Manipulating Sulfur in Agricultural Soils to Stimulate Sulfur-Based Autotrophic Denitrification and Other Changes in Microbial Community Composition

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Abstract

Nitrate contamination and eutrophication in aquatic systems is a worldwide issue. One way to overcome nitrate pollution is by stimulating denitrification. *Thiobacillus denitrificans* is commonly found in soils and capable of sulfur-based autotrophic denitrification, a process that reduces nitrate concentrations. *T. denitrificans* couples the oxidation of inorganic sulfur, using nitrogen compounds as the final electron acceptor. In a system modeling agricultural drainage, the aims of this study were to investigate soils amended with sulfur and those without the addition of sulfur to determine whether or not microbial community composition, particularly the presence of *Thiobacillus denitrificans,* could influence nitrate removal. To study the microbial community, community DNA was extracted from samples of sulfur-based soil, unaltered soil, and effluent water with high nitrate removal efficiency. PCR was used to amplify the V4 region of 16S ribosomal RNA gene, which was sequenced to further analyze the microbial community, diversity and alterations of bacterial populations within the samples. The sequenced data was paired; filtered using an average Phred score above 30, with an average Expected Error of 0.5, and truncated to 253 base pairs. Data analyses are currently on-going. Based on a review of the literature, it is predicted that *T. denitrificans* will be present in the soils amended with sulfur. We anticipate differences in the microbial community composition of the soil to correspond to the treatments. This data analysis may provide implications for new agricultural tiling methods to reduce nitrate runoff, preventing eutrophication.

Introduction

Eutrophication caused by excess nutrients is characterized by harmful algal blooms leading to decomposition of algae and hypoxic conditions. Agriculture is responsible for most modern-day eutrophication. Farming uses an excessive amount of nutrients, such as inorganic nitrogen, which runs off into large bodies of water (1). Nitrate pollution is a problem in freshwater and marine systems worldwide. Ground water is one of the most susceptible bodies of water to nitrate pollution the main source of human drinking water (2). Other anthropogenic factors contributing to the rise in nitrate concentrations in aquatic systems includes runoff from urban areas, industrial and sewage discharge, and the increased use of fertilizers containing nitrogen (3). All these factors introduce nitrate in high concentrations that confer degradation to overall water quality.

Denitrifying bacteria may minimize eutrophication by removing nitrate from the system through its ability to convert NO_3^- to N_2. *Thiobacillus denitrificans* is a proteobacteria that denitrifies in sulfur-rich aquatic ecosystems through coupling the oxidation of inorganic sulfur with the reduction of nitrogenous compounds. Other proteobacteria such as *Paracoccus denitrificans* and *Pseudomonas aeruginosa* (4, 6) are also common denitrifiers found in soil, making up 10-15% of the microorganisms found in soil (5).

To determine if the addition of sulfur can effectively facilitate denitrification and ensure nitrate removal, microbial DNA was extracted from an experimental system with soils amended with sulfur. PCR was then performed on all samples to amplify the DNA of all microbial species present, and the resulting samples were sequenced and analyzed. It was predicted that *T. denitrificans* and other denitrifying proteobacteria would be present in the sulfur-based soil, as *T. denitrificans* relies on sulfur for energy production.

Methods

Experimental Design

- Two Bins of Soil were set-up, as shown in Figures 1 & 2.
  - Bin A— Drainage tubing was wrapped in sulfur (Sulfur Amended Treatment)
  - Bin B— Drainage tubing was not wrapped (Control)
- Sample Date 1 was taken during week one, when the bins were flushed with water.
- Sample Dates 2, 3, and 4 were collected approximately every other week during which time nitrate (NO_3^-) was added to the system by the Rain Simulator.
- Soil, Effluent Water, and Sulfur samples were collected (n=4)

DNA Extraction & Analysis

- DNA extracted using QIAGEN DNeasy PowerSoil Kit
- V4 region of 16S rRNA gene amplified via PCR
- Samples sequenced using Illumina sequencing
- Analysis of samples using QIIME
- Sequencing reads were paired, filtered, & truncated

