Assembling microbial communities: a genomic analysis of a natural experiment in neotropical bamboo internodes

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Microbes participate in ecological communities, much like multicellular organisms. However, microbial communities lack the centuries of observation and theory describing and predicting ecological processes available for multicellular organisms. Here, we examine early bacterial community assembly in the water-filled internodes of Amazonian bamboos from the genus *Guadua*. Bamboo stands form distinct habitat patches within the lowland Amazonian rainforest and provide habitat for a suite of vertebrate and invertebrate species. *Guadua* bamboos develop sealed, water-filled internodes as they grow. Internodes are presumed sterile or near sterile while closed, but most are eventually opened to the environment by animals, after which they are colonized by microbes. We find that microbial community diversity increases sharply over the first few days of environmental exposure, and taxonomic identity of the microbes changes through this time period as is predicted for early community assembly in macroscopic communities. Microbial community taxonomic turnover is consistent at the bacteria phylum level, but at the level of Operational Taxonomic Units (OTUs), internode communities become increasingly differentiated through time. We argue that these tropical bamboos form an ideal study system for microbial community ecology due to their near-sterile condition prior to opening, relatively consistent environment after opening, and functionally limitless possibilities for replicates. Given the importance of opened internode habitats as locations of transmission for both pathogenic and beneficial microbes among animals, understanding the microbial dynamics of the internode habitat is a key conservation concern for the insect and amphibian species that use this microhabitat.

1 **Assembling microbial communities: a genomic analysis of a natural experiment in**

2 **neotropical bamboo internodes**

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Abstract

25   Microbes participate in ecological communities, much like multicellular organisms. However,

26   microbial communities lack the centuries of observation and theory describing and predicting

27   ecological processes available for multicellular organisms. Here, we examine early bacterial

28   community assembly in the water-filled internodes of Amazonian bamboos from the genus

29   *Guadua*. Bamboo stands form distinct habitat patches within the lowland Amazonian rainforest

30   and provide habitat for a suite of vertebrate and invertebrate species. *Guadua* bamboos develop

31   sealed, water-filled internodes as they grow. Internodes are presumed sterile or near-sterile (ie,

32   containing small loads of endosphere-associated microbes) while closed, but most are eventually

33   opened to the environment by animals, after which they are colonized by microbes. We find that

34   microbial community diversity increases sharply over the first few days of environmental

35   exposure, and taxonomic identity of the microbes changes through this time period as is

36   predicted for early community assembly in macroscopic communities. Microbial community

37   taxonomic turnover is consistent at the bacteria phylum level, but at the level of Operational

38   Taxonomic Units (OTUs), internode communities become increasingly differentiated through

39   time. We argue that these tropical bamboos form an ideal study system for microbial community

40   ecology due to their near-sterile condition prior to opening, relatively consistent environment

41   after opening, and functionally limitless possibilities for replicates. Given the potential

42   importance of opened internode habitats as locations of transmission for both pathogenic and

43   beneficial microbes among animals, understanding the microbial dynamics of the internode is a

44   key conservation concern for the insect and amphibian species that use this microhabitat.

45

46

Introduction

47

48   Host-associated microbial communities are an integral part of terrestrial and aquatic

49   ecosystems, and are essential for the health of many animal and plant species, particularly with

50   respect to nutrient acquisition and exclusion of pathogens and parasites (Fitzpatrick et al. 2020;

51   Trivedi et al. 2020). Microbiomes comprise a biological community of bacteria and other single-

52   celled organisms which interact ecologically with each other and their host (Compant et al.

53   2021). Host-associated microbial communities assemble through the process of lineages settling

54   in a location and either establishing themselves or failing to do so. Over time, the survivors of

55   abiotic filtering processes and subsequent interactions between the host and microbial lineages

56   determine the assembly of the microbial community (Trivedi et al. 2020). The assembly

57   processes of these communities can be affected by the ability of some microbial lineages to

58   establish early (e.g., priority or incumbency effects) and by the ecological interactions among

59   taxa (Bittleston et al. 2020). This dependence on initial conditions, combined with the rarity of

60   truly sterile environments in nature, means that the deterministic processes behind bacteria

61   community assembly can be difficult to study in nature.

62   Classical colonization theory seeks to explain the processes by which newly accessible

63   habitats acquire a full complement of species (Horn 1974; Milner 1987; McCook 1994; Milner et

64   al. 2008). Our current understanding of the development and structure of novel communities is

65   informed by the step-wise processes observed in macroscopic organisms. First, a disturbance

66   occurs which makes new habitats or niches available. These newly empty habitats are often

67   characterized by their sparse distribution of resources, the presence of predators, and/or physical

68   environmental constraints (Shea 2002). A given species' physiological and ecological

69   characteristics (its functional traits) determine its ability cope with the challenges of the empty

70   habitats and therefore to be an effective colonizer (Bazzaz 1979; Shea 2002). Early successional

71   species establish themselves in new habitats and eventually change the environmental

72   composition through their combined metabolic processes (McCook 1994). Primary colonizers

73   are of special note because they are able to survive in nutrient-sparse environments and often

74   exhibit striking tradeoffs between competitive growth rates and strong colonizing traits (Cadotte

75   et al. 2006; Vellend 2010). Next, secondary colonizers recruit to the area and find it hospitable

76   due to the manipulation of resources and nutrients by the primary colonizers (Vellend 2010).

