Appendix B

GENE SEQUENCING-BASED ANALYSIS OF MICROBIAL MAT MORPHOTYPES, CAICOS PLATFORM, BRITISH WEST INDIES

Abstract

Active carbonate platforms provide modern analogs to study microbial-mat development and taphonomy in the sedimentary record. Microbial-mat descriptions and classifications for tropical tidal-flat environments have focused predominantly on morphological observations. This is exemplified by flat and biscuit-shaped mats, where the mat morphotypes are postulated to reflect different Cyanobacteria communities as the main mat-building taxa. To compare the total microbial communities of these two mat types and test this Cyanobacteria hypothesis, we applied optical microscopy and gene sequencing methods using samples from a tidal algal marsh on Little Ambergris Cay, Turks and Caicos, B.W.I. With gene sequencing we find that total diversity and community composition differs significantly between morphotypes; the biscuit mat is more diverse than the flat mat. Microscopy results support that Cyanobacteria populations colonizing the surface layer of these two mat types are responsible for much of the mat's structural elements; however, genetic data find the Cyanobacteria population is indistinguishable between the two mat types. The recovered Cyanobacteria populations fall predominantly into three taxa: *Scytonema*, *Halomicronema,* and *Crinalium*. We propose that the morphology of these two mat types is not controlled by the Cyanobacteria, but instead reflects a time-integrated microbial response to environmental factors, where the microbial community becomes more diverse with time since environmental disturbance.

Introduction

In carbonate strata of all ages, stromatolites — attached, lithified sedimentary growth structures, accretionary away from a point or limited surface of initiation (Grotzinger & Knoll 1999) — are widely interpreted as a record of the interaction of microbial communities (particularly Cyanobacteria) with carbonate sediments and cements (e.g., Frantz et al. 2015). Although it is unclear what processes determine the morphology of microbial mats, hypotheses include microbial community composition (Dupraz & Visscher 2005; Gerdes et al. 2000; Golubic et al. 2000; Noffke 2010; Shepard & Sumner 2010), metazoan and protistan grazing (Bernhard et al. 2013; Garrett 1970), hydrodynamics and sedimentation (Andres & Reid 2006; Gebelein 1969; Mariotti et al. 2014; Martin et al. 1993), and other environmental factors (Gerdes et al. 2000; Petroff et al. 2010; Wharton et al. 1983). In modern environments, microbial mats in tidal flats, sabkhas, and shallow subtidal zones are commonly considered analogs and possible precursors to stromatolites (e.g., Browne et al. 2000; Dupraz et al. 2009).

Modern microbial mats develop a variety of morphologies ranging from flat, laminar forms, to cone-like pinnacles, to small domal biscuit structures (Browne et al. 2000). While naming conventions and classification of microbial mats in active carbonate tidal environments vary, general descriptions have focused on distinctions between a flat, laminar mat type (Fig. 1D) and a raised, biscuit-mat type (Fig. 1C). In their description of storm-disturbed West Caicos microbial mats Wanless et al. (1988) suggested that the two morphologies were a result of different "algae" with different colonization strategies: the flat laminar mats were made by Schizothrix and the biscuit-type mats by Scytonema. Schizothrix was described as a rapid colonizer, forming a new surface mat layer within weeks of the storm, where sediment cover was millimeters thick. Scytonema was described as a slower-growing mat type that eventually colonizes areas previously colonized by Schizothrix on the order of months, in the absence of smothering sediment flux.

In contrast, Gebelein (1969) described what were termed Schizothrix mats composed of the same organism with different surface expressions based on sedimentation rates and water velocity. Additional observations of open marine microbial-mat structures from the Bahamas suggested that accommodation space (water depth) is another important factor in controlling growth morphology (Andres & Reid 2006). Finally, Golubic (1991) described mat types similar to the Bahamian mats

in the sabhkas of Abu Dhabi as “gelatinous laminated biscuits” and “low flat mats,” in the subtidal and mid-intertidal zones, respectively, distinguished both by their environmental context and by different Cyanobacteria communities, as determined by morphology. Therefore these forms, and the factors controlling them, may not pertain only to the Bahamas.

It is critical to note that for the prior studies cited above, and others (Freytet & Verrecchia 1999; Paerl et al. 2001), the names “Schizothrix” and “Scytonema” were used to define shapes of microorganisms found in the mats rather than the genetic identity associated with those classifications. This led to the description of entire mats by the names Schizothrix or Scytonema (e.g., “Schizothrix mats” and “Scytonema mats”; Wanless et al. 1988) based on microscopic morphological observation. While microscopy still holds substantial value for many aspects of microbial ecology, genetic identification provides an objective comparative-biology framework and is the current gold standard for taxonomic classification of microorganisms (Woese 1987). This is particularly important for Cyanobacteria, for which morphology may appear diagnostic but is homoplasic, particularly baeocystous and filamentous cells types (Shih et al. 2013). Formally the terms *Schizothrix* and *Scytonema* define different genera of Cyanobacteria; here we reserve the use of these terms solely to denote the genetic clades, not morphological attributions at either microscopic or macroscopic length scales. Consequently we use the terms biscuit mat and flat mat to describe the two most common morphotypes on the Caicos platform (Wanless et al. 1988) and test the implicit assumption that these different morphotypes reflect the mat-building activities of different Cyanobacteria by mapping between observed structures and the phylogenetic identity of the taxa within them. We labor under current Cyanobacteria nomenclature accepted by the 16S rRNA gene *SILVA* database maintained by the Microbial Genomics and Bioinformatics Research Group in Bremen, Germany (Quast et al. 2013) as the classification scheme for all microorganisms discussed in this work.

