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Effects of wildfire residues on nitrate and sulfate reduction in wetlands.

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Effects of wildfire residues on nitrate and sulfate reduction in wetlands

A thesis submitted in partial satisfaction of the

requirements for the degree Master of Science

in Civil Engineering

by

Shruti Indiresan

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ABSTRACT OF THE THESIS

Effects of wildfire residues on

nitrate and sulfate reduction in wetlands

by

Shruti Indiresan

Master of Science in Civil Engineering

University of California, Los Angeles, 2022

Professor Sanjay K. Mohanty, Chair

Wetlands provide many critical ecosystem functions including cycling of elements such as nitrogen, sulfur, and carbon while providing other functions such as water treatment and flood management in urban areas. Climate change and related climate extremes such as wildfires have the potential to alter the ecosystem balance provided by wetlands. For instance, many wetlands are located downstream of wildfire-affected areas and receive stormwater runoff carrying wildfire residues. The deposition of the wildfire residues may affect some of the functions of the wetlands. Yet, no studies to date examine the effect of wildfire residues on the capacity of

wetlands to reduce nitrate and sulfate. To investigate the effect of wildfire residues on microbially mediated denitrification and sulfate reduction, batch experiments were set up in the lab using wetland sediments, water, and wildfire ashes. Results reveal that the presence of wildfire residues accelerated both denitrification and sulfate reduction initially. While the denitrification rate remained consistent following repeated exposure to nitrate-rich water, sulfate reduction rates became slower following each exposure to sulfate-rich water. An increase in nitrate and sulfate reduction in the presence of wildfire residues was attributed to the potential change in water chemistry and microbial community. The presence of wildfire residues increased salinity, dissolved organic carbon, and the concentrations of nitrate and sulfate leached from wildfire residues— all of which could have affected the microbial reduction rate of nitrate and sulfate. Analysis of SUVA showed a possible increase in aromatic DOC in pore water. Analysis of functional genes also confirmed the higher abundance of denitrifying genes in wildfire batches following one week in a field experiment, but by three weeks, denitrifying genes were insignificant in both the field and the lab. Overall, the results suggest that wetlands could provide an effective barrier to removing excess sulfate and nitrate released from wildfire residues or other sources following the deposition of wildfire residues.

The thesis of Shruti Indiresan is approved.

Jennifer A. Jay

Shaily Mahendra

Sanjay K. Mohanty, Committee Chair

University of California, Los Angeles

2022

Table of Contents

List of Figures

Figure No. Page

List of Tables

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1. Introduction

Wildfires are becoming more intense and frequent with climate change. They burn down forests, a natural carbon sink, in the long term while increasing carbon emissions and degrading air quality in the short term (Abatzoglou and Park Williams 2016). The health effects of wildfire smoke also present an environmental justice issue, as outdoor workers and socially disadvantaged groups are disproportionately exposed (D'Evelyn *et. al.* 2022). In particular, the majority Black, Hispanic, or Indigenous are more vulnerable to the effects of wildfires than other groups (Davies *et. al.* 2018). The burden on these communities is expected to increase with climate change. Climate change has increased the aridity of forests in the western US by about 75% from 2000 to 2015, making forests highly susceptible to burning for 9 more days a year (Abatzoglou and Park Williams 2016). Climate modeling estimates that the increase in burned forest area between 1984 and 2015 was doubled due to climate change (Abatzoglou and Park Williams 2016). In addition, the feedback loops due to carbon emissions and loss of carbon sinks exacerbate this problem.

Wildfires can also have serious implications for water quality and the ecosystem of water bodies such as wetlands near the periphery of wildfire-prone areas. Runoff from wildfire-affected areas contains high concentrations of wildfire ash which can then be deposited downstream in surface water and wetlands (Valenca *et. al.* 2020). Following a wildfire, increased erosion and pollutant sources can increase the loading of sediments, nutrients, trace elements and metals, polyaromatic hydrocarbons (PAH), and other water quality impairments to surface waters. This may affect the potable water supply for communities that depend on surface waters originating from the catchments downstream of forests. (Smith *et. al.* 2011). A study in Canada estimated that rivers that receive runoff from burned areas receive 1.2 to10 times more nutrient

loading than those downstream of unburned areas (Smith *et. al.* 2011). Thus, it is critical to determine the ecosystem response to the runoff after wildfires.