77   Secondary colonizers tend to be stronger competitors than primary colonizers and will eventually

78   replace the primary colonizers as the most abundant organisms in the habitat. Each successive

79   wave of species has higher growth rates and will outcompete the previous successional stage and

80   dominate the new niche (McCook 1994). There is a vast body of evidence to support this

81   sequence in multicellular organisms, which has demonstrated predictive power for the changes in

82   species distribution, abundance, and dominance through early community assembly at the

83   macroscopic scale (Vellend 2010).

84   On the other hand, at the microscopic level, efforts to determine colonization and

85   succession patterns yield conflicting results (Nemergut et al. 2013). These studies are hindered

86   by the difficulty of drawing boundaries around microbial communities given the prolific, perhaps

87   global, dispersal of microbes (Nemergut et al. 2007; Fierer et al. 2010). Further, it can be

88   difficult to ascertain which taxa contribute meaningfully to the manipulation of the resources of a

89   new niche given the significant metabolic redundancy of microbial communities (Nemergut et al.

90   2007), and microbes' ability to remain dormant but viable when their local environment is not

91   beneficial to them (Rehman et al. 2016; Sorensen and Shade 2020). Despite these differences

92   from multicellular organisms, microbial communities have been observed to adhere to some

93   predictions from the classical ecological theories of colonization and community assembly.

94   Microbial communities tend to follow both classical abundance curves and species-time

95   relationship observations (Fierer et al. 2010; Nemergut et al. 2013; Dini-Andreote et al. 2015).

96   Most relevant to the process of colonization and succession is the observation that microbial taxa

97   may act as ecosystem engineers in early successional stages and alter the environment for future

98   taxa (Nemergut et al. 2007).

99   Host-associated microbiomes are compelling models to study the process of early

100 microbial succession because each new host provides the opportunity to track the entire process

101 of primary succession without waiting for natural disaster or inducing experimental disturbance.

102 In the microbial world, the process of colonization can take days or weeks, rather than years or

103 decades. Plant hosts are particularly interesting because of the interactions between their various

104 microbiota spheres, such as the root-associated bacteria, the leaf-associated bacteria, and bacteria

105 found within plant tissues (Compant et al. 2021). The plant microbiome is defined as any area

106 that houses microbiota above or below the soil, as well as around and inside the roots and seeds

107 (Trivedi et al. 2020). Host-associated microbes in plants play key roles in nutrient uptake from

108 the soil, tolerance to environmental stress, hormone production, pathogen protection and more

109 (Schlaeppi and Bulgarelli 2015; Compant et al. 2021).

110   The microbial communities that form in small pools of water (phylotelmata) interact with

111 their plant hosts in a variety of ways, including providing metabolic services. For example,

112 phototrophic bacteria in bromeliad tanks may provide nitrogen to the plant (Vergne et al. 2021).

113 Conversely, pitcher plants may actively structure their microbial communities in part by

114 providing digestive enzymes to break down prey. The readily available nutrients that result select

115 for microbial communities that can best utilize them (Grothjan and Young 2022). Across a range

116   of habitats, bacteria from the genera Proteobacteria and Firmicutes are often found in

117   phylotelmata (Vergne et al. 2021; Yourstone et al. 2021).

118   We present a study of early microbial succession in the water-filled internodes of the

119   Neotropical bamboos *Guadua weberbaueri,* which form large, nearly-monoculture stands within

120   the matrix of Amazonian lowland forest. These woody bamboos develop water-filled internodes

121   as they mature (Figure 1A). The internodes are near-sterile when intact, but most are eventually

122   opened by vertebrates or arthropods (Louton et al. 1996). Similar to the related *Guadua*

123   *angustifolia, G. weberbaueri* is a significant component of the Amazonian flora and ecosystem

124   (Griscom and Ashton 2006). *Guadua* internodes are typically exposed to the external

125   environment by foraging brown capuchin monkeys that bite through the walls of the bamboo

126   culm to access the internode (Davidson et al. 2006). Other organisms that regularly open the

127   walls of bamboo culms include Rufous-headed woodpeckers and weevils (Jacobs and von May

128   2012).

129   *Guadua* bamboo internode microbiota should be reflective of two main sources. First,

130   there will likely be some endogenous microbial colonization from the bamboo's rhizosphere.

131   Endospheric microbiota are generally a subset of rhizospheric microbiota (Turner et al. 2013;

132   Trivedi et al. 2020), resulting in a non-random bacteria community before initial opening of the

133   internode. After opening, bamboo internodes are often used as key habitats by various

134   vertebrates and invertebrates, such as the poison frog *Ranitomeya sirensis* (Figure 1C) and

135   mosquito and damselfly larvae (Louton et al. 1996; von May et al. 2009*b*). Additionally, the

136   scarab beetle *Enema pan* is known to shred and feed on *Guadua* bamboo sap and the shredded

137   portions of the bamboo stem attract many sap-feeding insects (Jacobs et al. 2012). These

138   organisms may be potential exogenous sources of microbial colonization along with air and

139   rainfall mediated dispersal and other environmental sources. The small internode pools are

140   therefore a location at which many animal species interact indirectly, and as such could be a

141   transmission point of either pathogens or beneficial bacteria between individuals and species.