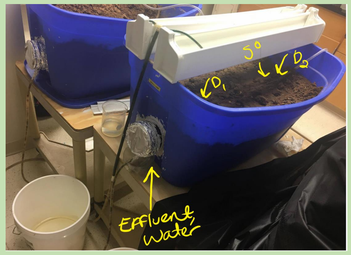

Figure 1: System modeling agricultural drainage. Picture shows sulfur manipulated soil indicating samples taken for DNA extraction: Soil (D1 and D2), sulfur embedded in the draining tubing, and effluent water.

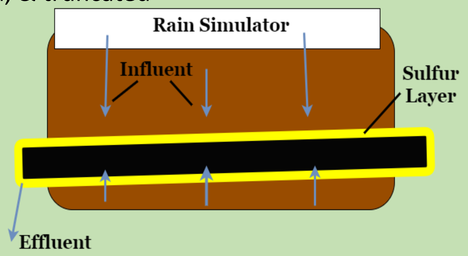

Figure 2: Model depicting agriculture drainage system. Influent is portrayed as water high in nitrate entering the soil and sulfur drainage. Effluent is represented by denitrified water.

Results

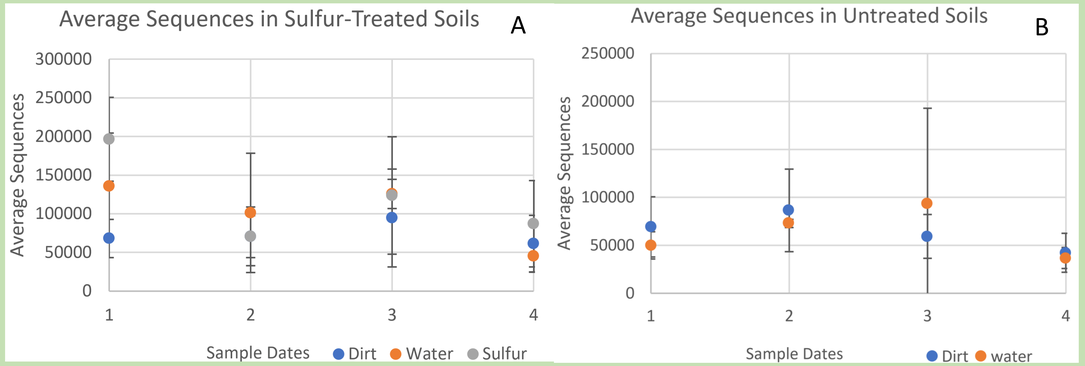

Figure 3: (A) Average prefiltered sequences in sulfur treated soils **(B)** in untreated soils comparing sulfur, dirt and water samples from a course of ten weeks with four sample dates.

Results (cont')

- Sequences filtered and trimmed at:
- Phred score above 30
- Average Expected Error 0.5
- Truncated to 253 Base Pairs

Table 1: Average number of sequences by sample types across all dates (n=16).

	Sulfur (Bin A)	No Sulfur (Bin B)
Sulfur	83,082.2	
Water	103,233.3	49,594.5
Dirt	66,855.7	63,407.7

Discussion

Based on the Phred score above 30, the sequences obtained from the samples are determined to be of high quality. In Table 1, the effluent water samples from Bin A have the highest average sequence count relative to all other samples. This may be due to expected enrichment by denitrifiers such as *T. denitrificans, P. denitrificans,* and *P. aeruginosa,* which are expected to be found in sulfur amended soils. It appears Figure 3A shows a higher average of sequences than Figure 3B as sample dates progressed. It is expected that future taxonomic analysis measuring beta-diversity (between sample diversity) will show higher populations of denitrifying microbes in the samples of sulfur-treated soils. This may be supported by the higher average amount of sequences found for sulfur treated soils than untreated soils. This study may provide a basis for new agricultural tiling methods to reduce nitrate runoff, preventing eutrophication.

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