, Figure 4, Figure 5). Finally, our hypothesis regarding changes in the community metabolic profiles over time were also supported because we could see clear shifts in functional group abundance over time (Figure 6, Figure 7). However, rather than identifying a single pattern, we identified two dominant patterns of metabolic acquisition, the second of which actually shows a loss of metabolic diversity, which may have implications for overall community metabolic function and redundancy (Figure 7).

To better understand the complex ecological dynamics and mechanisms at play in our internode communities, we can attempt to characterize the major taxa represented at each successional stage. Identifying the key traits of these taxa may allow us to infer information about nutrient-cycling and metabolic shifts in the community and identify potential selective pressures. The primary colonizers of these communities, genus *Halomonas*, is characterized by its distinct metabolic diversity, role in nitrogen cycling, and ability to thrive in high-saline, high-metal environments (Llamas, 2006; Desale, 2014). *Halomonas* has previously been isolated from plant rhizospheres and are able to survive in extreme and harsh environments (Llamas, 2006). These characteristics offer two insights into the endosphere microbiome of bamboo internodes. First, it offers support for our theorized mechanism of primary colonization of entry via root nodules. Second, it may infer a selective pressure, either at the level of the root or the internode, that is extreme in some manner but hospitable enough for *Halomonas* to thrive in.

Following the decline in *Halomonas* abundance, we see the rise in dominance of two other taxa, *Comamonadaceae* and *Acinetobacter* (Figure 2). *Comamonadaceae* is characterized by its ability to reduce nitrate in nutrient cycling and its ability to thrive in anaerobic conditions (Sadaie, 2007). It has also been proven to be an effective successor species with high growth rates in microbiome studies of sludge water (Sadaie, 2007). In previous studies, species of genus *Acinetobacter* have been shown to be unable to successfully colonize plant hosts unless introduced in the presence of a host pathogen (Kay, 2002). Kay *et al*. (2002) hypothesized that *Acinetobacter* species needed excess nutrients and sugars to successfully grow that were only present when released by the pathogen. Further, this study demonstrated that *Acinetobacter* species have strong growth traits and dominate other taxa that they are grown in parallel with, regardless if *Acinetobacter* incubation concentration is high or low (Kay, 2002). This view supports our observation of *Acinetobacter* as a dominant successor taxon in our progressive time series (Figure 2).

Our study adhered to classical colonization and successional theory in many ways. One of the best studied cases of colonization at the macroscopic level is the succession of plants following the 1980 Mount St. Helens' volcanic eruption (Wood, 1987). Comparisons to this iconic disturbance and succession will better inform how our results adhere to macroscopic trends. Following the Mount St. Helens' eruption, it was found that lupines were consistent primary colonizers of the disturbed area (Moral, 1993). These taxa were most effective at facilitating future succession after their death and the associated release of nutrients (Moral, 1993). This pattern requires the complete loss of primary colonizers, and subsequent successors, between successional stages. However, in our study we do not see a complete loss of primary microbial colonizer *Halomonas*, but rather a shift in dominance and relative abundance (Figure 2). When investigating species richness and diversity in Mount St. Helens' plots, it was found that there was an increase in both over time (Moral, 1993). This observation is consistent with our observed results of increased taxonomic density and diversity (Figure 3, 4, 5). Finally, in terms of metabolic analyses it was found that, following the eruption, plots were lacking in nitrogen (Bishop, 2002). Species that were able to fix nitrogen recruited to the area and metabolically altered the environment (Bishop, 2002). This pattern is consistent with our results that demonstrated increases in functions related to metabolism over time (Figure 7), This also coheres with our current understanding of the metabolic capabilities of *Halmonas* as potential nitrogen fixators in nutrient cycling (Llamas, 2006). Overall, our results

cohered largely with established plant-oriented macroscopic successional theory, with the exception of complete loss of primary colonizer taxa.

Compared to animal microbiome colonization and succession studies, our results align with general trends regarding taxonomic density and diversity. In human infant microbiome studies, it was shown that infants with younger microbiomes had simpler communities, with fewer unique taxa, compared to their mother (Chu, 2017). This suggests a positive taxon density versus time relationship similar to the trend we observed within our dataset (Figure 3). Further, a longitudinal infant microbiome case study found that phylogenetic diversity of microbiome communities increased steadily over time, with marked shifts in taxonomic groups that often corresponded to major health and diet changes (Koenig, 2010). Finally, patterns of taxonomic succession have been demonstrated through the emergence of discrete clustering of early sampling and late sampling communities when applying dissimilarity matrices (Koenig, 2010). These observations cohere with our results regarding increases in taxonomic diversity over time and clear patterns of successional taxon turnover (Figure 3, Figure 4, Figure 5). Overall, our results are consistent with these observations regarding changes in taxonomic density and diversity over time, along with changes in specific taxon dominance over time.