142   We experimentally opened the *Guadua* bamboo internode environment to initiate a

143   bacteria community assembly process (Figure 1B). We sampled internode water at the time of

144   opening and across a seven-day time series to characterize the early succession of bacteria

145   species colonizing the novel habitat patches. First, we tested whether each internode had a

146   similar set of initial microbial taxa at the time of opening. Second, we tested for taxonomic

147   turnover with time and assessed whether there was an observed loss of initial bacteria OTUs

148   (Operational Taxonomic Units). We hypothesized that there would be taxonomic turnover given

149   previous studies that have documented similar observations (Nemergut et al. 2013). In addition,

150   we measured the distance between bacteria communities at each time step to indicate whether

151   stochastic or deterministic processes govern succession in this system (Dini-Andreote et al.

152   2015).

153

154   **Materials & Methods**

155   *Sample Collection*

156   We collected our samples at Los Amigos Biological Station in southern Peru starting on 30

157   November 2016 under research permits from the Peruvian National Forest and Wildlife Service

158   (Servicio Nacional Forestal y de Fauna Silvestre) R.D.G. No 029-2016-SERFOR-DGGSPFFS,

159   R.D.G. 405-2016-SERFOR-DGGSPFFS, and R.D.G. 116-2017-SERFOR-DGGSPFFS. Los

160   Amigos is located in lowland tropical rain forest adjacent to the Madre de Dios river, an Amazon

161   river tributary. We collected water samples from eight bamboo stalks of the species *Guadua*

162   *weberbaueri* that were distributed across two plots in upland terra firme forest (Figure 1A). We

163   sampled four stalks from each plot. We determined the following eligibility for internode

164   sampling: the internode must be 1 to 2 meters above the ground, contain water, and be closed.

165   We opened the walls of bamboo internodes using a bleach-sterilized blade to cut a 20 – 40 mm

166   notch horizontally near the top of the node, above the water level. Then we pulled back a section

167   of the internode's external skin using the edge of the blade (Figure 1B). We chose this method

168   because it mimics the actions of foraging brown capuchin monkeys (Davidson et al. 2006;

169   Jacobs and von May 2012).

170   At the time of opening, we collected 0.5mL of water from each bamboo internode using

171   sterilized transfer pipettes (Figure 1B). We preserved these samples in 1.5mL of CTAB buffer.

172   This buffer has been used in similar studies of microbial communities in other phytotelmata

173   (e.g., pitcher plants; (Bittleston et al. 2016)). Our subsequent sampling occurred 24 hours after

174   opening, 48 hours after opening, and 168 hours after opening.

175

176   *Nucleic Acid Extraction:*

177   We extracted the total metagenomic DNA from the samples by incubating 200 microliters of

178   CTAB and water mixture with 400 microliters of buffer ATL from Qiagen's DNEasy Blood and

179   Tissue kit and 10 microliters of proteinase K. We incubated the samples at 56℃ for 24 hours. We

180   then performed a phenol-chloroform-isoamyl alcohol extraction following Barker 1998. We

181   chose the phenol-chloroform approach to minimize contamination by plant secondary

182   metabolites that could inhibit PCR or sequencing reactions. We included a negative control in

183   the extraction and processed and sequenced the control along with our samples. Samples were

184   amplified using primers specifically developed to target the V3-V4 hypervariable region of the

185   bacterial 16S rRNA gene (Kozich et al. 2013). We ran 25 PCR cycles with an annealing

186   temperature of 55 C. Sequencing libraries were created with the NEBNext DNA Ultra prep kit 2

187   (cat# E7645L) with barcoded dual-index primers.

188   Purified and eluted DNA amplicons were stored at 4℃ before being sent for sequencing.

189   We standardized all PCR products using a Qubit measurement and pooled equimolar quantities

190   of each separate PCR well for the final run. Each sample, taken from a single bamboo on a

191   sampling day, was individually barcoded prior to DNA sequencing. Libraries were sequenced

192   using the Illumina MiSeq platform. Data from this project can be found on NCBI's Short Read

193   Archive under BioProject PRJNA766623.

194

195   *Bioinformatic pipeline*:

196   We used the program mothur v.1.48.0 to identify OTUs (Schloss et al. 2009). Following the

197   mothur miseq SOP, we made contigs from our sequences, removed fragments that were not

198   within 8 base pairs of our 292 base pair target length, and removed sequences with ambiguous

199   base calls or homopolymers over 8 bp long. We removed sequences that did not align within an

200   80% similarity cutoff to a 16S bacteria sequence in the SILVA v.138 database (Quast et al.

201   2012). We identified the unique sequences in the dataset and removed chimeras. We matched

202   each of our sequences to the RPD Classifier database using the mothur classify.seqs and

203   removed any sequence that aligned most closely with a non-bacteria reference. We then clustered

204   sequences at a 0.03 distance threshold. Finally, we found the consensus classification for our

205   OTUs using the RPD Classifier database v18. The RPD Classifier lists some chloroplast

206   sequences under their "Bacteria" kingdom designation, using the phylum-level labels

207   "Cyanobacteria/Chloroplast." Since we were likely to have bamboo DNA in our extractions, we

208   removed any plant contamination from our sequences, resulting in the removal of two OTUs.