Among different water bodies, natural wetlands provide a variety of ecosystem services including water treatment, carbon sequestration, harboring biodiversity, and cultural services in the form of recreation (Moore and Hunt 2012). Wetlands have the ability to mitigate flooding and peak runoff rate while aiding the natural recharge of groundwater. Wetlands are also key players in greenhouse gas emissions. Although they are able to remove atmospheric CO_2 using vegetation, greenhouse gases including methane may be generated or released from the sediments (Moore and Hunt 2012). Wetlands contribute to the biodiversity of plants, microbes, and invertebrate and vertebrate animals (Moore and Hunt 2012). Wetlands remove sediments, nutrients, pathogens, and other common water contaminants through physical, chemical, and biological processes (Moore and Hunt 2012). They are often known as the "Earth's kidneys" due to their high and long-term water filtration capacity (Sharifi *et. al.* 2013).

Biogeochemical cycling is key to maintaining a balance of elements on Earth. The nitrogen cycle is one of the core biogeochemical cycles on Earth, maintaining a balance between pools of N_2 gas, ammonia, and nitrate. Globally, the nitrogen cycle has exceeded the planetary boundary due to anthropogenic applications of fertilizers and biological fixation. This can lead to weakened natural carbon sinks and cause feedback loops (Steffen *et. al.* 2015). Large-scale disruptions to nitrate cycling can lead to losses in biodiversity, harm the health of the climate and humans, and cause food shortages in parts of the world (Stevens 2019). Anthropogenic deposition of reactive N exceeds nature's capacity to remove it, and this could shift biodiversity, promote eutrophication, and affect human health (Martinez-Espinosa *et. al.* 2021). Excessive nitrate in watersheds can have health implications for children, lead to algal blooms, and cause

municipalities to have to spend a lot of money to mitigate the problem (Martinez-Espinosa *et. al.* 2021). Nitrate pollution in drinking water is associated with many health issues including blue baby syndromes (Knobeloch *et. al.* 2000), cancer, and birth defects (Schaider *et. al.* 2019). Anthropogenic activity has increased the nitrate flux in rivers and receiving waters over time. Furthermore, climate change increases the flux of nitrate due to the variability in discharge from rivers. This leads to increased primary productivity of phytoplankton, causing marine eutrophication and hypoxia. In aquatic ecosystems, this is an issue as hypoxia causes dead zones, kills fish, and therefore alters the ecology and food webs in these environments and hurts biodiversity (Justić *et. al.* 2003).

Similar to nitrate, sulfate concentration also increases during wildfire and can have an impact on the sulfur cycle. High S can lead to the formation of methylmercury in wetlands which poses risks to human health and wildlife (Hermes *et. al.* 2021). Methylmercury formation in wetlands is primarily driven by sulfate-reducing bacteria, as the methylation of Hg is coupled with the sulfur cycle. The deposition of sulfate pollution increases the activity of sulfate-reducing prokaryotes and therefore MeHg formation and export. Furthermore, wetlands commonly export water to ecosystems where fish live, leading to the bioaccumulation of MeHg up the food chain (Jeremiason *et. al.* 2006). Sulfate pollution in drinking water has also been linked to observable, albeit minor, gastrointestinal issues (Heizer *et. al.* 1997). The sulfate cycle is also closely linked with methane emissions, as sulfate-reducing microbes can use either methane or organic carbon as substrate (La *et. al.* 2022). Sulfate-driven anaerobic oxidation of methane is the major process that both removes sulfate and prevents methane emissions from wetlands.

Wildfires can affect nitrogen and sulfur cycling in surface water, wetlands, soils, and sediments. California watersheds already have high levels of nitrate deposition from agricultural

sources, and wildfires could exacerbate the problem (Gustine *et. al.* 2021). Post-fire nitrate depositions often cause exceedances of the EPA's water quality standards. In Mediterranean climates, deposition of N is decoupled from the growing season of vegetation that would normally consume excess nitrogen. Furthermore, wildfires can exacerbate these effects by depositing more nitrate while killing the vegetation that would normally consume it (Gustine *et. al.* 2021). Following burns in forests, the proportion of denitrifying bacteria increases, while nitrogen fixers decrease (Kennedy and Egger 2010). Denitrifying bacteria tend to benefit from anaerobic conditions created by burns. Fire can also eliminate the problem of a carbon-limited system, increasing the activity of denitrifiers. This may lead to increased N_2O emissions from incomplete nitrate reduction, which can contribute to climate change and feedback loops (Niboyet *et. al.* 2011).