To compare the microbial populations of the flat and biscuit mat morphologies, we collected microbial-mat samples of both morphotypes from tidal flats of the Caicos platform, Turks and Caicos, B.W.I. (Fig. 2). We examined the mats using microscopy and NGS iTag technologies. iTag sequencing is particularly valuable for profiling and comparing microbial diversity in complex samples because it focuses on a short, hypervariable region of the 16S rRNA gene — a classic marker used in phylogenetic studies (Caporaso et al. 2012). This technology is able to

produce hundreds of thousands of sequencing reads per sample, and is therefore the currently preferred technique to access and compare the microbial diversity of a wide range of environmental samples. Our results showed high similarity in Cyanobacteria populations between morphotypes. Holistic community analysis showed differences between morphotypes and suggested that other factors have greater influence on determining mat morphology on the Caicos platform than the Cyanobacteria.

Methods

The mats studied occur in a tidal marsh in the center of Little Ambergris Cay, West Caicos, B.W.I., visited in February, 2014 (Fig. 2). Both mat samples were collected during midday. The flat-mat sample was collected near the main tidal channel connecting the lagoon to the Caicos platform interior (Fig. 1A), and the biscuit mat sample was collected in the more interior part of the lagoon. The portion of the lagoon surveyed by foot and unmanned aerial vehicle (UAV) contained large regions of biscuit mats, intermixed with areas of flat laminar mats, and dynamic sediment-filled channels with no mat development (Fig. 1A, B). Individual biscuits ranged up to 20 cm wide and 10 cm high (Fig. 1C, E). Samples were collected from two representative locations on each mat morphotype (Fig. 1C, D), but importantly none of the microscopic and macroscopic visualization of the two mat types (n = 10) gave any indication of compositional differences between the Cyanobacteria observed in each morphotype.

Sampling was accomplished by aseptic coring (upper ~ 3 cm of mat) with sterile 50 ml polypropylene conical centrifuge tubes. Samples were kept at 4^{o}C until processed. A subset of the two mats was sectioned visually by pigment layer under a dissection microscope and then preserved in paraformaldehyde. These samples were washed and stored in ethanol at -20 ^{o}C. Preserved samples were vortexed to disaggregate the mat layers before pipetting onto slides used for microscopy and micrographs.

DNA was extracted from a thin (~ 3 mm^2 cross-sectional area), vertical section of each mat (~ 1 g total biomass) removed by sterile razor. Samples were mechanically lysed in a bead beater (FastPrepFP120, ThermoElectronCorp.) for 45 s at setting 5.5. DNA was extracted using the Power Soil DNA extraction kit (Mo Bio Laboratories, Inc.). iTag samples were prepared with

Earth Microbiome Project primers (515f and 806r) and recommended reagents 5 Prime Master Mix; (Caporaso et al. 2012). An initial amplification of 30 cycles with primers lacking the barcode, linker, pad, and adapter was performed for all samples, in duplicate. All samples yielded PCR amplicons when viewed on a gel after initial pre-barcoding PCR (30 cycles). Duplicate PCR reactions were pooled and reconditioned for five cycles with barcoded primers. PCR negative controls, substituting PCR water for DNA template, were amplified for 40 cycles total and also sequenced.

Resulting iTag sequences were processed using the *mothur* (Schloss et al. 2009) Standard Operating Procedure (SOP) for Illumina MiSeq sequencing of the 16S rRNA gene V4 region (accessed online May 2015). A concatenated file of the *mothur* version of separate archaeal and bacterial SILVA version 119 databases was used for alignment and taxonomic classification of sequence reads (Quast et al. 2013; Schloss & Westcott 2011; Schloss et al. 2009). Any taxa in the PCR negative control sample were removed from the resulting microbial-mat taxon database. iTag sequences have been submitted to the SRA under Bioproject: PRJNA316900.

Assessment of sampling depth was made with Good's Coverage — a common ecological approach that estimates the percent of the total species in an environment that were recovered in the sampling of that environment, equal to 1-[number of operational taxonomic units (OTUs) that have been sampled once, divided by the total number of all individuals sampled] multiplied by 100 (Good 1953). Alpha diversity was estimated using the Inverse Simpson metric (1/D) where D is a measure of the number of times an OTU is observed (species richness) divided by the total number of individuals in a community (species evenness) (Hill 1973; Simpson 1949). We used the UniFrac distance metric (Lozupone & Knight 2005) to assess the microbial community phylogenetic similarity. This method determines phylogenetic trees from the sequences in each sample and computes the branch length that is unshared between the each sample's tree, effectively quantifying how dissimilar the two communities are. All statistics were calculated using scripts in *mothur* and are reported at the unique sequence, 99%, and 97% OTU similarity levels.

