16S rRNA Gene Sequencing of Bacterial Communities in Varying Depths within Freshwater Stream Sediment

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Science / Biology

Abstract

The distribution of bacterial communities in stream sediment profiles could provide insight to the ecological function of bacteria in first-order streams. In this study, bacterial communities in sediment from varying depths obtained from three first-order streams in West Central Georgia were characterized using 16S rRNA gene sequencing. Bacterial 16S rRNA gene abundance showed that the phyla Proteobacteria and Firmicutes had the highest relative abundance in both surface and subsurface sediments. Sediment depth was found to influence the average relative abundance of bacterial phyla based on aerobic or anaerobic abilities as well as the abundance of organic matter. More aerobic taxa were found in the surface sediment, while more anaerobic taxa were found in the subsurface sediment. These results give insight into the microbial processes in surface and subsurface sediments that may impact the compositions of bacterial communities in first order steams.

Introduction

Microorganisms are associated with sediment and water in aquatic ecosystems and contribute greatly to the health of these systems. Exploring the diversity of freshwater sediment microbial communities can provide insight into distinct microbial processes that contribute to the natural purification of aquatic systems (Kumar et al., 2019).

A previous study by Kumar et al. (2019) suggested that in-stream nitrification may occur primarily in stream sediments, as well as degradation of organic matter and transformation of metal compounds. This study found that bacteria were the most abundant microbe in stream sediment, particularly the phyla Nitrospirae, Aquificae, Proteobacteria, and Firmicutes (Kumar et al., 2019). A comparison of microbial communities at varying depths in sediment could be insightful as to which microbial processes occur at different locations within the sediment depth profile.

In a study by Zhou et al. (2017) exploring bacterial and archaeal communities in mangroves, bacterial 16S rRNA gene abundance was found to decrease as sediment depth increased. Researchers concluded that sediment depth was an influential factor for the bacterial community composition and diversity. Aerobic bacterial taxa were largely found in the surface layer, while anaerobic bacterial taxa were largely represented in subsurface layers. Researchers concluded that oxygen availability and the distribution of other terminal electron acceptors along the depth profile shaped bacterial community distribution patterns. The surface layer had the lowest observed number of bacterial species. The largest percentage of observed surface layer species included the phyla Cyanobacteria and Proteobacteria. The largest percentage of observed subsurface phyla included Chloroflexi and Proteobacteria (Zhou et al., 2017).

In the current study, bacterial communities were compared at different sediment depths and channel positions in three streams found in West Central Georgia using 16S rDNA amplicon sequencing. The goal of this study was to gain a better understanding of bacterial community composition and diversity patterns in stream sediment in relation to depth in the stream bed. In doing so, this research could provide insight into the ecological functions of bacterial taxa in lower-order streams. Based on the results of Zhou et al. (2017), we hypothesized that we would see more aerobic taxa in surface sediment and more anaerobic taxa in subsurface sediment.

Materials and Methods

Study Site Description and Sampling Procedure

Sediment samples were collected from three lower-order streams in Troup County, Georgia (Fig. 1a): Long Cane Creek, Oseligee Creek, and Park Creek. Oseligee and Park Creeks are located within the city of LaGrange, GA and were considered to have a more urban land use in the surrounding area. The land use surrounding Long Cane Creek, located just outside the city of LaGrange, was forested with agricultural inputs, as it was located within a cow pasture. A variety of in-

Figure 1. a) Sediment core collection and b) sediment core taken at Long Cane Creek

stream parameters were quantified at each sampling site. These parameters included stream water pH, conductivity, dissolved oxygen, temperature, and channel width. These stream water measurements were collected using Extech ExStik meters (Extech Instruments, USA).

Three replicate sediment samples were collected at each site using a multi-stage sediment sludge sampler (AMS, Inc., USA). The three replicates per site were collected from middle, left, and right positions in the stream channel. For a given replicate, an approximately 0.3-m deep sediment sample was retrieved (Fig. 1b), and the surface and subsurface portions of the sediment core (approx. 8 cm from the top and bottom of the core) were collected in a sterile Whirl-Pak bag. Samples were placed on ice and returned to the lab, where they were frozen at -20ºC until processing.