Despite key roles of wetlands in the cycling of N and S, few studies have looked into the effects of wildfires on nitrate and sulfate removal in wetlands. The objective of this study is to examine the effect of wildfire residue deposition on the ability of wetlands to reduce nitrate and sulfate. The central hypothesis of the study is that wetlands would remove excess nitrate and sulfate following wildfire residue deposition by increasing their reduction rates. To test the hypothesis, batch experiments were conducted with natural wetland sediments exposed to wildfire residues, and nitrate and sulfate removal rates were measured. In situ field experiments were set up to monitor the effect of wildfire residues on the microbial communities and the expression of functional genes involved in nitrate and sulfate reduction.

2. Materials and Methods

2.1 Wetland site description and sampling methods

Wetland water and sediments were sampled at the Ballona Freshwater Marsh (33°58'10.4"N 118°26'03.4"W) within the Ballona Wetlands Ecological Reserve in Playa del Rey, Los Angeles, California, USA. It is the only wetland in the Los Angeles area, but 80% of land upstream is developed (Bergquist *et. al.* 2012). Efforts to restore the wetland began to expand the area of the wetland, which had been depleted to under 600 acres at its minimum, to its original 2000 acres (California Department of Fish and Wildlife). The purpose of the restoration project is to provide a habitat for native species, treat stormwater, and create flood protection and infiltration capacity in the watershed (Tsihrintzis *et. al.* 1996). The marsh receives water from Ballona Creek and discharges to the Pacific Ocean.

Natural wetland water and submerged soil were collected at this site to be used in experiments. To collect the wetland water, clean resealable plastic bags were used. The water sample was transported back to the lab and kept in a 4^{o}C cold room. The submerged soil, also referred to as wetland sediments, was collected using a shovel and placed in clean resealable plastic bags. The sediments were placed in the -80^{o}C freezer to halt microbial activity. When ready to use, the sediment was thawed and sieved using sieve #10 (2 mm opening) to keep small particles (< 2.0 mm) and remove large debris (> 2.0 mm). The wetland soil was placed in 50-mL centrifuge tubes, and centrifuged for 10 minutes at 5,300 rpm. The water supernatant was removed using a 10-mL electronic pipette, and the processed samples were stored in centrifuge tubes at -80^{o}C.

2.2 Wildfire site description and sampling methods

The Pacific Palisades fire took place in Topanga Canyon and was a brush fire that started on May 14, 2021 and was 100% contained by May 26, 2021. The fire burned 1202 acres of land and threatened 710 structures (Stewart, Los Angeles Fire Department, 2021). Four sampling locations were selected in the burned area based on the sites that had the potential to be carried with stormwater runoff. The wildfire residue samples were collected from the top 10 cm of soil using a sterilized spatula and stored in a cold room at 4^{o}C. The wildfire residues were sieved to remove debris larger than 0.833 mm.

2.3 Leaching of nitrate and sulfate from wildfire residues in stormwater

A 24-hour leaching experiment was performed to assess the levels of nutrient leaching from wildfire residues. 4.0 g of wildfire residues were added to 40 mL of DI water and shaken for 24 hours in an automatic wrist shaker (Wrist Action Shaker, Burrel Scientific). The mixture was centrifuged at 5300 rpm for 5 minutes. The supernatant was centrifuged again, and syringe filtered (0.45 μm). The electrical conductivity was measured. 0.1 mL of supernatant was mixed with 0.9 mL of DI water for ion chromatography analysis to measure levels of NO_3^- and SO_4^{2-} leaching.

2.4 Effect of wildfire residues on denitrification and sulfate reduction in wetland microcosms

To analyze denitrification and sulfate reduction in wetland sediments, 200-mL glass flasks with rubber caps were used (Figure 1). 30 g of wetland sediments was added to 60 mL of wetland water in flasks using a sterilized spatula. In preliminary experiments, triplicate batches

were made to represent controls with no wildfire residues, and batches that contained 10% wildfire ash by weight (3.0 g). To create mostly anoxic conditions in the batch experiments, argon gas was purged for 10 minutes using a fine syringe. Argon was used as opposed to nitrogen to avoid the gas purge affecting nitrogen cycling (Park and Lee 2020). The flasks were immediately closed with rubber caps and closed with a metal seal. Before placing the batches in the incubator, 1 mL of water from each batch was removed using a syringe and needle to represent time zero. Every time the liquid sample was removed, argon gas was purged through a Tedlar bag to avoid formation of a vacuum while also not pressurizing the batches. The batch experiments were kept in an incubator at 120 rpm and 30^{o}C. Nitrate and sulfate concentrations were assessed periodically by obtaining 1 mL of sample with a syringe, filtering it to remove particles greater than 0.45 µm, and measuring in the IC in a 1:4 dilution.