CONCLUSION

This study serves as a proof of concept for using bamboo as a model system for microbial studies. The unique structure of bamboo internodes allows for a natural, but controlled, closed environment that supports many unique interactions between microbes, hosts, vertebrates and invertebrates. Further, it has great potential to study processes of microbial community assembly because of the presumed sterility of the internode freshwater (Jiang, 2008). This may be particularly informative given new perspectives that assert that the sterile womb hypothesis of infant microbiomes may not be an accurate model (Perez-Munoz, 2017). Given this, bamboo may have the potential to serve as a viable, less complex, replacement for studies regarding microbial colonization. Further, studies regarding infant microbiomes are susceptible to interference from host immune response and external events, such as diet and illness, that the water in bamboo internodes is not exposed to (Mueller, 2015; Perez-Munoz, 2017). Finally, this study is important as a contributor to the characterization of a significant component of the Amazonian ecosystem (Griscom, 2007).

Guadua weberbaueri has been present in the southwestern Amazonian basin for at least 45,000 years and plays an important ecological and evolutionary role (Von May, 2012). This species holds economic importance in the construction of weapons, foresting, construction, artisanship, and cooking in Amazonian communities (Von May, 2012). A more complete understanding of this species and its associated microbiota may lay important groundwork for future studies regarding bamboo conservation and health.

Future directions of this study might include exploring the unique interactions within and around bamboo internodes. A complete assessment of these relationships might include the metagenomics sequencing of bamboo-associated organisms, particularly the external surfaces of internode-associated insects and amphibians. Other investigations might focus on the metagenomic sequencing of bamboo roots and soil. This would facilitate better understanding of the bamboo rhizosphere microbiome and the endosphere as a subset of the rhizosphere microbiome.