209   We used a custom script in R 4.1.1 to parse the output 'mcc.shared' sample by OTU matrix and

210   'mcc.0.03.cons.taxonomy' file into an OTU-by-sample matrix in which each cell of the matrix

211   represented the number of reads of an OTU in a specific bamboo-day sample (R Core Team

212   2021). Both input files and the code used for processing are available Dryad repository

213   (doi:10.5061/dryad.4qrfj6qd1). To account for possible contamination, we removed any OTU

214   represented by more than 10 sequences in our negative control. Finally, we set all OTUs with a

215   read depth of 10 or less to zero to account for index hopping during sequencing. To account for

216   possible amplification failure, we removed all days of any bamboo stems that had zero OTUs

217   during any day besides the first. After these steps, we retained seven stems, 19-5, 19-7, 19-8, 19-

218   10, 24-3, 24-6, and 24-9.

219

220   *Initial colonizers, taxon turnover, and community diversity over time:*

221   To visualize taxon turnover through time, we subsetted the OTU-by-sample matrix by internode

222   and sampling day. To assess patterns of species turnover during early succession, we identified

223   the proportional abundance of each bacteria family in each bamboo stem. We found the mean

224   proportional abundances of all bacteria families grouped by day, and selected the two most

225   abundant in each day, for a total of six families. We plotted the mean abundances of the six focal

226   families across each sampling day. To quantify the species-time relationship, we calculated the

227   Shannon diversity index of each stalk at each sampling point using the R package 'vegan'

228   (Oksanen et al. 2018). We tested for significant differences in the diversity values over time

229   using t-test between the distribution of diversity values calculated for each sampling day.

230

231   *Community differentiation over time:*

232   To identify patterns of community similarity in our dataset, we analyzed beta diversity as a

233   function of time and internode. To do so, we created a Bray-Curtis distance matrix using the R

234   package 'vegan.' To visualize the relative differences between our samples, we performed a

235   nonmetric multidimensional scaling (NMDS) ordination on the OTU-by-sample matrix using the

236   'vegan' command 'metaMDS.' For this analysis, we again used the proportional abundance of

237   OTUs. The algorithm calculates pairwise distances between all sets of communities (in this case

238   each community is a sample from a single internode sampled at a particular timepoint), then

239   represents those pairwise relationships in two-dimensional space while maximizing distances

240   between communities. Since this method is impacted by missing data, we removed samples with

241   fewer than 50 OTUs, thereby removing all of our day 1 samples.

242   We calculated both the location of each community in NMDS space and the weighted

243   'species score' for each OTU, representing that OTU's average location in NMDS space. Since

244   many OTUs appear in more than one community, the score represents the location of the

245   centroid of that community weighted by the relative abundances of the OTU. A pattern of

246   samples from individual bamboos grouping together in NMDS space would be consistent with a

247   process of community assembly determined by initial conditions. In contrast, a pattern of

248   samples grouping by sampling day would provide evidence of a more predictable pattern of

249   succession similar to those observed in macroscopic communities. Using the NMDS axes, we

250   plotted the locations of each OTU, and visually identified the OTUs belonging to the four most

251   common bacteria taxa. We overlaid the OTU plot with points representing the centroids of each

252   sampled community in NMDS space.

253   We tested for significant community differentiation through time using a PERMANOVA

254   approach, focusing on the relative importance of sampling time and sampling location. Sampling

255   location refers to the individual bamboo stem from which internodes were sequentially sampled

256   through our experimental procedure. Briefly, this is a non-parametric approach for MANOVA-

257   like analyses that estimates the proportion of variance in a multivariate dataset explained by each

258   of several factors. PERMANOVA uses a pairwise distance matrix between observations that are

259   grouped according to potential explanatory variables. The approach compares distances between

260   centroids (the mean position of all included points on the axes) of the pre-defined explanatory

261   groups to distances between centroids of randomly sampled observations. The p-value derived

262   from the approach represents the proportion of randomly sampled groups that have centroids that

263   are more separated than those of the explanatory groups. The approach is well-suited to zero-

264   inflated community data because it makes no distributional assumptions about the underlying

265   data (Anderson 2001, 2017; McArdle and Anderson 2001). We implemented the PERMANOVA

266   test using the 'adonis2' function in 'vegan.'

267   We applied a square root transform to the OTU-by-sample community matrix, then used

268   the Bray-Curtis index to create a pairwise distance matrix between all sampled communities. We

269   performed 999 permutations of the matrix to find the significance values of location (e.g.,

270   bamboo stem) and sampling day as potential explanatory variables for community

271   differentiation. Finally, we found the pairwise Bray-Curtis dissimilarity between all samples

272   within each timepoint and plotted those values against a time axis. We performed this analysis to

273   determine whether internodes tended to converge toward a stable community structure over time,

274   or whether they tended to have idiosyncratic community development trajectories at the level of

275   individual bacteria OTUs.

276

Results

278

279 *Early successional stages and taxon turnover:*

280 At the time of opening, only one stem had detectable bacteria, with the two most abundant taxa

281 belonging to the Proteobacteria families *Xanthomonadaceae* and *Acetobacteraceae*. Only two

282 stems had detectable bacteria at this stage. By 24 hours after opening the internode (day 2),

283 members of the Proteobacteria families *Enterobacteriaceae* and *Burkholderiaceae* being the

284 most abundant. By 48 hours after opening, *Moraxellaceae* had replaced *Enterobacteriaceae* as

285 the most abundant taxa. By seven days after opening, the two most abundant bacteria taxa were

286 *Comamonadaceae* and *Arcobacteraceae* (Figure 2A). We found that bacteria community

287 Shannon diversity steadily increased over time (Figure 2B). Shannon diversity differences were

288 statistically significant between day 1 and day 3 (p=0.0012, t= -5.069, df=7.3814), day 1 and day

289 7 (p=0.0013, t= -6.144, df=5.326), day 2 and day 7 (p=0.0168, t= -3.0121, df=7.997), and day 3

290 and day 7 (p=0.0323, t= -2.740, df=6.261).