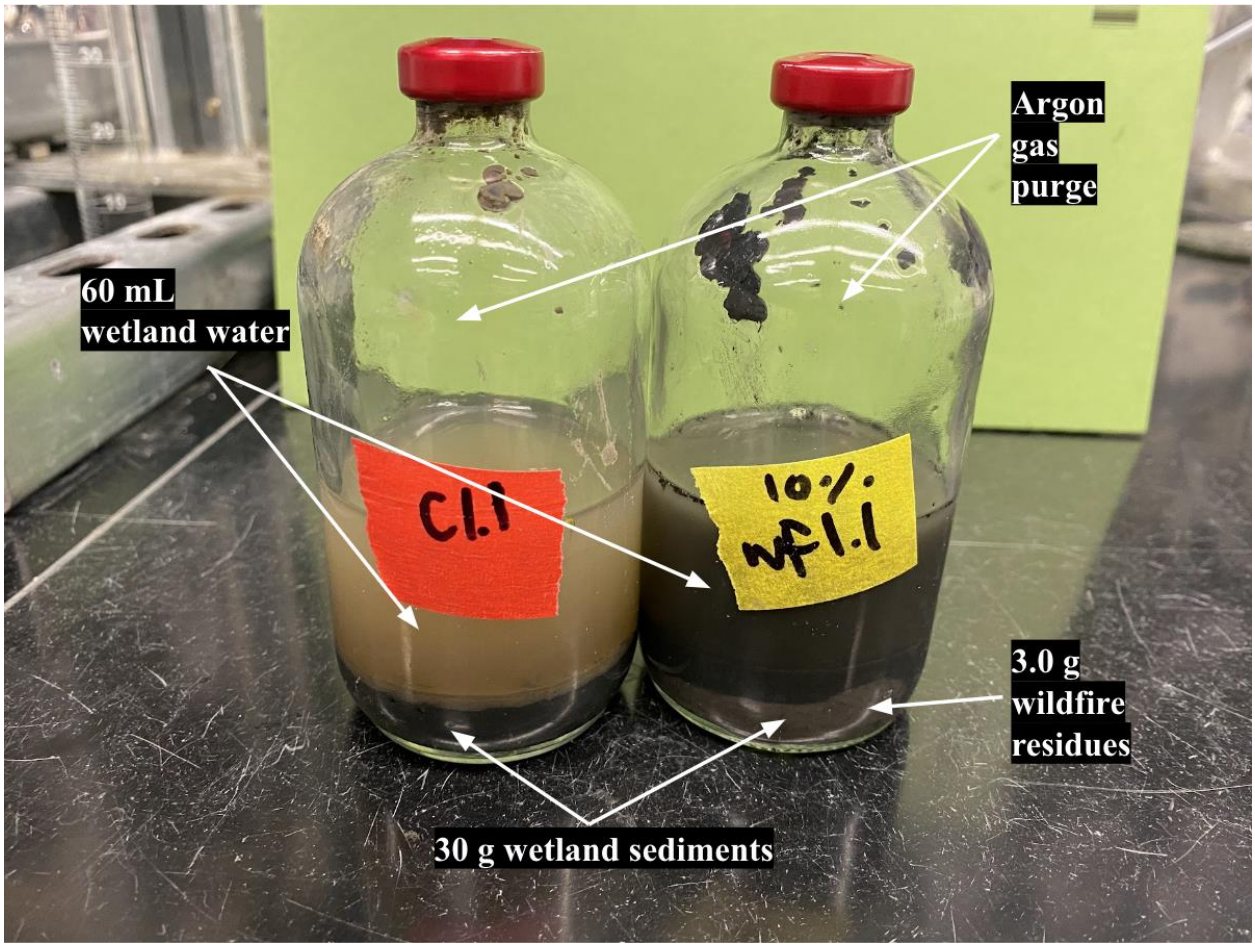


Figure 1: Setup of the laboratory batch experiments.

The same methods and conditions described in the initial experiment were used to analyze microbial metabolism in the batches following nutrient spikes. Preliminary data showed that almost all nitrate in the batch was consumed within 24 hours. Consequently, to better analyze the effect of wildfire residue deposition on denitrification, nitrate was spiked after each cycle of 24 h to measure change in reduction rates following each exposure. Batch experiments were set up as described before with 4 controls and 4 samples with 10% wildfire residues, and time zero samples were taken. The batches were then allowed to equilibrate for 3 days. Following the 3-day equilibration, samples were spiked so they would contain 100 ppm of nitrate. To achieve this, 6 mL of air was removed from the headspace of the batches using a

syringe and needle, and then 6 mL of 1000 ppm nitrate stock was added into each batch. Concentrations were assessed periodically to monitor the reduction of nitrate.