Figure 1: A) UAV photo mosaic from north shore of Little Ambergris Cays over tidal marsh. Red star indicates the sampling location of flat mats and the yellow star in darker region marks the sampling location of biscuit mats. Orange star indicates sediment-filled channel with no mat growth, and red arrow highlights a person for scale. White dots numbered 1 - 4 orient the mosaic with the following GPS coordinates: 1) 21.306231° N, 71.675926° W; 2) 21.301820° N, 71.686693° W; 3) 21.305593° N, 71.691211° W; 4) 21.297430° N, 71.725451° W. B) Close-up of contact between and examples of flat mats (lower half of image) and biscuit mats (upper half). Black bar is approximately 0.5 m. C) Close-up of biscuit mats in the sampled region with hand for scale. D) Close-up of the flat mats in the sampled region with hand for scale. E) Vertical cross-section through a biscuit mat showing shape and internal structure with hand for scale. F) Vertical crosssection through a biscuit mat showing annotated pigmentation layers (G = green, P = purple and pink, B = brown). Black bar is approximately 1 cm. G) Vertical crosssection through flat mat showing annotated pigmentation layers (G = green, P = purple and pink, B = brown). Hand is for scale.

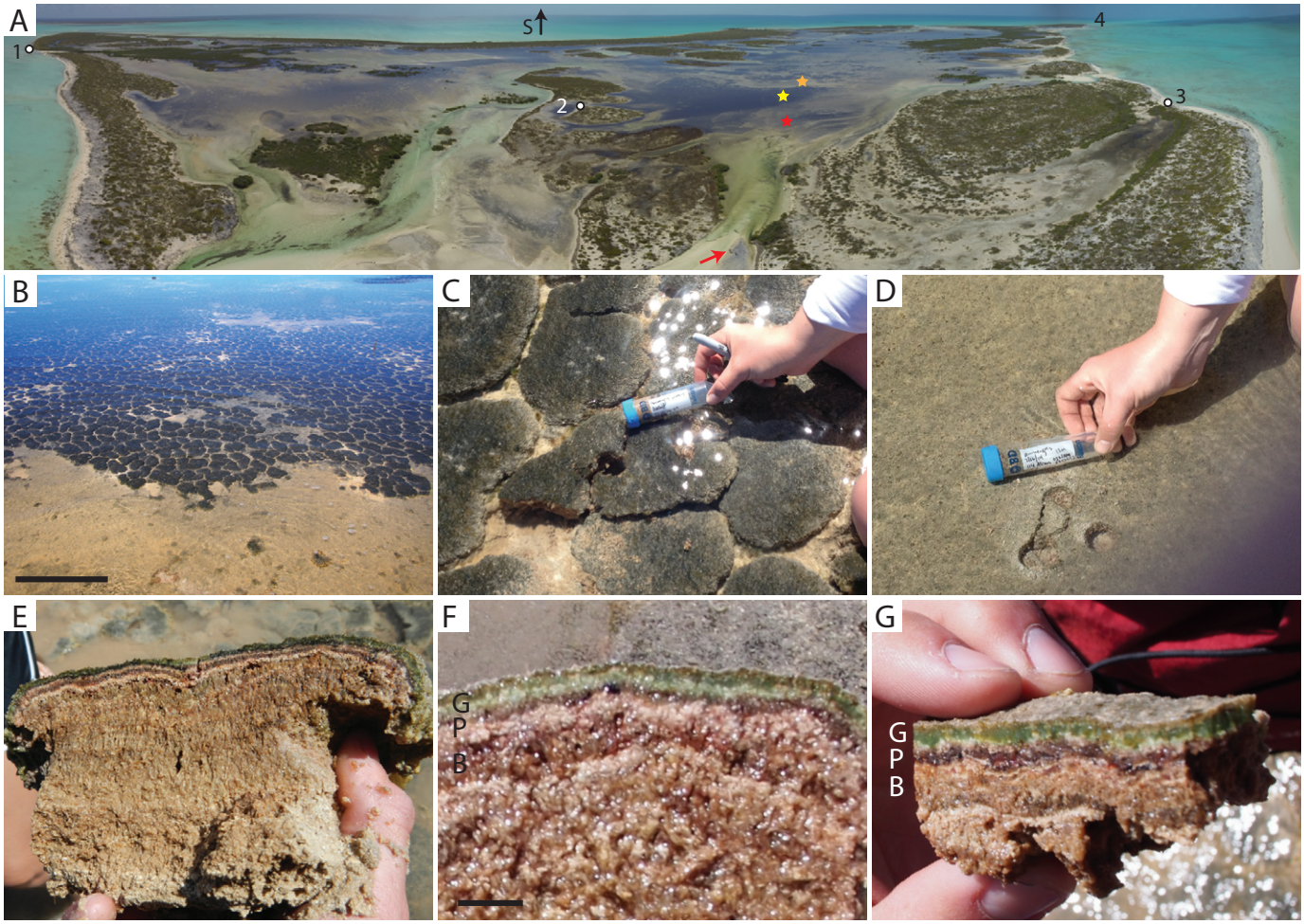


Figure 2: Location of study on Little Ambergris Cay within the Caicos platform. Inset shows location of Turks and Caicos with respect to the Bahamas, and neighboring Caribbean countries.