291

292 *Community differentiation over time:*

293 Our sample centroids show a steady progression from day one to day seven across our NMDS

294 axes, with samples from each day generally clustering in NMDS space along axis 1 (Figure 3A).

295 The PERMANOVA test of sampling day and stem (e.g., individual bamboo stem) as explanatory

296 variables for changes in community composition differentiation found evidence for significant

297 sampling day but not stem (sampling day: p = 0.001, F=3.011, df=3; stem: p = 0.168, F=1.180,

298 df=4). We had four sampling days and seven stems, for a total of 28 samples. Proportionately

299 more variance in community composition was explained by sampling day relative to stem ($R^2 =$

300 0.415 versus $R^2 = 0.217$). Inspection of Bray-Curtis distances between bacteria communities

301 showed that were high throughout the sampling period, with the exception of our single pairwise

302 comparison at day 0 (Figure 3B, 3C).

303

304 **Discussion**

305

306 In this study, we were motivated to identify how patterns of early microbial succession compared

307 against theoretical expectations derived from macro-scale organisms and previously studied

308 bacteria community microcosms. Particularly at higher levels of taxonomic resolution, our

309 results reflected classical predictions about early succession derived from macro-scale

310 communities (Horn 1974; Milner 1987; McCook 1994; Milner et al. 2008). Our hypotheses

311 regarding common primary colonizers and taxon turnover were supported by the consistent

312 primary colonization by *Enterobacteriaceae* and the subsequent domination by other taxa over

313 time (Figure 2A). We also found that taxon density increased significantly over time, and there

314 was a significant difference in taxonomic diversity between initial and final sampling time

315 (Figure 2B). This suggests a clear separation in taxonomic diversity and identity between our

316 initial sampling and our final sampling. Recapitulating the turnover analysis performed on OTU

317 counts, we see specific taxonomic groups of bacteria associated with sampling-day clusters along

318 the NMDS axes. Other research has led to similar conclusions that large-scale ecological

319 processes are replicated in phylotelmata microbiomes. For example, tree hole microbial

320 communities show a shift from r- to K-selected species over time (Pascual-García and Bell

321 2020).

322   Our PERMANOVA test showed that communities were more differentiated by the day of

323   sampling than by individual bamboo stem, further indicating a predictable successional

324   progression. We found that while the composition of higher taxa in the internodes followed a

325   predictable progression (Figure 2A, 2B). However, the Bray-Curtis distance between stems

326   within a day was high throughout the sampling period. This result indicates a high degree of

327   stochasticity in community assembly during early succession in bamboo internodes (Figure 2C).

328   The combination of these results implies a strong cumulative interaction between individual

329   bamboo stem and time that resulted a community structure at the OTU level that was heavily

330   influenced by contingent events in early community assembly despite a much more predictable

331   successional pattern at the bacteria phylum level. This may reflect seeding of later microbial

332   successional stages by insects or other animals using the open internodes, as has been observed

333   in other phylotelmata systems (Gebuhr et al. 2006). As a caveat, our work likely recovered only

334   a portion of the total microbial community. First, we did not use a bead-beating approach during

335   our DNA extraction, meaning that DNA from some bacteria with cell wall chemistries that were

336   not vulnerable to our digestion enzyme may not be represented in our sample. Second, we

337   focused on only bacteria rather than viruses, Archaea, or Eukaryotes. Finally, we sampled only

338   the water in the bamboo, while some microbes likely attach to the plant walls rather than

339   occurring in the water column.

340   Our work has implications for the ecology of the Amazon rain forest in addition to

341   bacteria community succession processes. Woody bamboo species in the genus *Guadua* are a

342   major component of Amazonian flora and ecosystem (Nelson 1994; Griscom and Ashton 2006)

343   and have been present in southwestern Amazonia for at least 45,000 years (Olivier et al. 2009).

344   Species in this genus play important ecological and evolutionary roles in the forest community

345   (Kratter 1997; Griscom and Ashton 2006; Jacobs et al. 2012), and hold economic importance in

346   the construction of weapons, forestry, construction, artisanship, and cooking (Jacobs and von

347   May 2012). A more complete understanding of this species' associated microbiota may be an

348   important starting point for future studies regarding bamboo conservation and health. Further

349   studies should focus on subsequent successional stages of the microbial community given that

350   open bamboo internodes can hold water for more than three months (von May et al. 2009*a*).

351   Future directions of study might include exploring the unique interactions within and

352   around bamboo internodes, such as opening by Brown capuchin monkeys (or Rufous-headed

353   woodpeckers or weevils) or their use in the life cycles of insects, and how these external factors

354   may contribute to the characteristics of the internal microbiome. A complete assessment of these

355   relationships may include the metagenomic sequencing of bamboo-associated organisms,

356   particularly the external surfaces of associated insects and amphibians. Another important aspect

357   of the internal endosphere microbiome is its potential interactions and sourcing from the

358   rhizosphere (Trivedi et al. 2020; Compant et al. 2021). Further investigations of this system

359   might include the metagenomic sequencing of the soil and the internal and external roots of the

360   bamboo to better identify sources of primary colonization and give context to the selective

361   pressures within the bamboo internode.