DNA Analyses

In order to examine the bacterial community composition within the sediment, genomic DNA was extracted from 0.25 grams of sediment from each replicate using the E.Z.N.A. Soil DNA Kit from Omega Bio-tek (Norcross, GA). Final extracts were eluted in 50 µl of elution buffer and stored at -20ºC until processing.

DNA extracts were quantified via Nanodrop (ThermoFisher, USA) and submitted to MR DNA Labs (Shallowater, TX) for 16S rRNA gene sequencing of the bacterial communities associated with stream sediment samples. The average DNA concentration from extracts was 3.81 ± 0.781 ng/ µl. Two DNA extract concentrations were low and therefore not submitted for sequencing. DNA sequence data was processed according to the analysis pipeline used by MR DNA Lab for 16S rRNA data, specifically using the 515F/ 806R primer pair targeting the V4 hypervariable region (Fig. 2).

Statistical Analyses

The relative abundance of various bacterial taxa was compared between the surface and subsurface sediment samples using an independent t-test ($\alpha = 0.05$). Sequence data were processed via a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX, USA) and Operational Taxonomic Unit (OTU) clustering at 3% divergence and therefore a 97% similarity. Taxonomic classification of the OTUs was completed using BLASTn against a curated database from GreenGenes/RDP/NCBI. The statistical program used for data analysis was jamovi (2019), and significance reported for any analysis was defined as $p<0.05$.

Results

The average measures for each instream measure are presented in Table 1. Park Creek and Oseligee Creek both had higher conductivity measures than Long Cane Creek, and Long Cane Creek was moderately wider than Oseligee Creek and

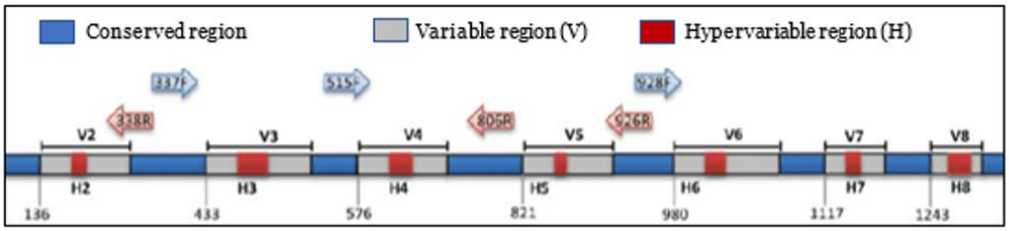

Figure 2. Illustration of conserved and variable regions within the 16S rRNA gene showing the 515F/ 806R primer pair.

Table 1. Instream water measurements for each site. Values represent the mean +/- 1 SE.

Site	pH	Conductivity (µS)	Dissolved Oxygen (mg/L)**	Temperature (°C)	Channel Width (m)
Long Cane Creek*	6.75	80.6	8.69	14.9	8.50
Oseligee Creek	7.57±0.0882	242± 0.577	9.09	14.8± 0.0333	2.30± 0.208
Park Creek	7.50± 0.0577	145± 43.9	3.69	12.1± 0.318	2.87± 0.533