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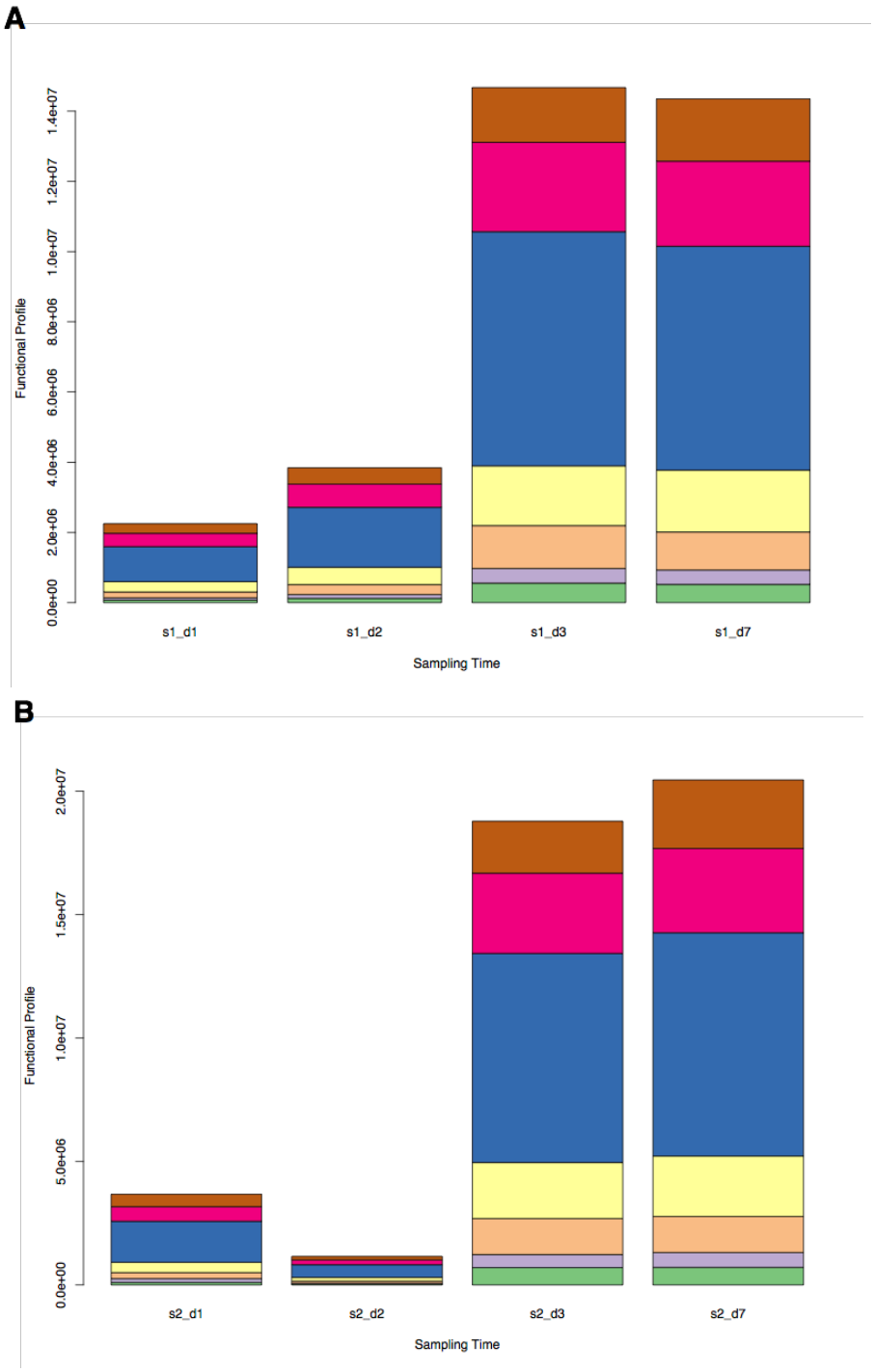
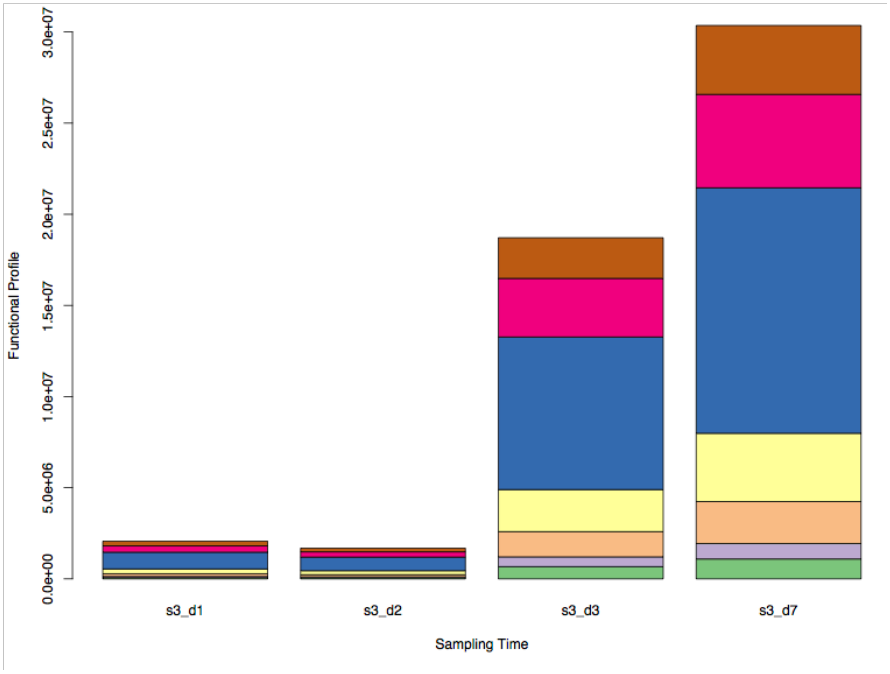
Supplementary Figures:

Supplementary Figure 1: Comparative progression of functional profiles of internodes over a period of 168 hours. The functional pathway profile of each internode was determined using PICRUSt and plotted as a stacked bar plot over time. **A:** Internode 1 metabolic function profile over time. **B:** Internode 2 metabolic function profile over time. **C:** Internode 3 metabolic function profile over time. **D:** Internode 6 metabolic function profile over time. **E:** Internode 7 metabolic function profile over time.



