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Biomat Resilience to Desiccation and Flooding Within a Shallow, Unit Process Open Water Engineered Wetland

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Supplemental Information

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Citation: Brady, A.R.; Vega, M.A.; Riddle, K.N.; Peel, H.F.; Lundeen, E.J.; Siegmund, J.G.; Sharp, J.O. Biomat Resilience to Desiccation and Flooding Within a Shallow, Unit Process Open Water Engineered Wetland. *Water* **2021**, *13*, 815. https://doi.org/10.3390/w13060815

Academic Editor: Pedro N. Carvalho
Received: 24 February 2021
Accepted: 12 March 2021
Published: 16 March 2021

1.1. USGS Hydrographs from Selected Santa Ana River Locations During Flood Event

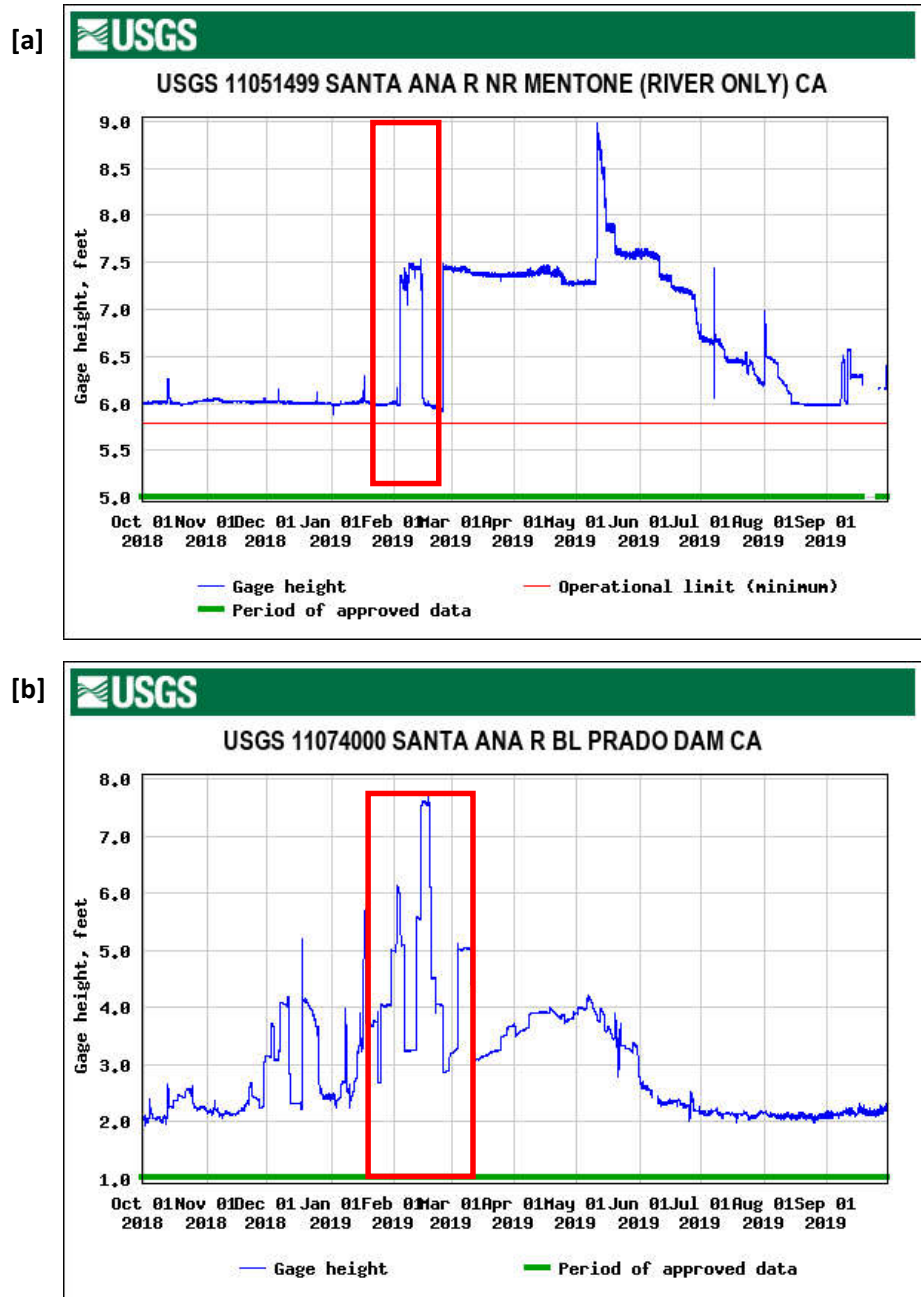

Figure S1. USGS hydrographs from Santa Ana River near Prado Wetlands demonstrate high flows during period of flood event. [**a**] Illustrates the February high flow period was not higher than the following months. [**b**] Demonstrates the gage height at the Prado Dam, illustrating the confluence of events that occurred during the time period (high storage pool) and high flows that resulted in the levee break inundating the Prado Wetlands. The rebuilt levee held in the following months, demonstrating the need for proactive design and implementation.

1.2. DNA Extraction, Sequencing, and Analysis

For 16S rRNA gene sequencing, PCR amplification was performed using 2 µL template DNA in a 25µL reaction using 5PRIME HotMasterMix (Quanta Biosciences, Beverly, MA, United States) with a nearly universal bacterial and archaeal primer set as per Kraus et al. (2018); the forward primer 515F-Y with the M13 primer (bolded) and gene specific

forward primer (underlined) (**GTA AAA CGA CGG CCA G C**CCG TGY CAG CMG CCG CGG TAA-3′) [1] while the reverse primer 926R (5′-CCGYCAATTYMTTTRAGTTT-3′) was unmodified from Parada et al. (2016) [2].

