The soil microbiome in stormwater natural treatment systems: influence of pollutant buildup and environmental conditions

INTRODUCTION

Natural treatment systems (NTS), such as biofilters, rain gardens or bioswales, are engineered systems which combine sand or soil-based media, plants and soil microorganisms to reduce stormwater runoff volume and improve stormwater runoff quality (Payne et al. 2014a). Pollutants, including sediments, heavy metals, and nutrients such as phosphate and nitrate (Davis et al. 2001a) are removed via sedimentation, sorption, plant uptake, chemical transformations, and microbial-mediated processes (Hsieh and Davis 2005; Davis et al. 2006).

For NTS to be effective in the long term, pollutants should be continuously removed and not be significantly released in subsequent storm events or as the system ages. This is relevant to nitrogen in the influent or stored within the NTS, and particularly nitrate, a critical and mobile water pollutant linked to eutrophication of surface waters and groundwater contamination. Nitrate may enter the NTS in influent stormwater and is also internally produced via nitrification, which involves microbe-mediated ammonia oxidation to nitrite and then nitrate. Nitrate may be temporarily assimilated by plants and microbes, or it may be transformed to gaseous forms and permanently removed via denitrification (Payne et al. 2014b). The denitrification pathway includes the reduction of nitrate to nitrite, which is further reduced to nitric oxide and nitrous oxide, and finally to nitrogen (Zumft 1997).

Denitrification in NTS has been extensively studied as a means to enhance permanent N removal, for example by including a saturated zone providing favorable denitrifying conditions (Dietz and Clausen 2006; Blecken et al. 2009, Gilchrist et al. 2013, Zinger et al. 2013, Payne et al. 2014c). However, the microorganisms responsible for N transformation processes have seldom been directly examined. An evaluation of nitrate removal and denitrification genes in a five year old bioretention cell revealed that denitrification gene abundances were positively correlated with average inundation time (Chen et al. 2013). Also, metagenomic analysis of microbial communities in wet and dry basins revealed that wet basins are better poised to denitrify (Morse et al. 2017).

The preceding studies highlight the importance of hydrologic conditions for denitrification and broadly nitrate removal. However, the potential effects of sequestered pollutants on microbes dwelling within NTS have not been studied. It is uncertain if retained pollutants could affect the microbial community in ways that would hinder nitrification and denitrification. The proposed research focuses on heavy metals, because they are effectively removed from stormwater runoff and retained in NTS media (Davis et al. 2001b; Hsieh and Davis 2005; Hunt et al. 2006; Sun and Davis 2007). The negative effects of high metal concentrations on soil microorganisms are well documented, including reduced taxa richness and increased unevenness (Singh et al. 2014), and diversity loss based on the Shannon Index (Wang et al. 2007). Transient loss in denitrification function when soil microorganisms are exposed to 120 mg/kg dry soil of Zn and 80 mg/kg of Cu has also been demonstrated, mostly as reduced ability to transform nitrous oxide into nitrogen (Holtan-Hartwig et al. 2002).
If the final step in denitrification is absent or inhibited, this may result in the release of nitrous oxide, a potent greenhouse gas contributing to climate change (Stocker et al. 2014) and playing a role in the destruction of ozone in the stratospheric layer (Ravishankara et al. 2009). Release of nitrous oxide from NTS can also occur during nitrification. The relative contributions of nitrification or denitrification to nitrous oxide release remain unclear and may vary with environmental conditions such as the water filled pore space (WFPS) (Bateman & Baggs 2005).

This study regards how the microbiome in NTS responds to environmental changes or perturbations, such as heavy metal pollution from stormwater runoff, and how this affects N cycling, particularly considering nitrate removal and release of trace gases such as N$_2$O. This knowledge is needed to inform optimal design and propose suitable maintenance activities. The questions to answer are: 1) to what extent do heavy metals that are sequestered in NTS soil media alter the NTS microbial community, potential for nitrate leaching and release of nitrous oxide from these systems?, 2) what explains the type and extent of alterations?, and 3) what is the role of the microbial community vs. environmental controls in regulating nitrous oxide release?