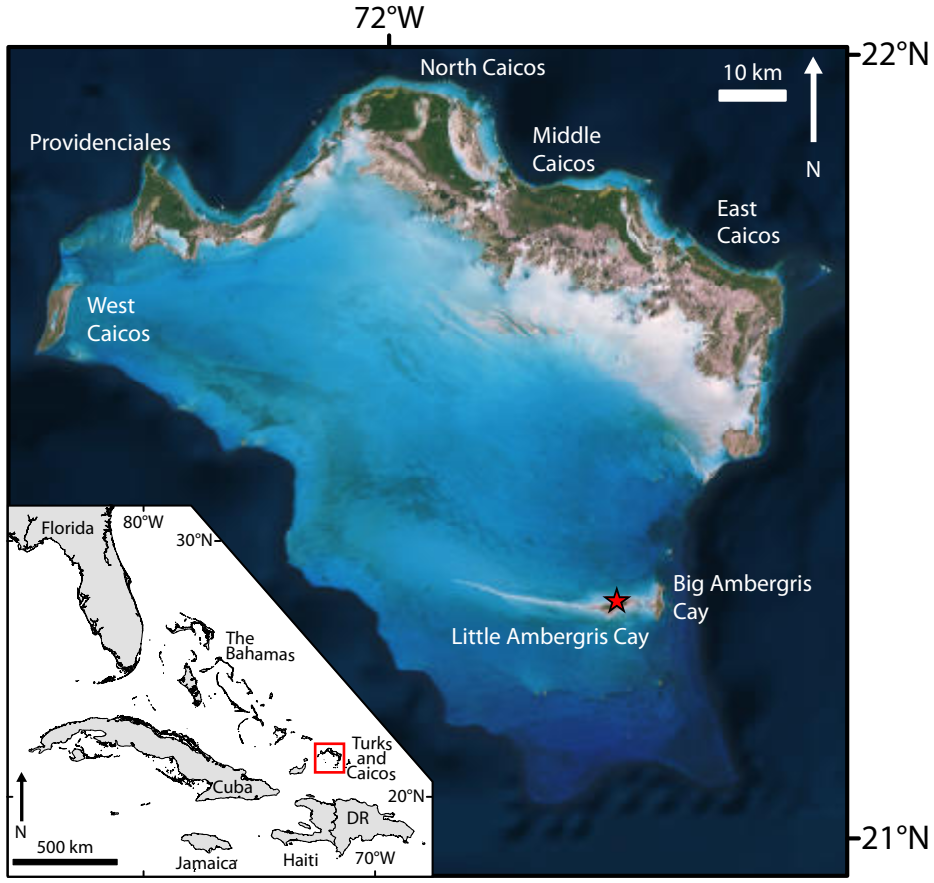
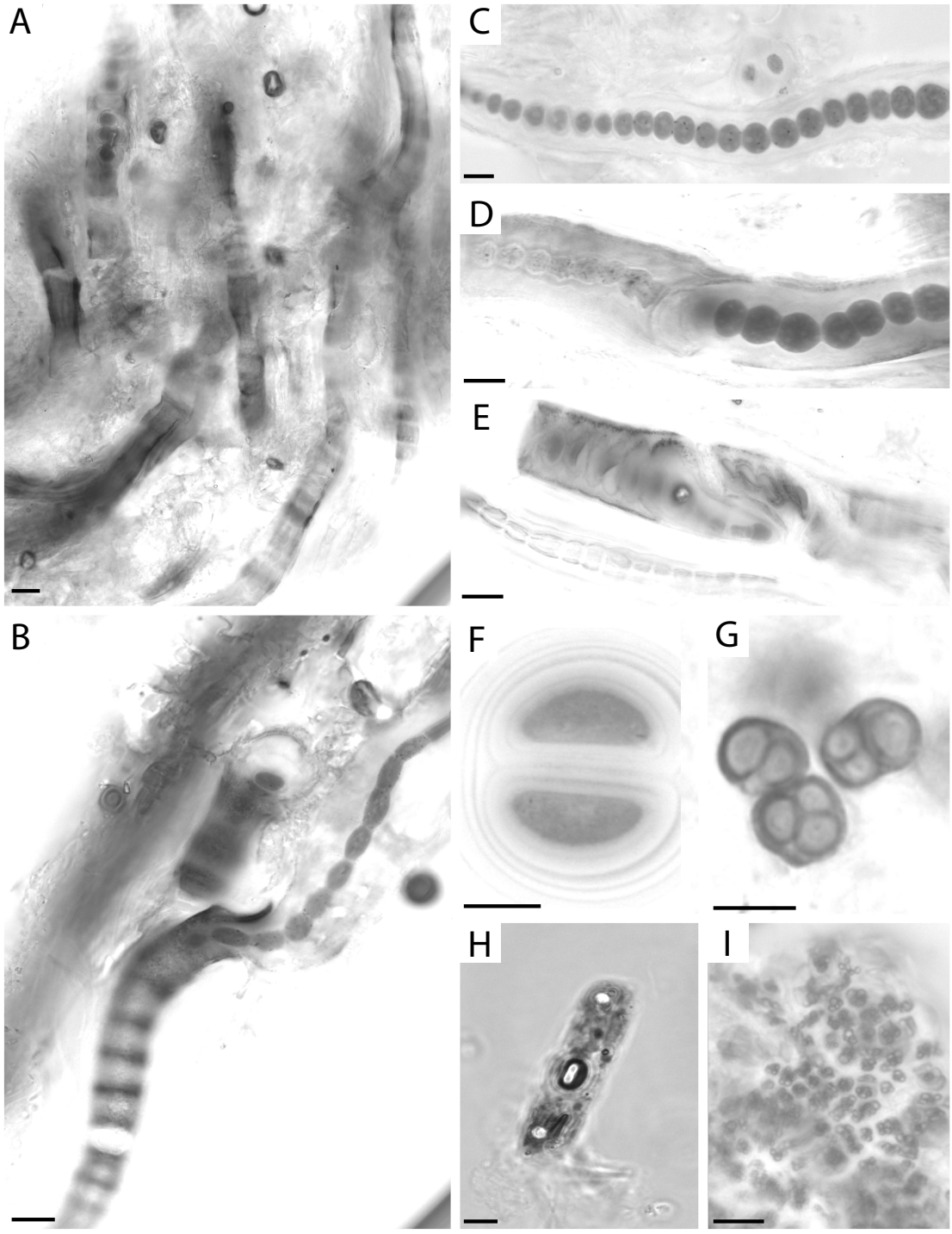


