Microbial Diversity, Biomass, and Community Structure Differences among Restored and Natural Saltwater Marshes, Louisiana

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EARTH & PLANETARY SCIENCES

RESTORE SCIENCE PROGRAM

Introduction

Tidally influenced saltwater marsh construction projects are being completed in Louisiana to combat coastal erosion and land loss, restore critical fisheries, and counteract ecosystem injuries after the Deepwater Horizon oil spill. In natural saltmarsh systems, ecological communities develop in response to tidal inundation, salinity fluctuations, sedimentation, and carbon storage.

The objective of this research was to monitor the marsh soils at restored sites created within the past ten years and compare soil community compositions with those from nearby natural systems. Evaluation of successful created marshes involves assessing aboveground biomass, survival of planted vegetation, and recruitment of local endemic versus invasive species. Here, we compare the microbial communities responsible for cycling nutrients within natural and created marsh soils.

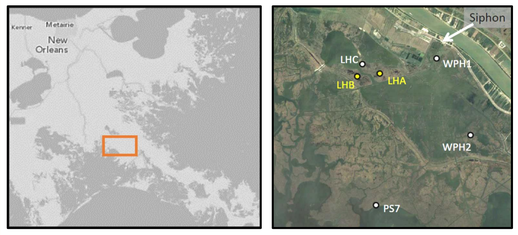

Figure 1. Left, site location in south Louisiana, within Bay Batiste of Barataria Bay, along and near to the Mississippi River, in the Lake Hermitage (LH), West Point a la Hache (WPH) area. Right, Google image of created marshes (LHA and LHB) and natural marshes (LHC, PS7, WPH1, and WPH2).

Materials and Methods

- The Lake Hermitage Marsh Creation Project made two marshes:
  - LHA filled between Aug 2012 – Oct 2013
  - LHB filled between Dec 2013 - May 2014
- Soil cores from two depths (0-2 cm and 8-10 cm) were collected from 1 m, 10 m, & 100 m inland, and from sediment from 1 m off the marsh edge.
- Soil organic carbon content and soil pH were also measured.

Figure 2. Left, native *Spartina alterniflora* at a representative nature marsh site. Right, natural marsh soil cores revealed a dense network of grass roots and rich black soils.

Figure 3. Left, non-native grasses and other vegetation at one of the created marsh sites. Right, created soil cores exhibited little root structure, organic matter, and had lighter sandy soils.

PLFA Extraction Workflow

- Microbial community profiles were determined using phospholipid fatty acid (PLFA) and gas chromatography/mass spectrometry (GC/MS) techniques and complimented by 16S rRNA gene profiles generated by Illumina sequencing.
- The modified Bligh and Dyer method was used to extract intact total lipids, which included solvent extraction and phase separation of polar lipids for PLFA recovery.

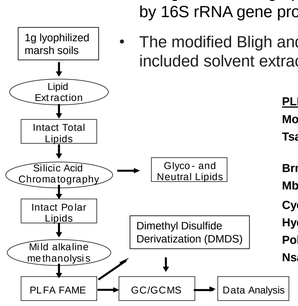

Figure 4. PLFA extraction workflow.