**Only one replicate was recorded for Long Cane Creek.*

*** Dissolved oxygen was only measured once at each site.*

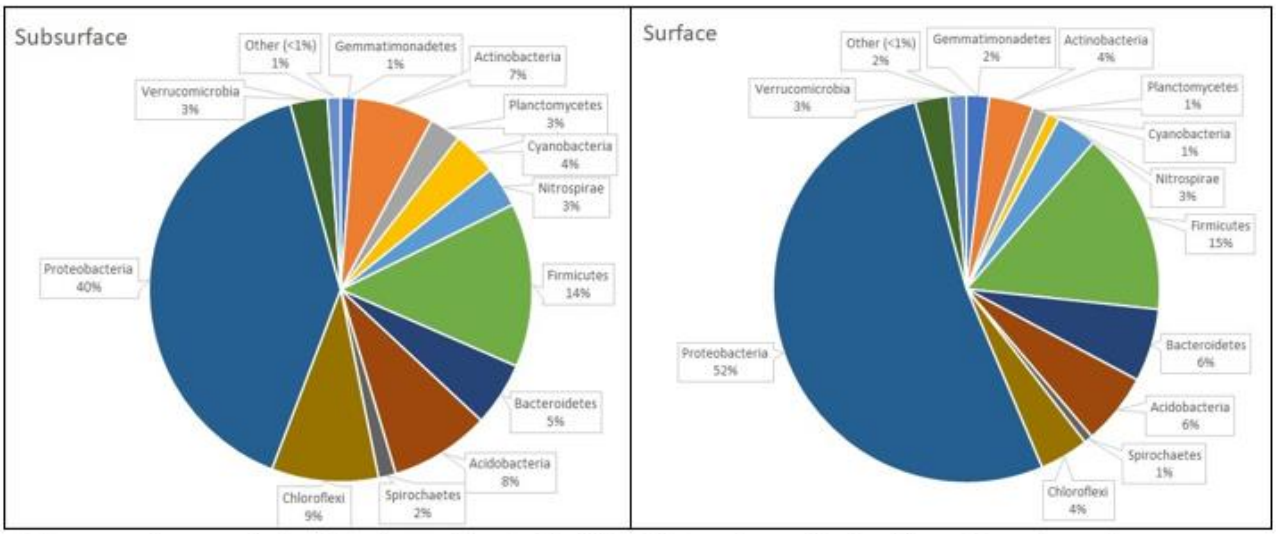

Figure 3. Average relative abundance of bacterial phyla within surface and subsurface sediment samples.

Park Creek. Stream water pH, temperature, and dissolved oxygen concentrations were relatively similar among all sites.

The composition of bacterial communities found in the surface and subsurface sediment samples from Oseligee Creek, Long Cane Creek, and Park Creek is shown in Figure 3. The phyla Proteobacteria and Firmicutes had the highest relative abundance in both surface sediments and subsurface sediments. In the subsurface sediment, the phyla Proteobacteria was found in less abundance, 40%, compared with the surface, 52% (Fig. 3). With the Proteobacteria, the classes delta-, beta-, alpha-, and gamma-proteobacteria were the most dominant. Subsurface sediment samples contained significantly higher relative abundance of Actinobacteria ($p = 0.025$) compared to surface sediment samples. Figures 3 and 4 both summarize the average relative abundance of various bacterial taxonomic levels from surface and subsurface sediments. Of the 118 bacterial classes observed in the sediment samples, 6 of them were significantly different in terms of relative abundance in surface versus subsurface sediment samples ($p<0.05$) (Fig. 4).

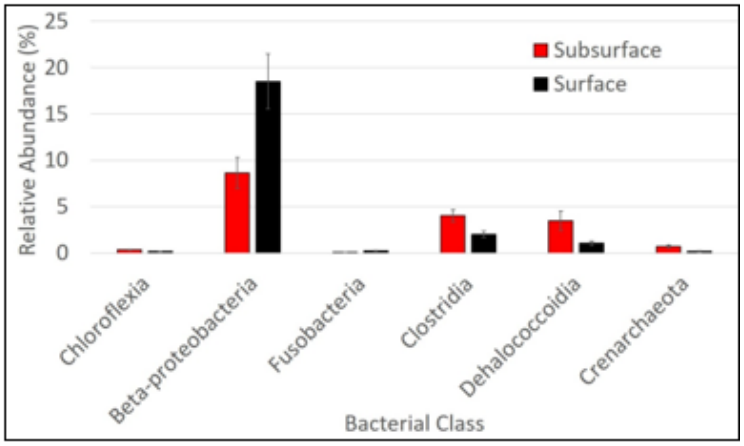

Figure 4. Average relative abundance of six bacterial classes within surface and subsurface sediment samples that were significantly different ($\alpha = 0.05$) between the two depths sampled.