Figure 3: High-contrast black-and-white photomicrographs of the most dominant microbial morphologies found in both flat and biscuit mats from Ambergris Cay, West Caicos, BWI. A) Abandoned light brown sheaths dominant in the lower layers of the biscuit mat. B) Cyanobacteria with brown sheaths can also be found in flat mat. C - E) Images of bright green filamentous Cyanobacteria enclosed in sheaths. Sheaths grade from clear in C to light brown in D. Light and dark granules or occlusions are visible in single Cyanobacteria cells. F) Green and G) brown coccoid cells. H) Micrograph of putative single-celled green algae. I) Purple colonial microbes. Images A, C, D, E, and I are from biscuit mat. Images B, F, G, and H are from flat mat. Black bar is 10 μm.



Results

Mat Description

Flat mats (Fig. 1D) are characterized by lateral continuity of surface mat laminae (Fig. 1G), whereas biscuit mats (Fig. 1E) are present as discrete quasi-polygons with mat layering oriented normal to the convex-up surface of the quasi-polygon, curving away from the horizontal on quasi-polygon margins. We observed no clear differences in the relative abundances of grazers (only rare millimeter-scale cerithid gastropods) in the region surveyed. The general compositions of both flat and biscuit mat types follow an expected vertical progression of mm-scale pigmented zonation, from a dark surface layer to lighter green layers to purple and pink layers near the bottom of the pigmented mat section (Fig. 1F, G). These visible color changes in pigments are indicative of canonical photosynthetic microbial-mat communities stratified by light penetration and anaerobic biogeochemical processes (Stal 2012; Stolz 2000; Van Gemerden 1993). Below the bright, pigmented portion of the mat are several centimeters of brown organic material and carbonate sediment (ooids and mud), at times creating visibly inter-bedded laminae (Fig. 1E). Overprinting these layers is a palisade-type texture consisting of the empty sheaths of Cyanobacteria (Fig. 3A) that no longer contain the bright, green living Cyanobacteria cells seen in high contrast in Fig. 3B-E. These sheaths are the dominant structural component of the biscuit-type morphology. Brown (Fig. 3G) and green (Fig. 3G) coccoid bacteria, putative single-celled green algae (Fig. 3H), and maroon coccoids (Fig. 3I) were also observed in the pigmented regions of both mat types.

Sequencing

Cyanobacteria.--- Twenty-two Cyanobacteria taxa were recovered between the two mats (Table 1). The same three taxa predominate in both mat types: *Scytonema* (0.94% relative abundance in flat mat, 0.89% relative abundance in biscuit mat), *Halomicronema* (0.42% relative abundance in flat mat, 0.57% relative abundance in biscuit mat), and *Crinalium* (0.06% relative abundance in flat mat, 0.15% relative abundance in biscuit mat) (Table 1, gray). While sequences belonging to *Schizothrix* were not observed in our samples, *Halomicronema* and *Crinalium* were classified in the same morphological Subsection and Family (Subsection III; Family I), and share similar

morphologies. While the dominant Cyanobacteria are effectively the same between the two mat types, the biscuit mat has higher alpha diversity than the flat mat. In 1,000 random *in silico* subsamplings to equal depth of the Cyanobacteria populations in each sample, the same number of unique operational taxonomic units (OTUs) were observed.