Raw reads were demultiplexed with AdapterRemoval [3] and imported into R for quality visualization and processing using DADA2 [4]. Adapters were manually trimmed with forward and reverse reads truncated to 239 and 251 base pairs, respectively. The respective maximum expected errors were limited to 2. Filtered and trimmed reads were then dereplicated, denoised and merged. Chimeric sequences were identified and removed based on identification by consensus.

1.3. Prado Wetlands Analog Water

Table S1. Analog water recipe based on Prado Wetlands influent without NO_3^- or NO_2^- [5]. *$NaNO_3$ utilized for specific NO_3^- concentration (15 mg/L – N).

Salt	MW (g/mol)	g/L
$MgSO_4$-$7H_2O$	246.5	0.12
$CaCl_2$	147.0	0.24
K_2HPO_4	136.09	0.01
$NaHCO_3$	84.01	0.30
Na_2SO_4	142.02	0.05
KCl	74.55	0.025
NaCl	58.4	0.064
*$NaNO_3$ (15 mg/L – N)	85.0	0.091
*$NaNO_3$ (30 mg/L – N)	170.0	0.182

2. Results

2.1. Adonis and Beta-Dispersion Results for Microbial Ecology Comparison

Table S2. The post-flood biomat community was significantly different from mature and nascent biomats, as seen in the adonis results below. Additionally, the rehydrated biomat microbial communities were significantly different from mature, nascent, and fresh biomat communities.

Comparison	Adonis			Beta Dispersion	Distance from Centroid				
	Distance Matrix	R^2	P-Value	P-Value	Nascent	Mature	Flood-Impacted	Rehydrated	Fresh
Mature : Flood-Impacted	Weighted UniFrac	0.50	0.001	0.38	n/a	0.12	0.098	n/a	n/a
Mature : Flood-Impacted	UniFrac	0.29	0.001	0.015	n/a	0.36	0.47	n/a	n/a
Rehydrated : Mature	Weighted UniFrac	0.50	0.002	0.021	n/a	0.12	n/a	0.050	n/a
Rehydrated : Mature	UniFrac	0.46	0.002	0.025	n/a	0.36	n/a	0.26	n/a
Nascent : Rehydrated	Weighted UniFrac	0.49	0.002	0.007	0.15	n/a	n/a	0.050	n/a
Nascent : Rehydrated	UniFrac	0.34	0.002	0.001	0.4725	n/a	n/a	0.26	n/a
Nascent : Mature	Weighted UniFrac	0.41	0.001	0.311	0.15	0.12	n/a	n/a	n/a
Nascent : Mature	UniFrac	0.25	0.001	0.04	0.47	0.36	n/a	n/a	n/a
Rehydrated : Fresh	Weighted UniFrac	0.91	0.034	0.11	n/a	n/a	n/a	0.050	0.031
Rehydrated : Fresh	UniFrac	0.51	0.034	0.05	n/a	n/a	n/a	0.26	0.31
Rehydrated : Flood-Impacted	Weighted UniFrac	0.56	0.008	0.038	n/a	n/a	0.098	0.050	n/a
Rehydrated : Flood-Impacted	UniFrac	0.34	0.008	0.001	n/a	n/a	0.47	0.26	n/a