Discussion

Proteobacteria (17.2%), Actinobacteria (12.3%), and Firmicutes (7.4%) were the dominant phyla found in the Kumar et al. study (2018). These phyla were found at lesser amounts than our study, due to a larger percentage of unclassified sequences (36%) compared to 1-2% in our study. Actinobacteria were found in greater abundance in the Kumar et al. study (2018). However, Actinobacteria were found in greater abundance in water samples compared to the sediment samples, possibly because most of its members are aerobic (Kumar et al., 2018). Actinobacteria are often involved in the decomposition of organic matter, which increases as sediment depth increases (Kumar et al., 2018). Actinobacteria can be aerobic or anaerobic, and they can form spores, which could also account for the increasing abundance with depth.

In the Zhou et al. study (2017), the phyla Acidobacteria, Chloroflexi, Dehalococcoidia, Planctomycetes, Nitrospirae, and Spirochaetae were found in greater abundance at deeper sediment depths. Acidobacteria abundance in subsurface sediment samples (8%) was greater than its abundance in surface sediment samples (6%) (Fig.3). Chloroflexi abundance was greater in subsurface sediment (9%) than in surface sediment (4%) (Fig. 3). Dehalococcoidia abundance in subsurface sediment samples was significantly greater than its abundance in surface sediment samples (Fig. 4). Dehalococcodia are well known for their anaerobic respiration on oxidizing hydrogen by halogenated organic compounds, which could account for the significant difference in its abundance between the sediment depths (Zhou et al., 2017). Planctomycetes abundance was greater in subsurface sediment samples (3%) than in surface sediment samples (1%)

(Fig.3). Spirochaetae abundance was greater in subsurface sediment samples (2%) than in surface sediment samples (1%) (Fig.3). However, the phylum Nitrospirae was found in equal abundance in both sediment depths (3%) (Fig.3). Chloroflexi may have been found in greater abundance in subsurface sediment samples due to the utilization of organic matter in subsurface sediments, and it is facultatively anaerobic (Zhou et al., 2017). Spirochaetae are often facultative anaerobes. Clostridia are predicted to metabolize aromatic compounds in anaerobic conditions which may explain why it was found in significantly greater abundance in subsurface sediment than in surface sediments (Kumar et al., 2018) (Fig.4). There is a correlation with sediment depth and oxygen tolerance, and the average relative abundance of anaerobic bacterial phyla was shown to be higher in subsurface sediment samples.

The average relative abundance of Beta-proteobacteria was found to be significantly greater in surface sediment samples compared to subsurface sediment samples (Fig. 4). Our finding is supported by the Zhou et al. study in which the bacterial phylum Beta-proteobacteria was found only in the surface level depth which is likely due to its aerobic characteristics (2017). Also, Beta-proteobacteria can use nitrite as a terminal electron acceptor during denitrification. The concentration of Beta-proteobacteria is significantly higher in surface layers, due to the availability of nitrate (Nedwell et al, 1999). Bacteroidetes abundance was found to decrease with depth in both this study and the Zhou et al. study (2017). In the Zhou et al. (2017) study, Cyanobacteria were enriched in the surface layer sediment, and their abundance drastically decreased with sediment depth. The majority of Cyanobacteria are aerobic and photosynthesize, which would explain their abundance in surface layer sediment (Zhou et al, 2017). However, the surface sediment samples (1%) of Cyanobacteria were in lower abundance than the subsurface sediment samples (4%) (Fig.3). Kumar et al. (2018) reasoned that although nitrification is an aerobic process, it could be inhibited by sunlight, which may explain why Cyanobacteria abundance increased with sediment depth.

Conclusion

In conclusion, this study gives a comprehensive comparison of bacterial community composition in surface and subsurface sediment samples from three first-order streams. The two most abundant phyla, Proteobacteria and Firmicutes, remain stable in surface and subsurface sediment samples. With respect to the hypothesis, more aerobic taxa were found in surface sediments, while more anaerobic taxa were found in subsurface sediments. The distribution pattern of the bacterial community determined by this study may be influenced by the oxygen availability in surface and subsurface sediments. Nitrification, which is typically expected to occur in the water or surface level sediment, was implied to occur in subsurface sediments. Our results give insight into the microbial processes in surface and subsurface sediments that may impact the compositions of bacterial communities in first-order steams.

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