The nitrate spiking experiment was repeated weekly following a similar procedure. Each week, “time zero” samples were taken prior to spiking, and then samples were taken immediately after spiking. The batches were allowed to incubate. and samples were taken periodically to be measured using ion chromatography (IC).

Preliminary experiments showed that sulfate reduction appeared to occur slower in wildfire batches, but sulfate leaching from wildfire residues more than doubled the peak concentration of sulfate in the wildfire batches. To remove extra variables, the original batches were allowed to equilibrate over a period of 23 days such that almost all the sulfate leached from wildfire residues would be consumed before spiking sulfate to monitor the reduction rate. Prior to spiking the batches, “t=0” samples were taken, and 5 mL of air was removed from the headspace. Batches were then spiked with 5 mL of 1000 ppm sulfate stock to bring the batches to 100 ppm sulfate. Samples were taken immediately after spiking and then periodically over 2 weeks and measured in the IC. This experiment was repeated thrice following the same procedure.

2.5 Analysis of dissolved organic carbon (DOC) quality and quantity in pore water

Filtered samples from the preliminary leaching experiment were analyzed for total organic carbon (TOC) to estimate the quantity of organic carbon leached from wildfire residues. To analyze the effect of wildfire residues on DOC quality, new batches were set up and kept at the same conditions as described in Section 2.4. Water samples were taken from the batches periodically and assessed using a spectrophotometer set to 254 nm. To analyze the aromatic to

aliphatic ratio, composite samples were prepared from the triplicate batches and measured using SUVA254 in a 1:10 dilution.

2.6 In-situ experiment to track microbial community shift in wetland sediments after wildfire residues exposure

In-situ experiments were conducted at the Ballona Marsh to track possible shifts in the wetland microbial community following exposure to wildfire residues in the field. About 100 g of wetland sediments and 5 g of wildfire residues were added to 1 liter of natural wetland water in glass bottles. The bottles were closed with glass wool and submerged in the wetland inside mesh boxes. The glass wool allowed water to pass between the bottle and the wetland, but it kept all sediments inside the bottle. The mesh boxes kept the bottles in the same place. The bottles were submerged on August 20th and samples were collected after 1, 3, 10, and 21 weeks. The samples were transferred into 50-mL sterile centrifuge tubes and kept in the -80^{o}C freezer to be used to analyze the microbial community.

2.7 Microbial analysis in field and laboratory samples

To quantify the effect of added wildfire residues on denitrifiers and sulfate reducers, total nucleic acids were extracted from the sediments from batch and field studies. The previously frozen sediment samples from the field experiments were retrieved from storage in –80 ºC and brought to room temperature. For the lab experiments, wetland microcosm batches were opened up after 21 days of incubation and the water and sediments were put into 50 mL centrifuge tubes. 0.5 g of each sample was used to perform nucleic acid extraction. For the field experiment, the sample was homogenized from the frozen tubes; for the batch experiment, 250 mg of sediments and 250 uL of wetland water was used for nucleic acid extraction. Total nucleic

acid extraction was performed using a modified phenol-chloroform extraction as described in full in a previous paper (Gedalanga *et. al.* 2014). Briefly, the cells in sediments were first lysed chemically and mechanically by incubating at 65 ºC with a lysis buffer of sodium acetate and EDTA, SDS, phenol, and zirconia-silica beads for 2 minutes, then by bead-beating for 2 minutes. The tubes were then incubated for 8 minutes and underwent another 2-minute bead beating. Cells were centrifuged again for 5 minutes, and the supernatant was transferred to new nuclease free tubes. Nucleic acids were extracted twice using a phenol-chloroform-isoamyl alcohol extraction and then again with just chloroform and isoamyl alcohol, and then stored in isopropanol and sodium acetate at –20ºC overnight to precipitate. The precipitate was then collected, purified, resuspended in nuclease-free water, and measured for purity and concentration on a NanoDrop 2000C spectrophotometer. Following total nucleic acid quantification, an aliquot of each sample was obtained for cDNA synthesis. DNA was removed using DNase, and the RNA alone was synthesized into cDNA using the Lamda Biotech EasyScript Plus Kit according to the manufacturer's instruction, then quantified on the spectrophotometer. DNA and cDNA samples were stored in –20ºC.