362   After bamboo internodes have been opened, they become habitat patches in which a

363   variety of multicellular animals interact with microbial lineages. While we have focused on the

364   role of animals in seeding microbial lineages into the internode, the fitness of the animals is also

365   impacted by their interaction with microbial communities. Animals are dependent on their

366   microbiomes for protection from pathogens and parasites, and gut microbiomes help their hosts

367   digest food (Stough et al. 2016; Rosshart et al. 2017). In frogs, the skin microbiome can prevent

368   mortality due to the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) as many

369   groups of cutaneous bacteria produce antifungal compounds inhibiting the growth of *Bd* (Harris

370   et al. 2006; Brucker et al. 2008; Burkart et al. 2017). Recent studies conducted in southern Peru

371   have identified at least 20 cutaneous bacteria isolates that inhibit *Bd* growth (Catenazzi et al.

372   2018). In arthropods, the microbiome impacts host fitness and life history, notably by interacting

373   with parasitic infections (Hamdi et al. 2011; Hughes et al. 2014; Bouchon et al. 2016). The

374   bamboo internode microbial community may play a key role in the ecology of species inhabiting

375   *Guadua* islands in Amazonia as a source for host-associated microbiome lineages. For example,

376   eight species of frogs and a lungless salamander are known to use open bamboo internodes as a

377   retreat site (von May et al. 2009*a*; Whittaker et al. 2015). Additionally, two of these species, the

378   poison frog *Ranitomeya sirensis* (von May et al. 2009*a*, 2009*b*) and the tree frog *Osteocephalus*

379   *castaneicola* (Whittaker et al. 2015), are known to breed in bamboo internodes with their larvae

380   completing their development in the phytotelmata. Further research can clarify the role

381   internodes play in seeding bacteria into the various hosts that occur in the community.

382   In addition to its tropical biology applications, our study serves as a proof of concept for

383   using bamboo internodes as a model system for microbial studies. The unique structure of

384   bamboo internodes allows for a natural, but controlled, semi-closed environment that supports

385   ecological interactions between microbes, vertebrates and invertebrates. Bamboo internodes

386   provide a unique set of characteristics as a naturally occurring microcosm for studying bacteria

387   community dynamics. First, the physical structure of the internode changes little after opening,

388   removing a potential set of confounding variables present in other microcosm systems. Because

389   internodes receive little natural sunlight, bamboo internode communities are composed of

390   heterotrophic bacteria, much like soil communities or host-associated microbiome communities

391   (Fierer et al. 2010). Unlike soil communities, internodes have defined boundaries (Fierer et al.

392   2010; Nemergut et al. 2013). Unlike host-associated communities, internode communities should

393   be less directly impacted by host-immune driven top-down control (Jacobs and Braun 2014).

394   However, similar to host-associated communities, the animal occupants of the internode provide

395   the majority of the available nutrients to the system and likely the majority of the colonizing

396   bacteria lineages. As such, internode communities provide a natural model system in which

397   bacteria that are similar taxonomically to host-associated communities interact in an environment

398   without host immune regulation. This contrast could serve as an opportunity to investigate the

399   degree to which host immune regulation alters microbial community dynamics. The versatility of

400   the bamboo internode system as a model, as well as the system's evolutionary uniqueness as a

401   high-endemicity series of ephemeral islands in an Amazonian rainforest matrix, make future

402   work on the internode communities an exciting prospect.

403

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562

563   **Figure legends**

564   Figure 1: *Guadua weberbaurei* characteristics and associated animals. A) cross-section of a

565   closed internode partially filled with water (the phylotelmata), B) a representation of the

566   experimental opening and sampling of bamboo water, C) the frog *Ranitomeya sirensis*, which

567   lays its eggs in bamboo internodes, D) weevil feeding on bamboo sap. Panels A and B illustrated

568   by Karin von May, photo credit for C to Rudolf von May, photo credit for D to Marcos Ríos.

569

570   Figure 2: Species composition and community diversity through time. A) abundance of the top

571   four most common bacteria families in each sampling day. Triangles represent mean abundances

572   across bamboos, small circles are single bamboo measures. B) Measures of Shannon Diversity of

573   the bacteria community of each bamboo on each sampling day.

574

575   Figure 3: Community progression through time. A) OTUs of the four most abundant bacteria

576   taxa cluster (represented by x-shaped points) cluster in different regions of the NMDS space, B)

577   centroids of the communities (circular points) for each cluster more strongly by sampling day

578   than by bamboo stem, and C) pairwise distances between bamboo samples within sampling days

579   become larger over time.

Figure 1

Guadua weberbaurei characteristics and associated animals

A) cross-section of a closed internode partially filled with water (the phylotelmata), B) a representation of the experimental opening and sampling of bamboo water, C) the frog *Ranitomeya sirensis*, which lays its eggs in bamboo internodes, D) weevil feeding on bamboo sap. Panels A and B illustrated by Karin von May, photo credit for C to Rudolf von May, photo credit for D to Marcos Ríos.