2.2. Microbial Community UniFrac and DCA Ordinations

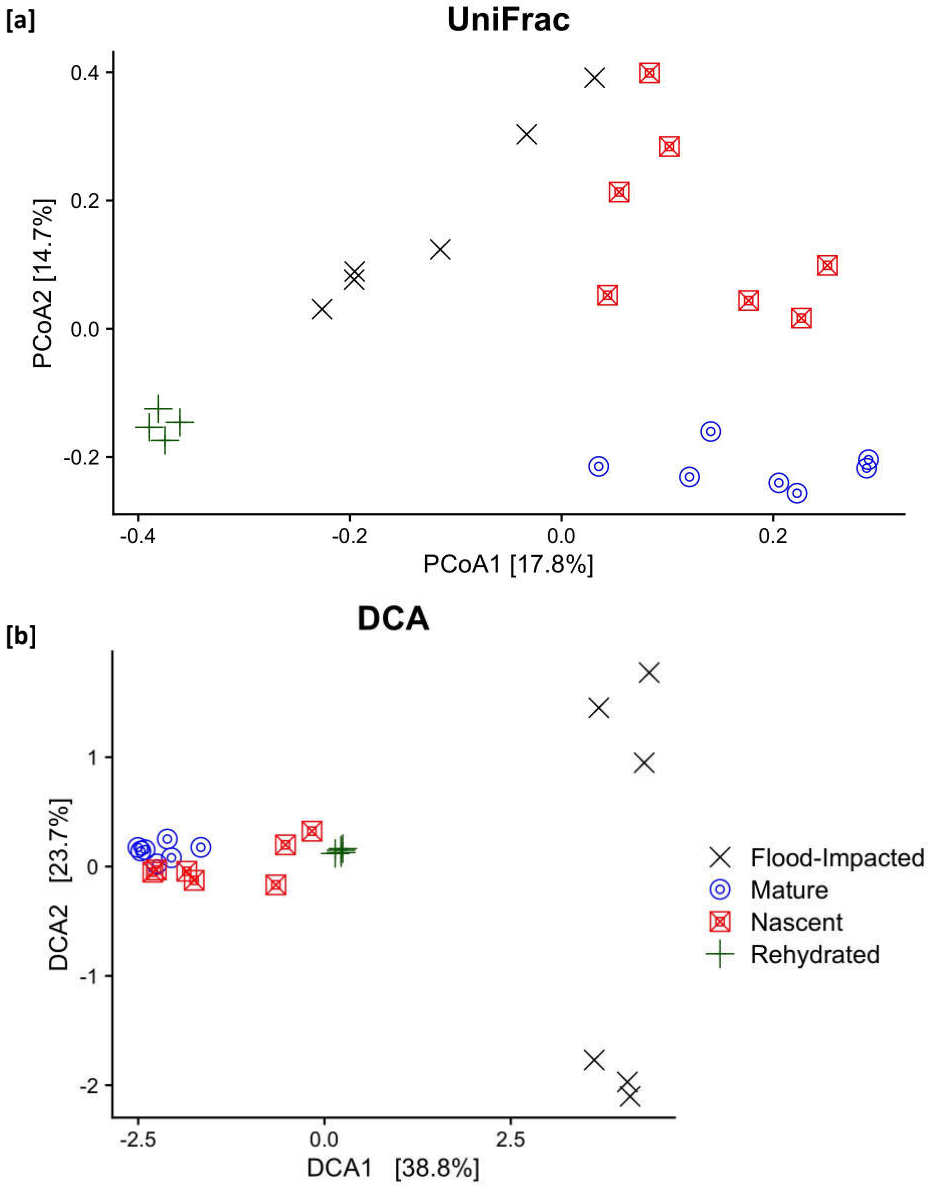

Figure S2. Ordinations demonstrate the changes in 16S rRNA gene microbial community structure by sample types. Though Weighted UniFrac distances were used in the primary analysis, a PCoA using UniFrac distances [**a**] and the Detrended Correspondence Analysis (DCA) [**b**] show the grouping of communities by sample type were evident in all visualizations.