Table 1. Relative abundances of the complete cyanobacterial taxonomic diversity of flat and biscuit microbial-mat samples. The only differences in Cyanobacteria diversity between mat types occur in rare taxa that are all less than 0.15% of the total relative abundance. Seven taxa were observed only in the flat-type mat [underlined], and six taxa were found only in the biscuit-type mat [italics]. Average and standard deviation for Number of OTUs and Inverse Simpson metric calculated for 1000 random subsamplings to equal depth of the Cyanobacteria population of each mat.

Cyanobacterial Taxonomy	Flat Mat Rel. Abund.	Biscuit Rel. Abund.
SubsectionIV;FamilyI;Scytonema	0.924%	0.893%
SubsectionIII;FamilyI;Halomicronema	0.424%	0.572%
SubsectionIII;FamilyI;Crinalium	0.062%	0.146%
SubsectionI;FamilyI;Cyanothece	0.036%	0.012%
SubsectionV;FamilyI;Hapalosiphon	0.030%	0.004%
SubsectionIII;FamilyI;Tychonema	0.018%	0.110%
SubsectionI;FamilyI;Chroococcus	0.015%	0.001%
SubsectionIII;FamilyI;Phormidium	0.013%	0.000%
SubsectionIII;FamilyI;Euhalothece	0.012%	0.000%
SubsectionIII;FamilyI;Geitlerinema	0.009%	0.004%
SubsectionII;FamilyI;Xenococcus	0.006%	0.000%
SubsectionIII;FamilyI;Rubidibacter	0.004%	0.000%
SubsectionIII;FamilyI;Prochlorothrix	0.002%	0.000%
SubsectionIII;FamilyI;Arthrospira	0.001%	0.000%
SubsectionIII;FamilyI;Spirulina	0.001%	0.027%
SubsectionII;FamilyI;Stanieria	0.000%	0.003%
SubsectionII;FamilyII;Pleurocapsa	0.000%	0.006%
SubsectionIII;FamilyI;Aerosakkonema	0.000%	0.001%
SubsectionIII;FamilyI;Haloleptolyngbya	0.000%	0.027%
SubsectionIII;FamilyI;Leptolyngbya	0.000%	0.126%
SubsectionIII;FamilyI;Trichocoleus	0.000%	0.002%
SubsectionIV;FamilyI;Cylindrospermum	0.000%	0.031%
Total Cyanobacterial Rel. Abund.	1.6%	2.0%
Top 3 Species Rel. Abund.	1.4%	1.6%
Top 3/Total Rel. Abund.	0.91	0.82
Avg. Number of OTUs	62.00	67.00
Std. Number of OTUs	0.00	3.79
Avg. Inverse Simpson	2.61	4.16
Std. Inverse Simpson	0.00	3.82-4.55

Total Diversity.--- In aggregate, we recovered 85,319 sequences for the flat-type mat and 101,610 sequences for the biscuit-type mat. More OTUs were also observed for the biscuit morphology (Table 2). We captured 98% of the microbial community for both samples based on the Good's Coverage statistic at the unique and 99% OTU level, and 100% at the 97% OTU level (Table 2), demonstrating that these differences are not due to differential community recovery during sequencing. Finally, the flat-mat morphology has half the Inverse Simpson diversity of the biscuit mat across all OTU levels within the 95% confidence interval (Table 2). The biscuit mat has more sequences, OTUs, and higher diversity than the flat mat.

Table 2. Number of observed OTUs, sequencing coverage (Goods Coverage), and species richness (alpha diversity - Inverse Simpson) for rarefied dataset.

OTU Clustering	Sample	OTUs Observed	Goods Coverage	Inv. Simpson	Inv. Simpson 95% Conf.
unique	Flat	3137	0.98	86	83-88
unique	Biscuit	3518	0.98	173	170-177
0.01	Flat	3092	0.98	85	83-88
0.01	Biscuit	3460	0.98	173	170-176
0.03	Flat	1472	1.00	68	66-70
0.03	Biscuit	1562	1.00	139	136-141

Based on UniFrac analysis, 31% of the phylogenetic diversity is unshared between the two microbial-mat samples. Seven of the top 10 taxa of both samples are not found in the other sample, most of which are from the phylum Proteobacteria (Table 3). *Gammaproteobacteria*; *Vibrionales*, *Holophagae*, *Alphaproteobacteria*; *Rhodospirllales*, *Deltaproteobacteria*; Sh765B-Tzt-29, *Alphaproteobacterales*; *Rhodobacterales* were all observed in the flat mat but not the biscuit mat. Conversely, *Alphaproteobacteria*; *Rhizobiales*, *Deltaproteobacteria*; *Desulfovibrionales*, *Gammaproteobacteria*; *Chromatiales*, *Planctomycetes*, *Bacteroidetes*, and *Deltaproteobacteria*; *Syntrophobacterales* were found in the biscuit mat but not in the flat mat.

Table 3. Top ten most abundant sequences in both flat and biscuit mat types with SILVA taxonomy. Highlighted taxa appear in both mat sample types.