Analyses of denitrifying and sulfate reducing genes were conducted of both DNA to estimate abundance and cDNA to estimate activity. Abundance was measured against 16s rRNA as a baseline, and activity was measured against the housekeeping gene *rpoD*. To analyze denitrifiers, the genes of interest selected were *nirS* and *napA*, enzymes involved in nitrate and nitrite reduction. Sulfate reducers were analyzed through the *dsrAB* gene, whose enzyme catalyzes the last step in sulfate reduction. A table of primer sequences and cycling profiles are listed in Table 1. RT-qPCR was run using a reaction mixture of primers, SYBR green, BSA, and the DNA or cDNA samples. Each sample for control and wildfire was placed in a ThermoCycler

StepOne Plus PCR instrument in triplicates along with controls for generating a standard curve made by serially diluting DNA from the pure culture and adding primers. For denitrification, the pure culture selected was *Pseudomonas stutzeri* and for sulfate reduction the pure culture was *Desulfovibrio vulgaris*. No template negative controls were also included to verify there was no extraneous contamination or residual nucleic acid. RT-qPCR was run with 10x master mix (SYBR Green), 0.3 µM primer, 10 ng RNA template, and 0.2 µg mL^{-1} bovine serum albumin (BSA) to mitigate inhibition in environmental samples.

Table 1: qPCR conditions for genes of interest

Gene	Primer Sequence (5' → 3')	Cycling Profile (40 cycles)	Reference
16s rRNA	F: CCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	15 min hold @ 95°C → 40x 95°C for 15 s, 60°C for 30 s, 72°C for 30 s	Cira *et. al.* 2021, Ji *et. al.* 2012
rpoD	F: GGGCGAAGAAGGAAATGGTC R: CAGGTGGCGTAGGTGGAGAA	Holding stage: 2 min @ 50°C, 10 min @ 95°C → 40x 95°C for 15 s, 60°C for 45 s	Polasko *et. al.* 2021
nirS	F:GTSAACGTSAAGGARACSGG R:GASTTCGGRTGSGTCTTGA	5 min hold @ 95°C → 40x 95°C for 60 s, 59°C for 60 s, 72°C for 60 s	Berger *et. al.* 2019
narG	F:TAYGTSGGGCAGGARAAACTG R:CGTAGAAGAAGCTGGTGCTGTT	5 min hold @ 95°C → 40x 95°C for 20 s, 59.5°C for 30 s, 72°C for 40 s	Berger *et. al.* 2019
dsrAB	F: ACSCACTGGAAGCACG R: GTGTAGCAGTTACCGCA	3 min hold @ 94°C → 40 cycles of 15 s at 94°C, 20 s at 54°C, 2 min at 72°C	Wagner *et. al.* 1998, Leloup *et. al.* 2004

2.8 Analytical methods

The pH of the leaching experiments was measured using an Ion-Selective Electrode (model #9107BN, Fisher Scientific) and electric conductivity was measured using a Two-Cell Accumet 92 Probe, Fisher Scientific. The concentrations of nitrate and sulfate in the water extracted from the batch experiments were assessed using an ion chromatograph (Dionex™ Integrion™ HPIC™ System, ThermoFisher). The TOC was measured with a Total Organic Carbon Analyzer (TOC-L, Shimadzu). The DOC was analyzed using a spectrophotometer set at 254 nm (PerkinElmer Lambda 365 UV-Visible Spectrophotometer). The extracted nucleic acid samples were analyzed using a NanoDrop 2000C spectrophotometer (Thermo Scientific) and genes were measured using qPCR on a StepOnePlus ThermoCycler (Applied Biosystems, Foster City, CA).

3. Results

3.1 Deposition of wildfire residues to wetland sediment accelerates denitrification and sulfate reduction

Leaching experiments showed that NO_3^- and SO_4^{2-} were both leached in significant quantities from wildfire residues. 8.49±0.41 ppm (mean ±one standard deviation) of nitrate was leached from 4.0 g of wildfire residues in 40 mL of DI water.. In contrast, higher amount (332.9 ±0.9.93 ppm) of sulfate was leached. Preliminary data showed that in the control batches without wildfire residues, denitrification was negligible as nitrate concentration was always low. In batches with wildfire residues, about 3 ppm of nitrate was leached at time 0, all of which was

reduced by 24 hours into the experiment (Figure 2). Data from the spiking experiments showed that nitrate concentration was depleted faster in wetland microcosm batches that contained wildfire residues, thereby implying that denitrification would be accelerated in wetlands following deposition of ash-laden stormwater runoff from wildfire-affected areas (Figure 3a). Furthermore, the exponential decay coefficient for each trial was similar, implying that denitrification occurs at similar rates following nutrient spikes (Figure 4a).

