Investigating the Response of Epiphytic Microorganisms to Nutrient Enrichment Using eDNA Metabarcoding

Anne C. Hurley, Dale A. Casamatta

Department of Biology, University of North Florida

Objectives

- **Evaluate the effects of nitrogen enrichment on the composition of epiphytic microbial communities on *Spartina alterniflora* in GTMNERR**, and determine whether or not these impacts differ between creekside plots and marsh interior plots.
- **Characterize the biodiversity of these epiphytic communities**, and assess how this diversity varies spatially and temporally (i.e., creekside vs. interior and over the course of the six-month sampling period).

Background

- **Diverse communities of epiphytic microorganisms colonize the surfaces of *Spartina alterniflora* and other marsh vegetation (fig. 2).** Autotrophic members include eukaryotic algae and cyanobacteria.
- **Epiphytic microbes contribute to the functioning of saltmarsh ecosystems.** For example, nitrogen fixation by cyanobacteria (fig. 3) can be a significant source of nitrogen input (Currin & Paerl, 1998). Additionally, as a food source for grazers (such as snails), epiphytic microalgae are a component of food webs supporting commercially valuable species (Jackson et al., 2009).
- **Nitrogen enrichment has been shown to alter the community structures of microalgae in estuarine settings.** Changes include a reduction in microalgal species diversity (Lemley et al., 2016).

Figure 1. Study site. Guana Tolomato Matanzas National Estuarine Research Reserve, St. Augustine, FL

Figure 2. Epiphytic algal growth on marsh vegetation.

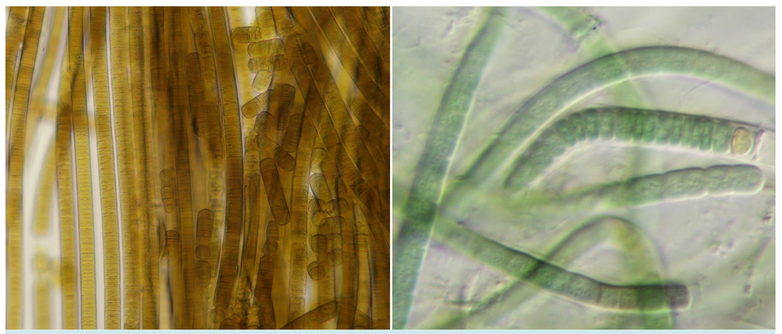

Figure 3. Micrographs of cyanobacteria cultured from epiphytic material.

Experimental Design

- Study site located in the southern section of GTMNERR (fig. 1).
- Twenty plots, located either creekside or in the marsh interior, and either enriched with nitrogen or untreated (fig. 4).
- Samples collected in April, July, and September of 2022.

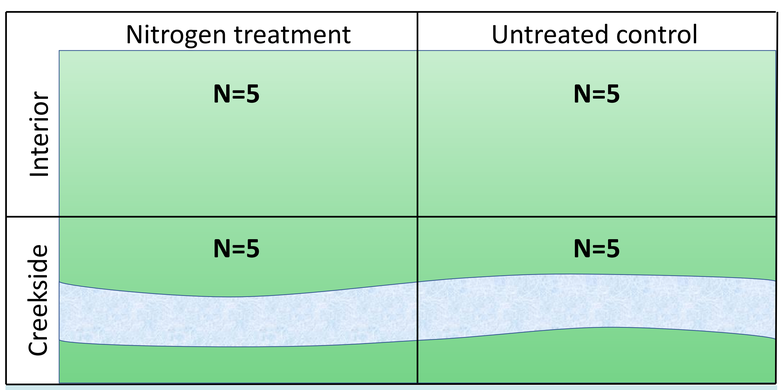

Figure 4. Schematic diagram of experimental setup. N=5 plots per experimental group.

Methods

- Three segments of *S. alterniflora* were collected from each plot.
- Sterile deionized water was added to each bottle containing plant segments, and epiphytes were dislodged by vigorous shaking. The resulting suspensions were aliquoted into microcentrifuge tubes and centrifuged to pellet epiphytic material.
- DNA was extracted from the pellets using the DNeasy PowerSoil Pro Kit (QIAGEN).
- Four barcode regions representing microbial groups of interest were separately PCR-amplified from extracted DNA (table 1).
- Amplicons were pooled by sample and sequenced on an Illumina MiSeq instrument to generate 300-bp paired-end reads.

Microbial Group	Barcode Region	Primers	Primer Sequence (5'-3')
Cyanobacteria	16S rDNA (V3-V4 region)	CYA359F CYA781R	GGGGAATYTTCCGCAATGGG GACTACWGGGGTATCTAATCCCWTT
Prokaryotes (including cyanobacteria)	16S rDNA (V4-V5 region)	515F 926R	GTGCCAGCMGCCGCGGTAA CCGYCAATTYMTTTRAGTTT
Diatoms	*rbc*L	Equimolar mix of Diat_rbcL_708F_1 Diat_rbcL_708F_2 Diat_rbcL_708F_3	AGGTGAAGTAAAAGGTTCWTACTTAAA AGGTGAAGTTAAAGGTTCWTAYTTAAA AGGTGAAACTAAAGGTTCWTACTTAAA
		Equimolar mix of Diat_rbcL_R3_1 Diat_rbcL_R3_2	CCTTCTAATTTACCWACWACTG CCTTCTAATTTACCWACAACAG
Eukaryotic algae (including diatoms)	Universal Plastid Amplicon (23S rDNA)	p23SrV_f1 p23SrV_r1	GGACAGAAAGACCCTATGAA TCAGCCTGTTATCCCTAGAG

Table 1. Primers used to amplify barcode regions from microbial groups of interest.

Next Steps

- Trim primer sequences from demultiplexed sequence data using CUTADAPT.
- Process reads and assign taxonomy to amplicon sequence variants (ASVs) using the DADA2 pipeline in R.
- Data analysis: test the effects of nutrient enrichment and plot location, as well as sample collection date, on epiphytic community structure.

References

Currin, C. A., & Paerl, H. W. (1998). Epiphytic nitrogen fixation associated with standing dead shoots of smooth cordgrass, *Spartina alterniflora* . *Estuaries*, 21: 108–117.

Jackson, G., Zingmark, R. G., & Lewitus, A. J. (2009). Modeling epiphytic community production. *Marine Ecology Progress Series*, 387: 61-70.

Lemley, D. A., Adams, J. B., & Bate, G. C. (2016). A review of microalgae as indicators in South African estuaries. *South African Journal of Botany*, 107: 12–20.

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