2.3. Rank Abundance of Biomat Types

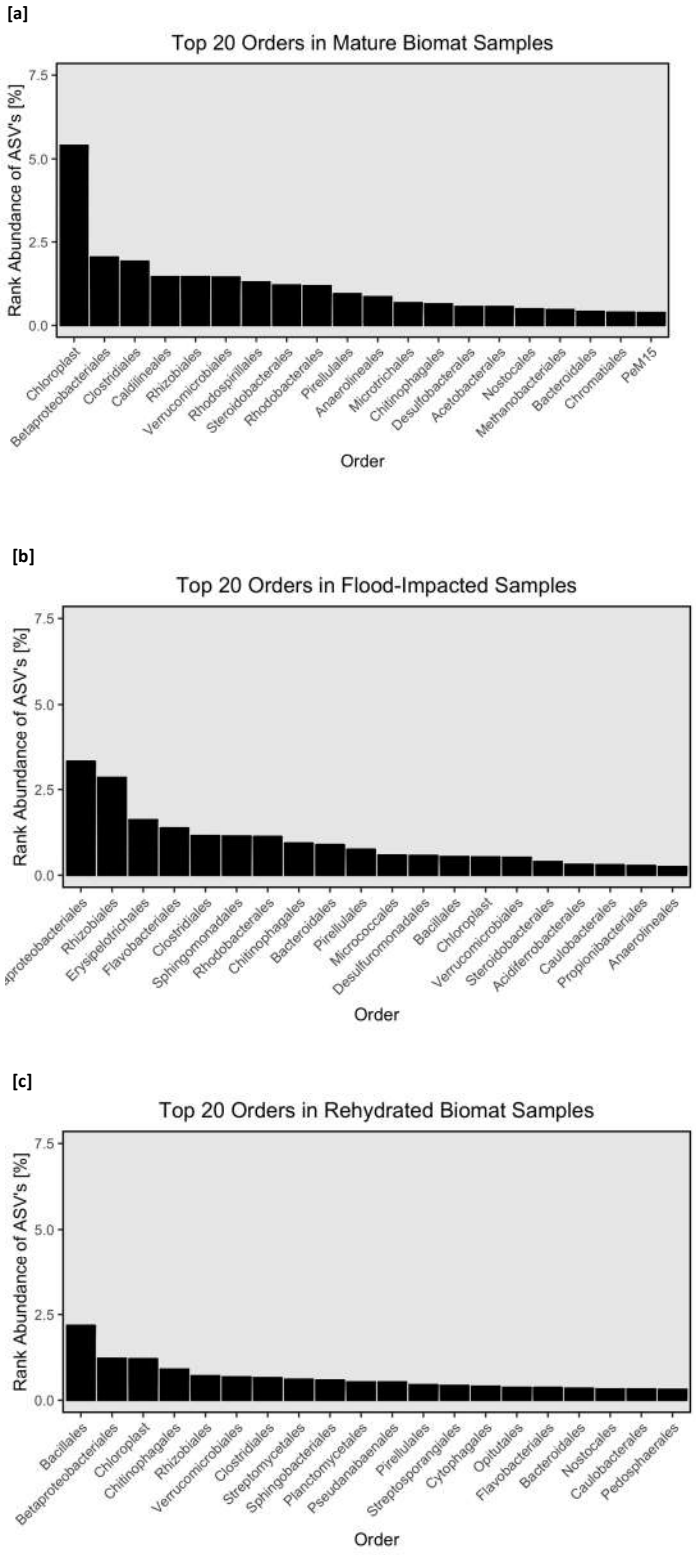

Figure S3. Rank abundance of the Top 20 Orders in each biomat type: mature biomat [**a**], flood-impacted biomat [**b**], and rehydrated biomat [**c**].

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