Sulfate reduction showed that over 100 ppm of sulfate was leached into the batches due to wildfire residues, making it hard to compare rates in the control and wildfire batches (Figure 2). The batches were allowed to equilibrate before and between each sulfate spike, and data obtained from the IC showed that sulfate was depleted faster in wildfire batches than in control batches (Figure 3b). However, unlike nitrate, the rate of sulfate reduction slowed down after each spike (Figure 4b). Even so, the wildfire batches were consistently reducing sulfate faster than the controls.

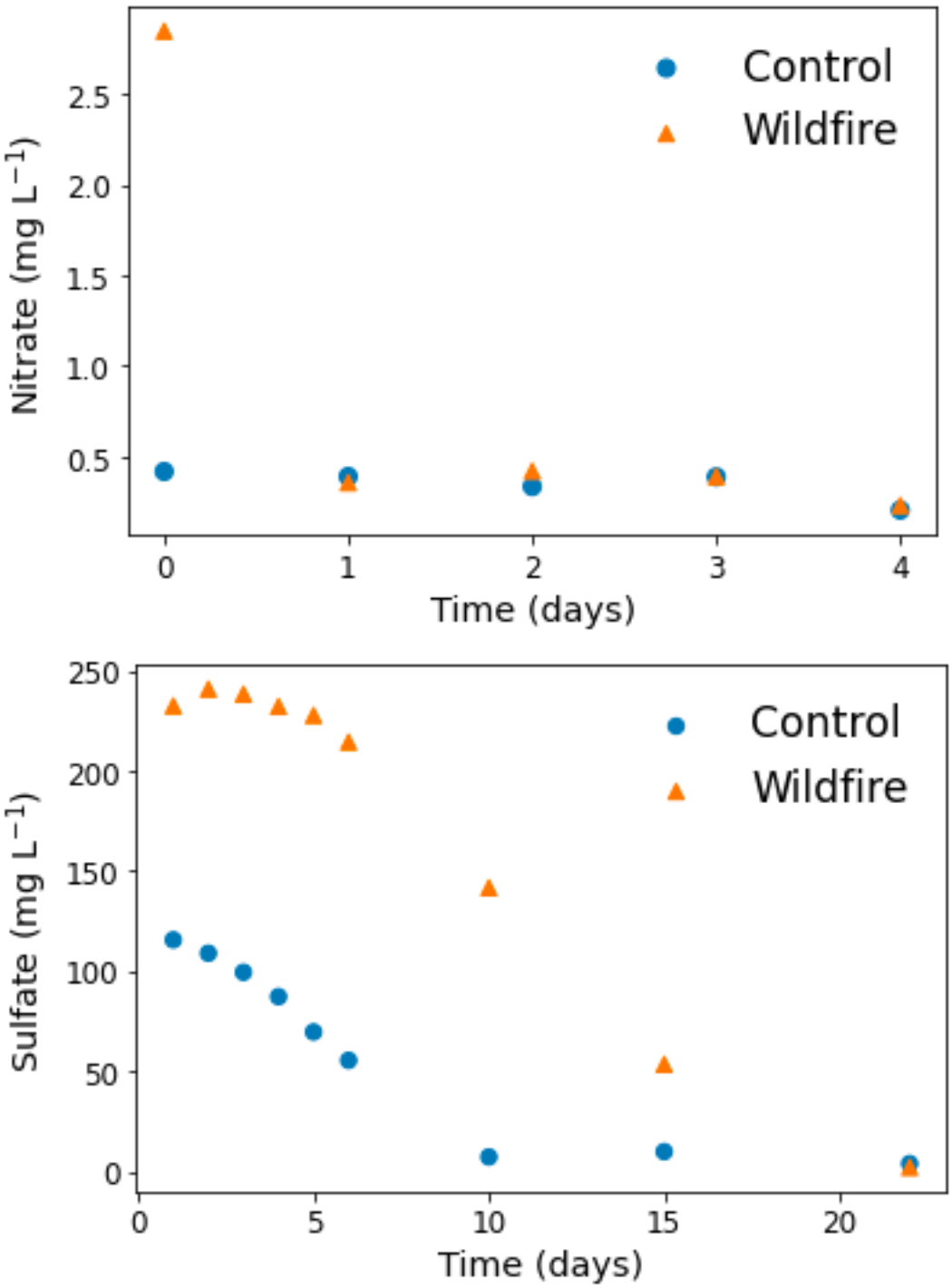


Figure 2: Results from preliminary batch experiments that show leaching and removal of nutrients. It is seen that all nitrate leached is brought down to control levels within 24 hours, while sulfate removal takes about 3 weeks until it is fully depleted. Furthermore, sulfate is initially leached in the wildfire batches before removal begins.