PLFA Structural Classes

Mono: Monounsaturates, 16:1ω7

Tsat: Terminally branched saturates, i15:0

Brmono: Branched monounsaturates, i17:1

Mbsat: Mid-chain branched saturates, 10me18

Cyclo: Cyclopropyl fatty acids, cy17:0

Hydroxy: Hydroxy saturates, 3-OH-12:0

Poly: Polyunsaturates, 18:2ω6

Nsat: Normal saturates, 14:0

General Community Classification

Gram-negative bacteria, Proteobacteria

Gram-positive bacteria, Sulfate-reducing bacteria (SRB), and Firmicutes

Metal and Sulfate Reducers

Actinomycetes and SRB

SRB and other anaerobic eubacteria

Several bacteria; i.e., *Pseudomonas*

Eukaryotes, fungi, algae, humans

Ubiquitous in nature

PLFA Profiles & Community Structure

- Soil pH values were from created marsh soils was significantly different than from the natural soils at all soil depths (p = 0.01).
- The created marsh soils pH was near neutral (7.05 ± 0.9) whereas the natural marsh pH values averaged ~6.35 (± 0.7), which was likely indicative of organic acid accumulation.
- The created marsh soils had average organic contents of <10%, which was significantly lower than natural soils that averaged >20% (p = 0.0001).
- Created soils were associated with *Paspalum* spp. (crowngrass) and *Schoenoplectus pungens* (common bulrush), whereas natural mashes were dominated with *Spartina alterniflora*.
- Biomass estimates ranged from below detectable levels(1-5 pmol PLFA gdw^{-1}) to 6 $x10^4$ pmol PLFA gdw^{-1}, with shallower soils exhibiting higher biomass (average 10^4 pmol PLFA gdw^{-1}) compared to deeper soils (average 10^3 pmol PLFA gdw^{-1}).
- For example, in 2018, created marshes had less total lipid biomass, and total biomass was significantly different at depth (p = 0.002).

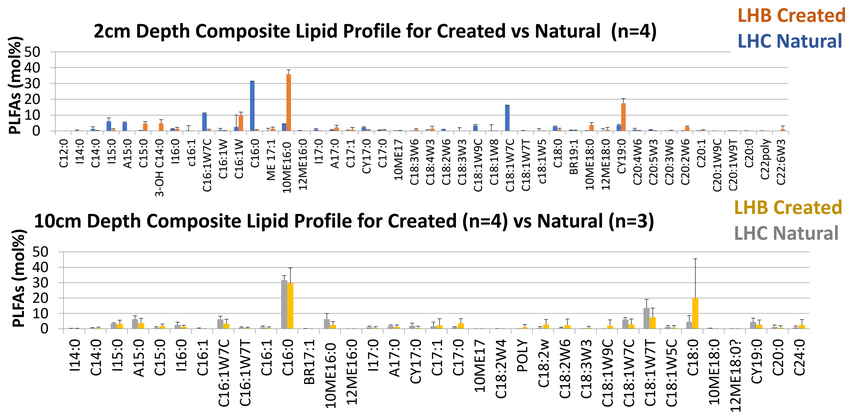

Figure 5. Lipid profiles for 2 cm depth (top) and 10 cm (bottom) depth from created and natural marsh soils, determined by GC/MS.

Overall PLFA Community Structure

- Diverse PLFA profiles were observed; shallow depths had more PLFA diversity.
- Surface soil samples had higher relative abundances and diversity of eukaryotic PLFA biomarkers (18:4ω3, 20:2ω6) than deeper samples were18:2ω6, indicative of fungi and animals dominated (Figure 5). Polys are displayed as blackish grey bars in Figure 6.
- PLFAs related to plants ω3 were more abundant in surface soils, particularly from natural soils (Figures 5 and 6). These eukaroyotic profiles are typical of marsh plants (e.g., *S. alterniflora*, *Juncus roemerianus*).
- In 2018, natural marsh soils had at least twice as much PLFA biomass than the created marshes at the shallow depth and 10X or more biomass at the deeper depth. But, in 2019, natural soils had 1.5X as much PLFA biomass than the created marshes at the shallow depth and 4X or more biomass at depth.

Community Structural Comparisons

- SRB as evidenced by lipids (a15:0, i17:0, & 10Me16:0) were detected in the 8-10 cm deep natural marsh soils, whereas actinomycetes and metal-reducers (i15:0, Me18:0's, Me17:0, br17:1, & Br19:1) were typical at the 0-2 cm deep.
- Cyclopropyl fatty acids, indicating anaerobic processes and/or the presence of nutritional stress, averaged 4 mol% in created marsh soils regardless of depth, but natural marsh soils had cyclopropyl fatty acid that averaged 6-8% of the lipid profile. Deeper soils had slightly higher percentages.
- Shallow natural marsh soils exhibited more mid-branched saturates, branched monounsaturates, and polyunsaturates, whereas shallow created marsh soils had more terminally-branched saturates.