Top 10 SILVA Taxa of Flat Mat	Rel. Abund.	Top 10 SILVA Taxa of Biscuit Mat	Rel. Abund.
Proteobacteria;GammaproteoVibrionales; Vibrionaceae;Vibrio	10.19%	Chloroflexi;Anaerolineae;Anaerolineales; Anaerolineaceae	14.81%
Chloroflexi;Anaerolineae;Anaerolineales; Anaerolineaceae	5.23%	Proteobacteria;Alphaproteobacteria; Rhizobiales; Hyphomicrobiaceae;Rhodomicrobium	4.86%
Acidobacteria;Holophagae;B276-D12	4.15%	Spirochaetae;Spirochaetes;Spirochaetales; Spirochaetaceae;Spirochaeta	3.69%
Proteobacteria;Alphaproteobacteria; ss1-B-07-44	3.57%	Proteobacteria;Deltaproteobacteria; Desulfovibrionales;Desulfovibrionaceae; Desulfocurvus	3.37%
Proteobacteria;Alphaproteobacteria; Rhodospirillales;MSB-1E8	2.44%	Proteobacteria;Gammaproteobacteria; Chromatiales;Chromatiaceae;Thiococcus	3.13%
Proteobacteria;Deltaproteobacteria; Sh765B-TzT-29	2.37%	Planctomycetes;Phycisphaerae;mle1-8	2.63%
Spirochaetae;Spirochaetes;Spirochaetales; Spirochaetaceae;Spirochaeta	2.23%	Bacteroidetes;SB-5	2.08%
Proteobacteria;Alphaproteobacteria; Rhodospirillales;Rhodospirillales_ Incertae_Sedis; Candidatus_Alysiosphaera	2.13%	Bacteroidetes;Sphingobacteriia; Sphingobacteriales; Saprospiraceae;Lewinella	2.05%
Bacteroidetes;Sphingobacteriia; Sphingobacteriales;Saprospiraceae; Lewinella	2.02%	Proteobacteria;Alphaproteobacteria; ss1-B-07-44	1.92%
Proteobacteria;Gammaproteobacteria; Pseudomonadales;Pseudomonadaceae; Pseudomonas	1.59%	Proteobacteria;Deltaproteobacteria; Syntrophobacterales; Syntrophobacteraceae;Desulfacinum	1.88%
Proteobacteria;Alphaproteobacteria; Rhodobacterales;Rhodobacteraceae; Tropicimonas	1.53%	TA06	1.70%
Four shared species	13.04%	Four shared species	22.47%
All top ten species	37.44%	All top ten species	42.11%

Discussion

Visual observation confirmed that the Cyanobacteria construct the main structural components of both microbial-mat types in the form of discarded sheaths. The biomass also contained interbedded layers of sediments indicative of past episodes of sedimentation, followed by recolonization of the substrate by the microbial community. Sequence analysis shows that the phylogenetic identity of the Cyanobacteria populations is extremely similar between the two mat types, where greater than 82% of the Cyanobacteria observed in both mat types belong to the same

three taxa. We therefore fail to reject the null hypothesis that the two morphotypes have the same Cyanobacteria population.

From sequence data, Cyanobacteria make up less than 2% of the total relative abundance of either mat community. However, it is important to note that sequence abundance cannot be directly correlated with population size, as iTag data can have biases in amplification efficiencies between different types of microorganisms (Parada et al. 2015). Our microscopic evaluation shows that Cyanobacteria are more than 2% of the microbial population by number, and certainly by biovolume, and therefore remain relevant, structure-building members of the mat community.

Though our sequencing efforts were limited to two representative samples, we also completed microscopic evaluations from a larger sample size (n = 10) collected at the same locations and times as the sequencing samples. These microscopic evaluations did not show any clear differences between the two morphotypes. As was observed in stromatolites at Highborne Cay (Foster et al. 2009), morphological observations did not completely capture the diverse and complex Cyanobacteria community diversity in these mat morphotypes.

The dominant Cyanobacteria present in these mat samples are members of the genera *Scytonema, Halomicronema,* and *Crinalium*. Members of *Scytonema* were also found in the thrombolite metagenome from Highborne Cay, Bahamas (Mobberley et al. 2013). Statistical analyses of the two microbial-mat samples show that we recovered the majority of the microbial community in our sampling (Good's Coverage 98% or greater) and that the alpha diversity of the biscuit mat is twice that of the flat mat. Whole-community diversity analysis (UniFrac) shows that about one third of the diversity in each mat sample is unshared.

The difference in diversity between the two microbial-mat morphotypes supports the hypothesis that mat morphology is defined mainly by time since mat colonization. Observations of our sampling site over time also corroborate this hypothesis, where flat mats were later found growing in locations previously containing biscuit mats after storm events (S. Bachtel, 2015, personal communication). In this scenario, faster-growing populations initially colonize the microbial mat. Then, with time, the more established mat would accumulate a more complex and diverse microbial population (Reid et al. 2000; Stal et al. 1985), concurrent with a development in mat

morphology from flat to biscuit. This is analogous to plant diversity in the development of a forest, where initial colonization is performed by a few rapidly growing or stress-tolerant species that are later joined or replaced by a more complex community (Sigler & Zeyer 2004). Successional diversity mechanics have also been suggested by other studies looking at a different set of Bahamas mats in Highborne Cay (Baumgartner et al. 2009).