As a consequence of sampling NTS for this study, opportunistic measurements will also be made for subsamples to assess antibiotic resistance genes (ARGs) by others (UCLA), and fecal indicator bacteria (FIB) by USCB. FIB concentrations are often high in stormwater, which can pose a challenge to widespread use of NTS if these systems are unable to consistently meet FIB criteria for surface waters (Rippy et al. 2015).

The proposed research for the summer was to develop a study plan and perform field sampling of representative natural treatment systems. The study plan has been completed and some preliminary field samples have been collected to aid in method development. Site selection is ongoing and is guided by previous research of metal buildup in bioswale soil at Manzanita Village, UCSB and site visits that have been performed at five Southern UC campuses (UCSB, UCLA, UCSD, UCI, and UCR). Field sampling will follow once site selection and method development are complete. A summary of the study plan is included.

**STUDY PLAN**

**Site selection**

- Site selection criteria are outlined in Figure 1.
- NTS surveys have been performed on five campuses (UCSB, UCLA, UCSD, UCI, and UCR) to collect data regarding the construction date, design (media layers, dimensions, and inlet and outlet structures), maintenance, vegetation, and drainage area characteristics.
- The site pre-selection is primarily based on the catchment to NTS area ratio and percent impervious cover, with higher values likely representing higher metal loading.
- The study will include five NTS across three Southern UC campuses (UCSB, UCI, and UCSD) to cover a gradient of metal pollution.
Soil sampling

- For each selected NTS, samples will be collected and analyzed at four time points to capture seasonal variation in moisture and carbon content, which is linked to microbial and plant activity.
- Environmental parameters relevant to microbial and plant growth, nitrification and denitrification, and metal bioavailability will be recorded at each sampling event.
- To capture spatial variation, four different locations within each NTS will be sampled.
- Each sample will be a composite of 3-4 soil cores of a 10 cm depth, representing the typical rooting zone depth. Soil at larger depth will not be collected since most microbial biomass and activity (Fierer et al. 2003) and pollutant accumulation (Blecken et al. 2009) is thought to occur near the surface. Prior to sample collection, the top layer (detritus) will be scraped off and plants will be cleared/trimmed to allow access to the soil layer.

Field measurements

- To understand nitrous oxide release from NTS, trace gas measurements will be performed immediately preceding soil sample collection using static chambers fitted with a gas analyzer or by collecting headspace samples into evacuated containers for analysis by gas chromatography.
- To characterize vegetation, the point-intercept method will be used to estimate total plant cover, percent cover of individual species, percent cover of bare ground, and to determine the total number of species (plant species richness) at each sampling location.
Field conditions will be recorded (air temperature, cloud cover, etc.)

**Soil processing, characterization and analysis**

- Soils will be sieved (2 mm) upon return to the lab and subsampled for analysis of parameters relevant to microbial growth and activity and metal bioavailability, including: gravimetric moisture content, soil water potential, pH, soil organic matter by loss on ignition, nutrients (nitrite, nitrate, ammonium, and phosphate), and dissolved organic carbon (DOC).
- A portion of soil will be allowed to air dry prior to sieving and storage at 4°C until further characterization (including TC, TN, particle size analysis, cation exchange capacity, electrical conductivity) at UC Davis Analytical lab.
- To evaluate metal content in soil, lab personnel at UCR will extract metals from soil subsamples using a sequential extraction procedure and analyze via inductively couple plasma – mass spectroscopy (ICP-MS).
- To measure the potential of the microbial community for denitrification and nitrification, enzyme activities will be evaluated via the potential nitrification and denitrification assays.
- To evaluate if NTS may be a potential hotspot for ARG proliferation, DNA from a soil will be extracted and qPCR of a known ARG will be performed by others (UCLA).
- A soil subsample will be eluted and tested for fecal indicator bacteria (FIB) using the Colilert and Enterolert assays (IDEXX Laboratories, Inc., Westbrook, ME).
- To understand the relationship between the bacterial community and N₂O release and nitrate accumulation, the bacterial community will be assessed for its diversity, and functional gene abundance and expression of nitrifiers and denitrifiers, as outlined below.