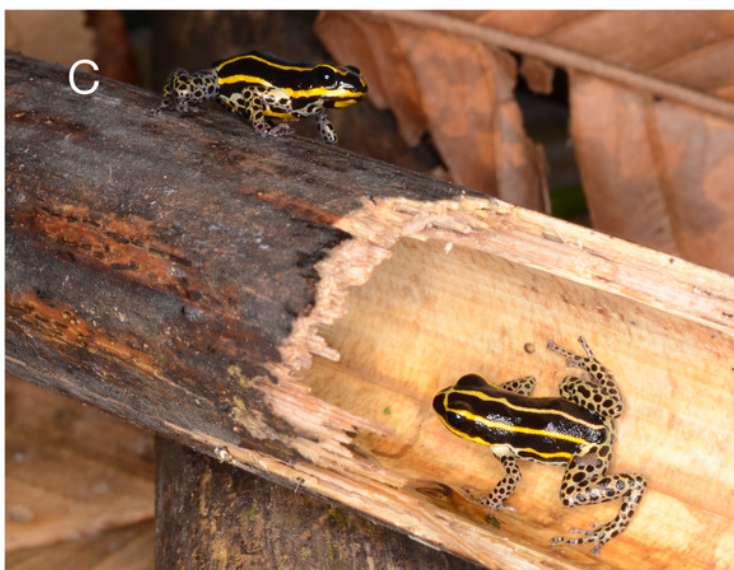
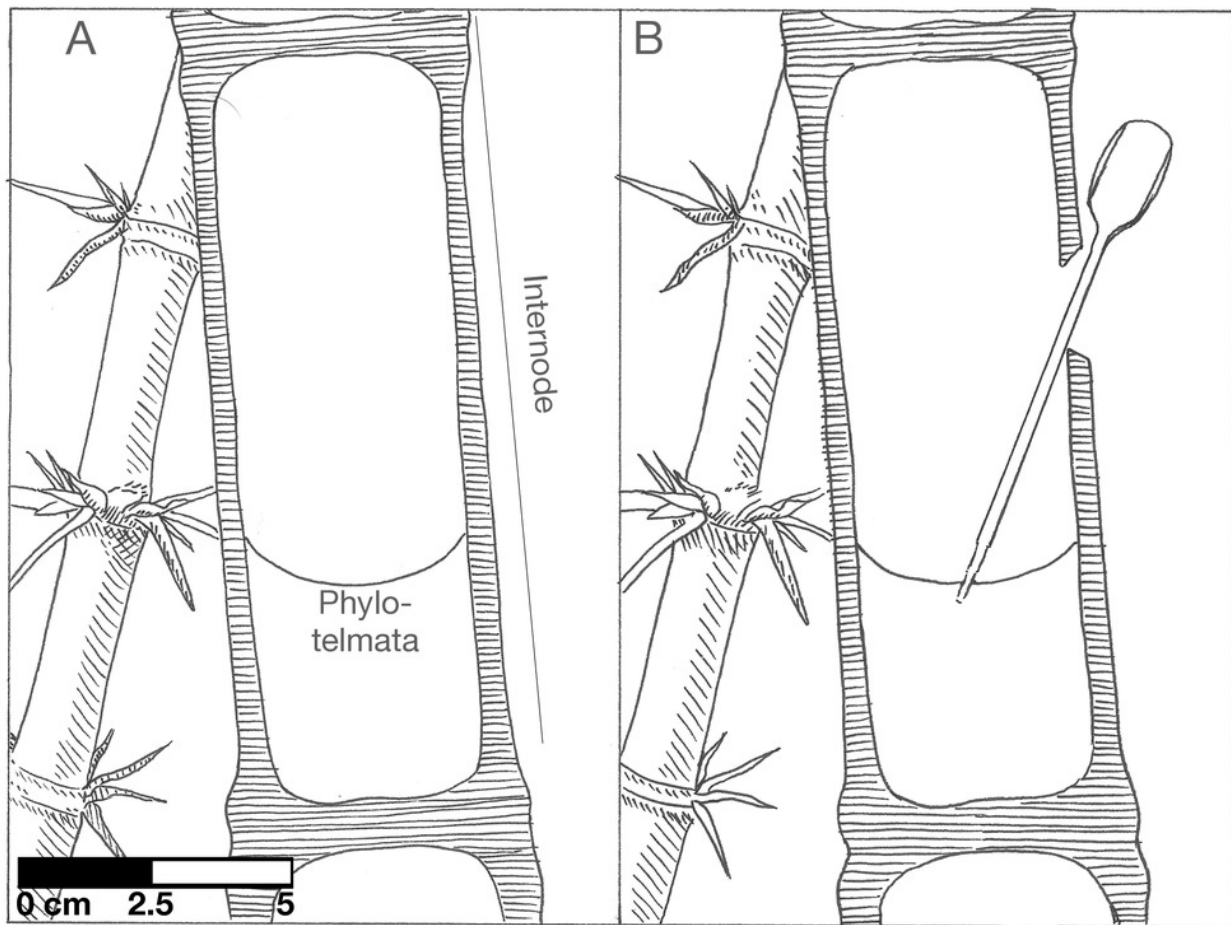