When examining the top ten most abundant taxa of each mat morphotype, most taxa that differ between the two occur in the bacterial phyla Proteobacteria. Deltaproteobacteria make up 2 - 3% of the microbial population in the two mats — these organisms are commonly capable of sulfate reduction, which is consistent with the view that sulfur cycling is important in these ecosystems (Baumgartner et al. 2006; Visscher et al. 2000; Visscher et al. 1998; Visscher & Stolz 2005). OTUs belonging to the anoxygenic phototrophic sulfide-oxidizing Gammaproteobacteria genus, *Thiococcus,* were present at about 3.1% abundance in the biscuit-type mat, likely using the photosynthetic oxidation of sulfide produced by sulfate reducers to drive carbon fixation. This type of anaerobic closed internal sulfur cycle has been demonstrated in siliclastic tidal marshes at mid-latitudes (Wilbanks et al. 2014).

Chloroflexi have not been the focus of previous Bahamas microbial mat studies, though it is possible that their filamentous morphology may have been mistaken for Cyanobacteria. Sequences belonging to the Chloroflexi are among the most abundant taxa in both mat types (Table 3; biscuit-type mat 14.81%, flat-type mat 5.23%). The Chloroflexi present in the Ambergris Cay mats fall into the class Anaerolineae, a group which is typically characterized by anaerobic, nonphototrophic heterotrophs (Yamada et al. 2006), though many appear to be capable of aerobic respiration (Hemp et al. 2015a; Hemp et al. 2015b; Pace et al. 2015; Ward et al. 2015). Thus we suggest that the Chloroflexi observed in these mats play an important role in both aerobic and anaerobic carbon cycling, breaking down biomass produced by Cyanobacteria and other photo- and chemoautotrophs. We also note that it has also recently been shown that Cyanobacteria themselves may play important roles as heterotrophs respiring organic matter in these systems (Stuart et al. 2015).

We observed members of the Rhodospiralles (typically facultative photoheterotrophs) present in the flat-type mat at 2.4% and 2.1% abundance, as well as *Tropicomonas* at 1.5%, a member of the

Rhodobacteraceae — a metabolically versatile group that includes aerobic and anaerobic heterotrophs as well as facultative photoheterotrophs. The biscuit-type mat had 4.9% of sequences corresponding to *Rhodomicrobium*, a photoheterotrophic Alphaproteobacteria. These anoxygenic photoheterotrophic organisms likely inhabit the base of the photic zone in the mat, below the Cyanobacteria, where they can utilize organic compounds from the breakdown of biomass from primary producers while also making use of light energy to generate ATP (Imhoff 1995; Overmann & Garcia-Pichel 2013). A high proportion of Rhodobacteriales Alphaproteobacteria were also found in thrombolitic microbial mats in Highborne Cay, Bahamas, by genetic sequencing (Myshrall et al. 2010) and lipid analysis (Edgcomb et al. 2013).

Both samples also contain high abundances of sequences corresponding to various aerobic and anaerobic heterotrophs. Included in this grouping are Spirochaeta, common saccharolytic bacteria likely breaking down algal or Cyanobacteria extracellular polysaccharides in the mat (Leschine et al. 2006), as well as the Bacteroidetes genus *Lewinella*, a group known to be capable of protein and polysaccharide breakdown (Khan et al. 2007) and likely responsible for degrading the organic polymers common to these microbial mats. The flat mat also contains high concentrations of widespread Gammaprotobacteria aerobic heterotrophs *Vibrio* (~ 10%) and *Pseudomonas* (2%). These organisms likely occur in the upper, aerobic layers of the mat, where they aerobically respire organic compounds produced by Cyanobacteria and other autotrophs.

Conclusions

Microscopic and genomic data reveal that the flat and biscuit microbial-mat types present on the tidal flats of Little Ambergris Cay are not distinguished by their Cyanobacteria communities. They contain Cyanobacteria of similar morphology (based on microscopy) and similar phylogenetic diversity (based on gene sequence identity), at similar relative abundances. Additionally, there is no evidence for differences in the relative abundances of metazoan grazers between them. The two microbial-mat types do, however, contain differences in their non-Cyanobacteria populations, and the biscuit mat has a more diverse microbial community than the flat mat.

If we assume that the biscuit-mat morphology developed from an initial flat-mat architecture, as repeated field observations have also suggested, this diversity difference could be explained by mat

communities becoming more diverse the longer they remain undisturbed by changes in environmental conditions, with the frequency of sedimentation or erosion events due to storms or proximity to tidal channels probably the most important among these. Thus the results of this study support the hypothesis postulated early by Gebelein (1969), and expanded on by others (Andres & Reid 2006; Mariotti et al. 2014; Martin et al. 1993), that environmental factors play a more fundamental role in microbial-mat morphology than the Cyanobacteria communities concentrated within their upper layers. If the results from the Caicos mats are more broadly applicable to mat morphologies observed elsewhere, within the limited degree to which these mat morphologies may display differential textural expressions in the rock record, morphological interpretations might more profitably focus on paleoenvironmental information rather than the signatures of different microorganisms (e.g., (e.g., Grotzinger & Knoll 1999).

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