**Microbial community analysis**

- DNA and RNA will be extracted from soil samples in duplicate and then pooled, using Power Soil RNA extraction and DNA elution kits (MoBio, Carlsbad, CA). The genomic DNA (gDNA) and RNA extracts will be purified and quantified using commercially available kits and then used as template for subsequent microbial community analysis.
- Microbial biomass (size) will be estimated via the substrate induced respiration method and by quantifying extracted soil gDNA.
- Using the extracted gDNA as template, polymerase chain reaction (PCR) will be used to amplify the 16S rRNA gene and functional genes (*amoA, nirK, nirS, and nosZ*). The amplicons will be sequenced using the Illumina platform to examine phylogenetic and functional diversity and composition of the entire bacterial community.
- The abundances of genes associated with nitrification and denitrification will be quantified via qPCR of functional genes (*amoA, nirK, nirS, and nosZ*). Additionally, gene expression will be assessed using a real time (RT)-PCR-based approach to significantly improve understanding of the active functional groups (*amoA* (bacterial and archaeal), *nirS, nirK*, and *nosZ*) in the NTS soil environment at the time of sampling.

**Quality control and quality assurance**
To ensure the validity of the analytical results, samples will be analyzed in triplicate for assays measuring properties with expected high sample heterogeneity, including water potential, water moisture, soil organic matter, substrate induced respiration, potential nitrification and potential denitrification. Further, additional samples will be prepared to assess potential contamination during lab processing including sample filtration (filter blanks) and lab analysis (analytical duplicates and laboratory blanks).

Nutrient extraction will include a filter blank in every extraction batch and one extraction blank per batch of extraction solution. The potential nitrification and potential denitrification assays, performed on triplicate samples, will require preparing daily standard curves, which will be assessed for linearity prior to required measurements. All materials used will be washed and rinsed with acid solution to remove any potential nutrient and contaminant residues.

FIB analysis will include an analytical duplicate in every batch, and a filter blank per batch of dilution water. RNA and DNA extraction, which are performed in duplicate, will include duplicate extraction blanks. PCR reactions, which are performed in triplicate to overcome potential amplification biases, will include negative and positive controls. Standard curves for DNA quantification will be analyzed in duplicate with every sample batch. Runs where the standard curve $R^2$ is < 0.99 will be aborted and a new set of standards will be analyzed. All materials used for microbial analysis will be pre-sterilized by the manufacturer or sterilized by autoclaving.

**Data Analysis**

The correlation between different studied metals will be investigated through pairwise Pearson’s correlation to reduce the number of variables and select a few representative metals for subsequent statistical analysis. The relationship between continuous variables will be investigated via simple regression. The effects of metals on single biological variables (e.g. substrate induced respiration, denitrification potential, nitrification potential) will be investigated by correlating concentrations of specific metals with the residual variability in the biological variables (after accounting for relevant environmental variables such as DOC, SOM, air temperature, soil moisture, and pH). Multivariate statistical techniques based on correspondence analysis will be used to investigate the relationship between microbial community data (qPCR and sequencing data outputs), metal concentrations and environmental variables. All analysis will be performed in R.

**ANTICIPATED RESULTS**

Upon project completion, anticipated results include new insights into the impacts of stormwater-derived pollutants on microbial communities and microbe-mediated processes such as nitrogen cycling in natural treatment systems. This research is encased within the larger Multi-campus Research Programs and Initiatives project “Fighting Drought with Stormwater: From Research to Practice”. While this project is focused on Southern California, results may inform other rapidly urbanizing regions which are shifting towards green stormwater management as a result of global water scarcity concerns.

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REFERENCES