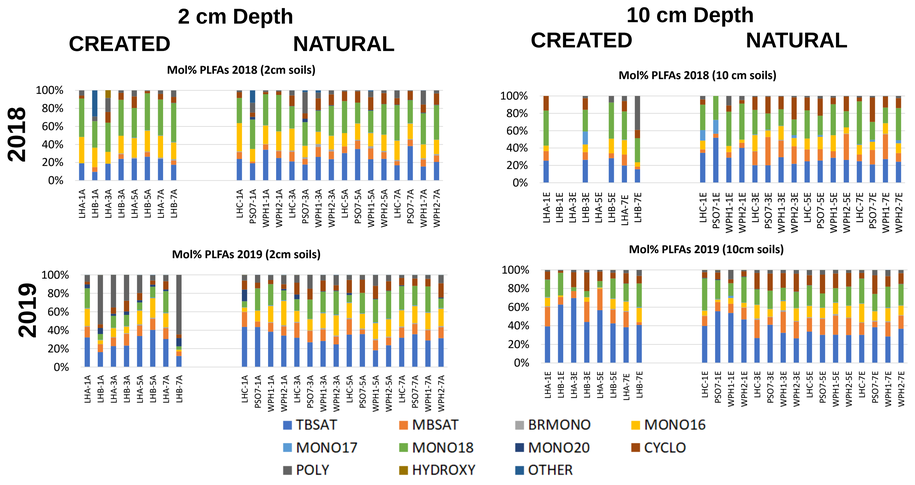

Figure 6. Lipid classes observed from created and natural marsh soils, separated by depth (2 cm vs 10 cm) & year (2018 vs 2019).

Methane-Oxidizing Communities

- Following DMDS derivatization and GC/MS analysis, the double-bond position of monounsaturated fatty acid can reveal Type I and Type II methanotrophs.
- Shifts in these communities can be compared to presence/absence data for putative methane-oxidizing populations within the soils from 16S rRNA genes.
- In a subset of samples, the created marsh soil (2019) sample LHA-5A had both Type I and II methane-oxidizers, but both types were not detected in LHA-5E, LHB-5A, and LHB-5E samples.
- In natural marsh soils, samples LHC-3A, LHC-3E, LHC-1A, and PS07-5E contained both types of methane-oxidizers.
- Differences in these communities define carbon cycling dynamics within the soils as the created marsh soils mature.

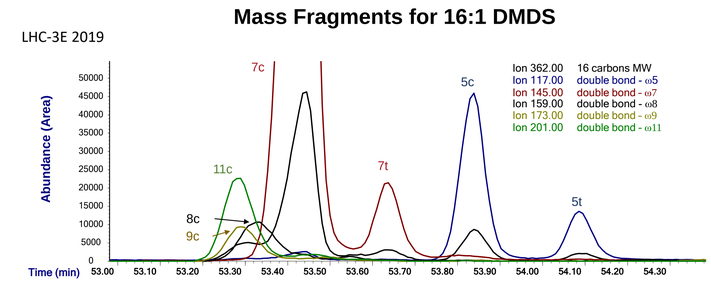

Figure 7. The double-bond of the ω8 position for 16 carbon is indicative of Type I methane-oxidizers.

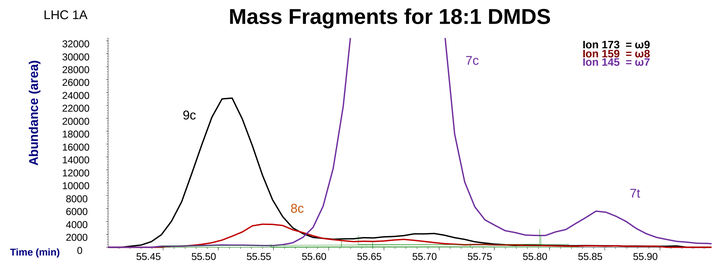

Figure 8. The double-bond of the ω8 position for 18 carbon is indicative of Type II methane-oxidizers.

Summary

These results show that created marsh soils are developing and there are changes in biomass, microbial diversity, and organic carbon content. After nearly 10 years, the physical and chemical nature of the created marsh soils remain significantly different than natural marsh soils, and the diversity and structure of the microbial communities also differ.

Natural marsh soils had higher organic carbon content than created soils, and lipids from the natural soils were mostly indicative of facultative and anaerobic bacteria that would be associated with anaerobic processes.

Microbial groups associated with methane oxidation, as detected by lipid biomarkers, had higher relative abundance from natural marsh soils compared to created marsh soils, likely due to the lower organic carbon content and the absence or minor production of methane in created marsh soils.

Created marsh shallow soils had distinct eukaryotic influences, likely due to the difference in vegetation.

Over the ensuing years, created marsh soils should begin to mimic natural marsh soils, which would be evidence for marsh restoration. Further monitoring of created marsh soils would be needed to fully understand the impact of microbial community succession on nutrient cycling and recruitment and sustenance of higher organisms like fish or crustaceans.

Acknowledgements

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