C


Functional Profile
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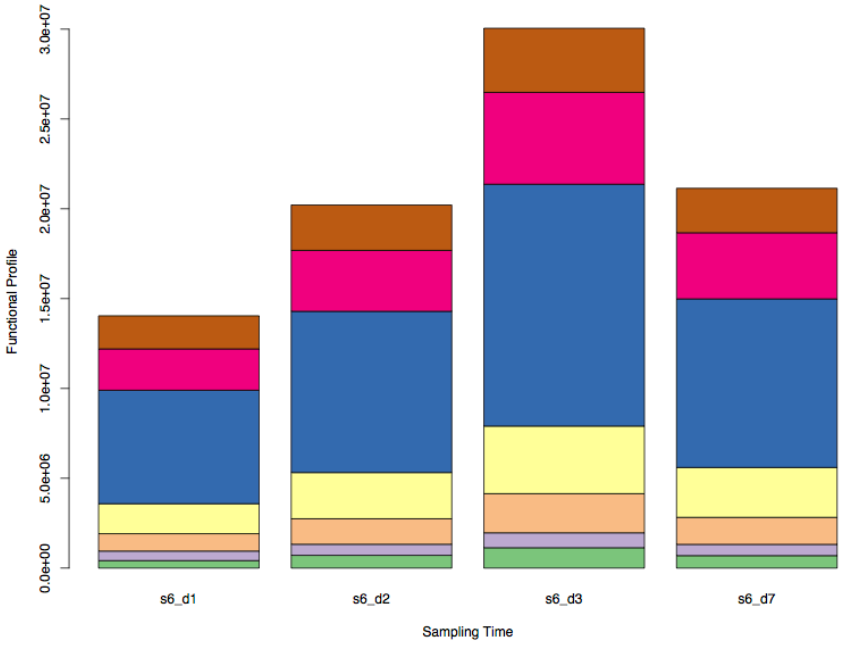
5.0e+06
1.0e+07
1.5e+07
2.0e+07
2.5e+07
3.0e+07
s3_d1
s3_d2
s3_d3
s3_d7
Sampling Time

D


Functional Profile
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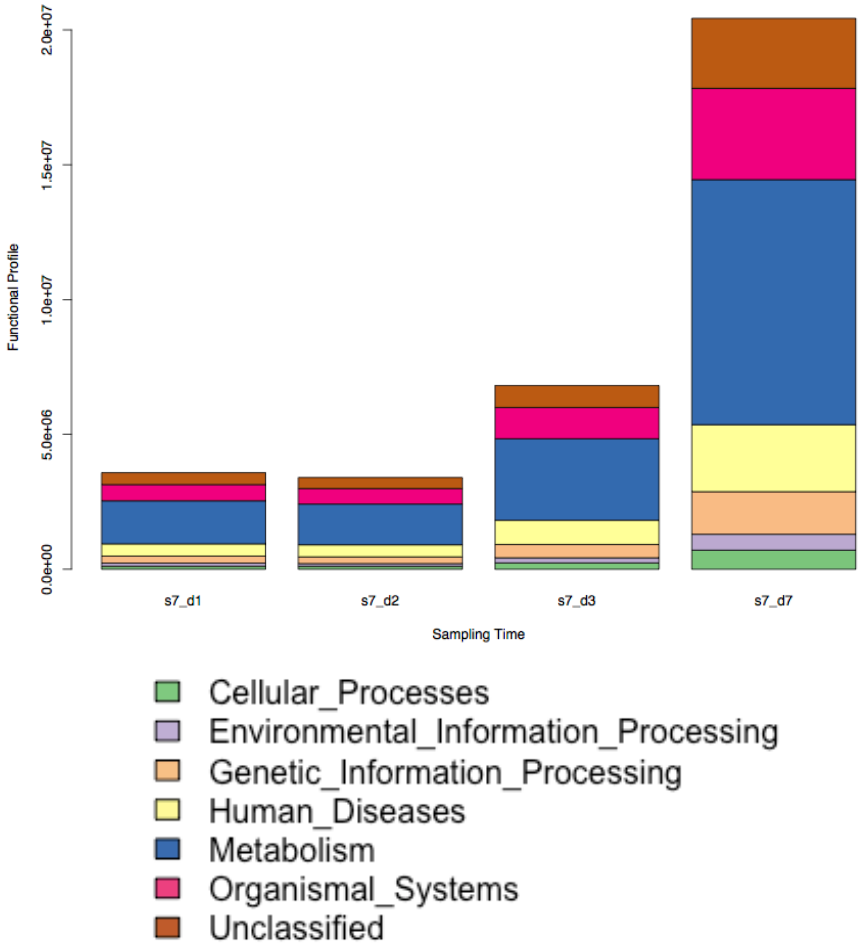
1.0e+07
1.5e+07
2.0e+07
2.5e+07
3.0e+07
s6_d1
s6_d2
s6_d3
s6_d7
Sampling Time

E


Functional Profile
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1.5e+07
2.0e+07
s7_d1
s7_d2
s7_d3
s7_d7
Sampling Time
Cellular_Processes
Environmental_Information_Processing
Genetic_Information_Processing
Human_Diseases
Metabolism
Organismal_Systems
Unclassified