Figure 2

Species composition and community diversity through time

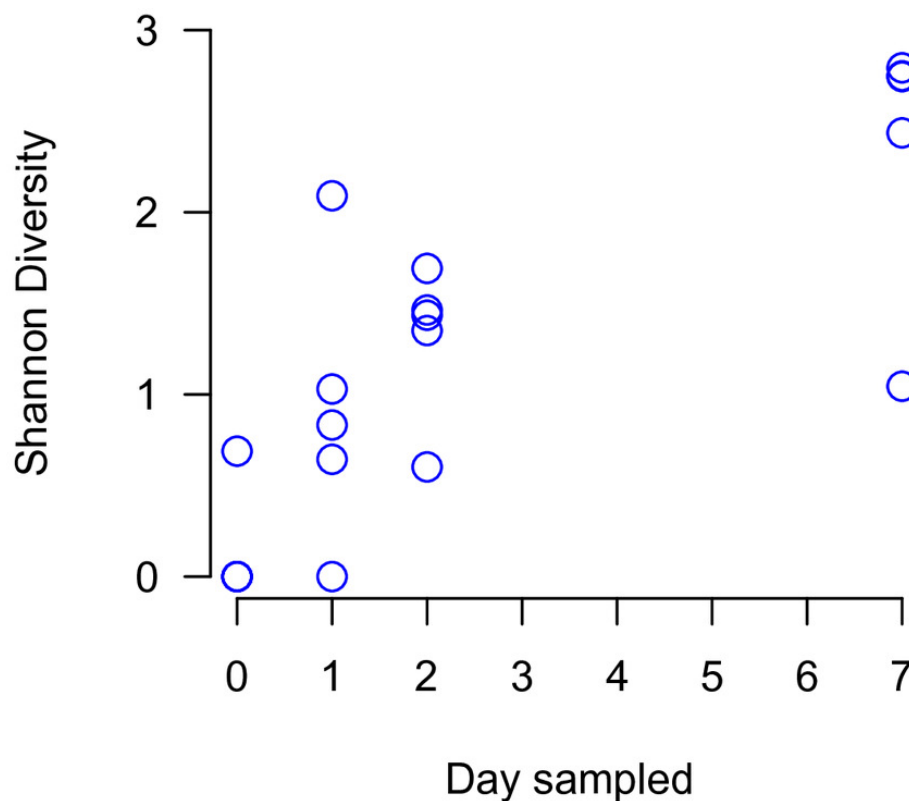
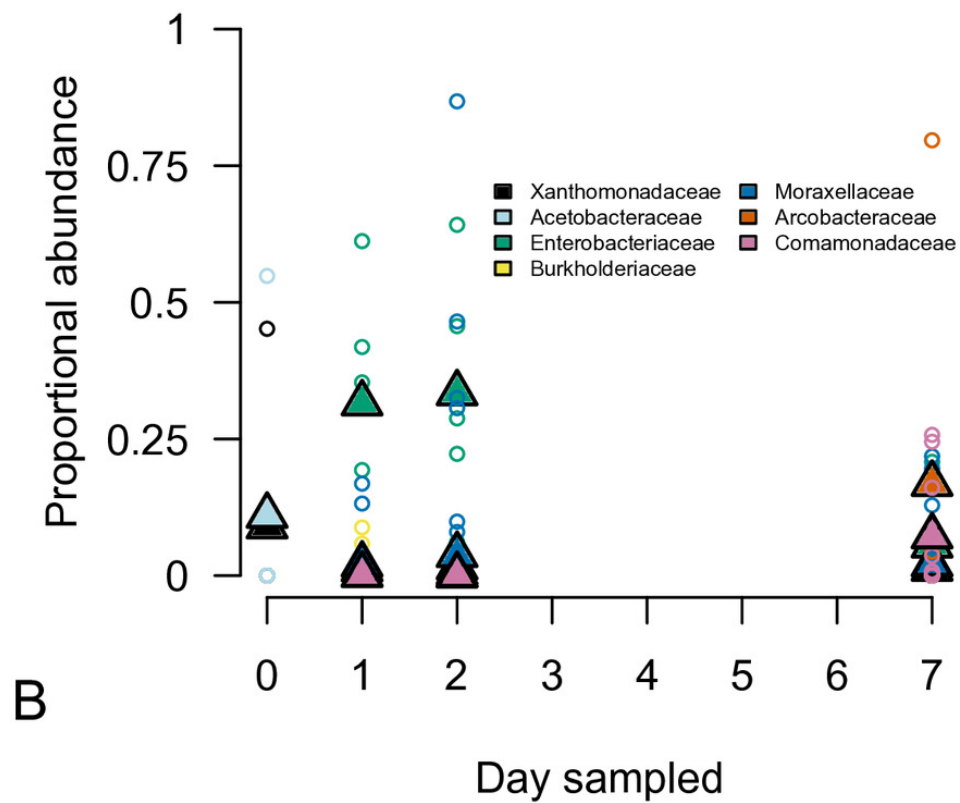
A) abundance of the top four most common bacteria taxa in each sampling day. Triangles represent mean abundances across bamboos, small circles are single bamboo measures. B) Measures of Shannon Diversity of the bacteria community of each bamboo on each sampling day.

B

Figure 3

Community progression through time

A) OTUs of the four most abundant bacteria taxa cluster (represented by x-shaped points) cluster in different regions of the NMDS space, B) centroids of the communities (circular points) for each sample cluster according to day, and C) pairwise distances between bamboo samples within sampling days become larger over time.