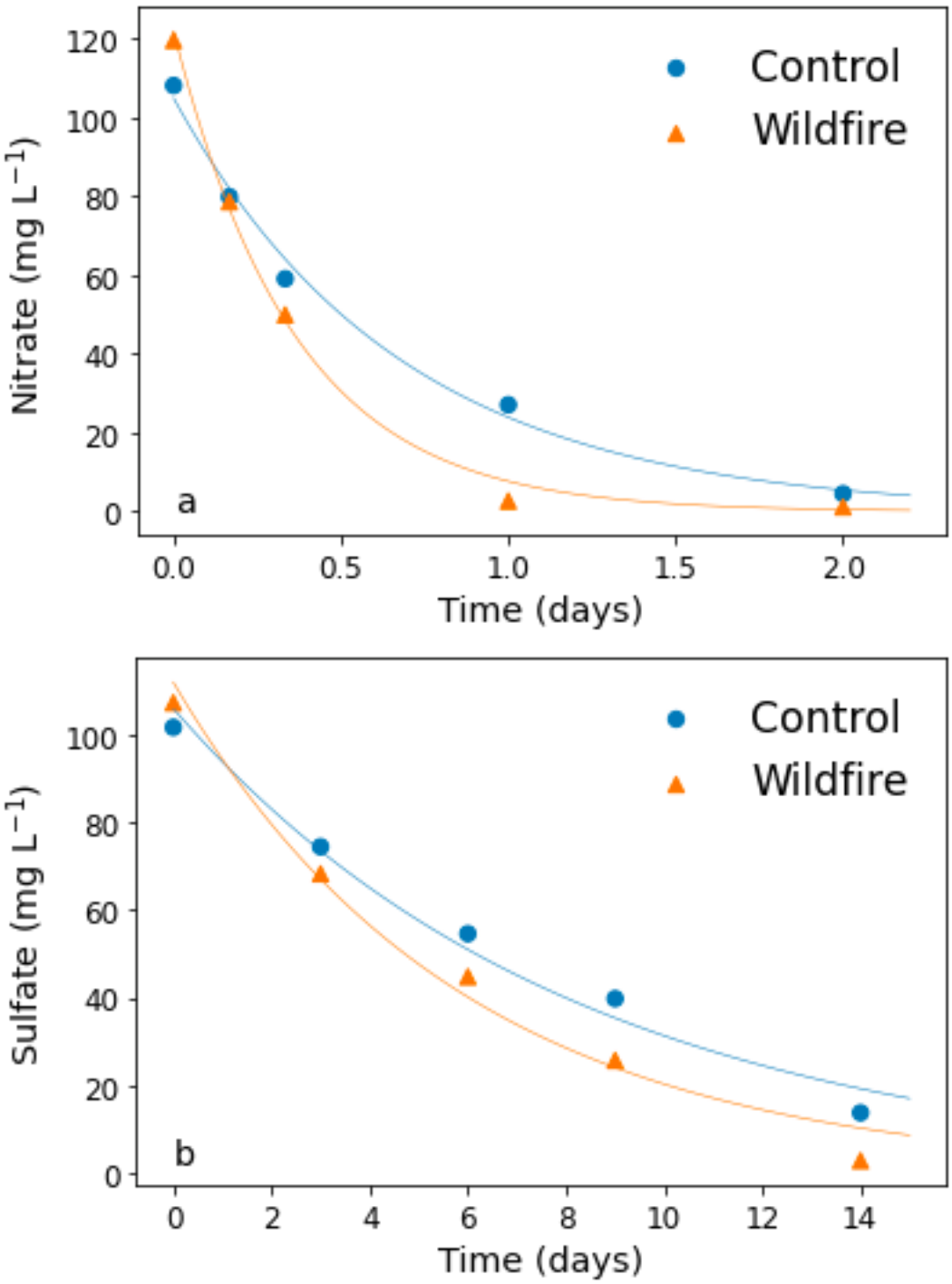


Figure 3: Example of kinetics of nitrate (a) and sulfate (b) reduction in batch experiments. It is observed here that nitrate reduction is a function of exponential decay, and the wildfire batches depleted nitrate faster than the control batches. Sulfate reduction also follows exponential decay and is accelerated in wildfire affected batches.

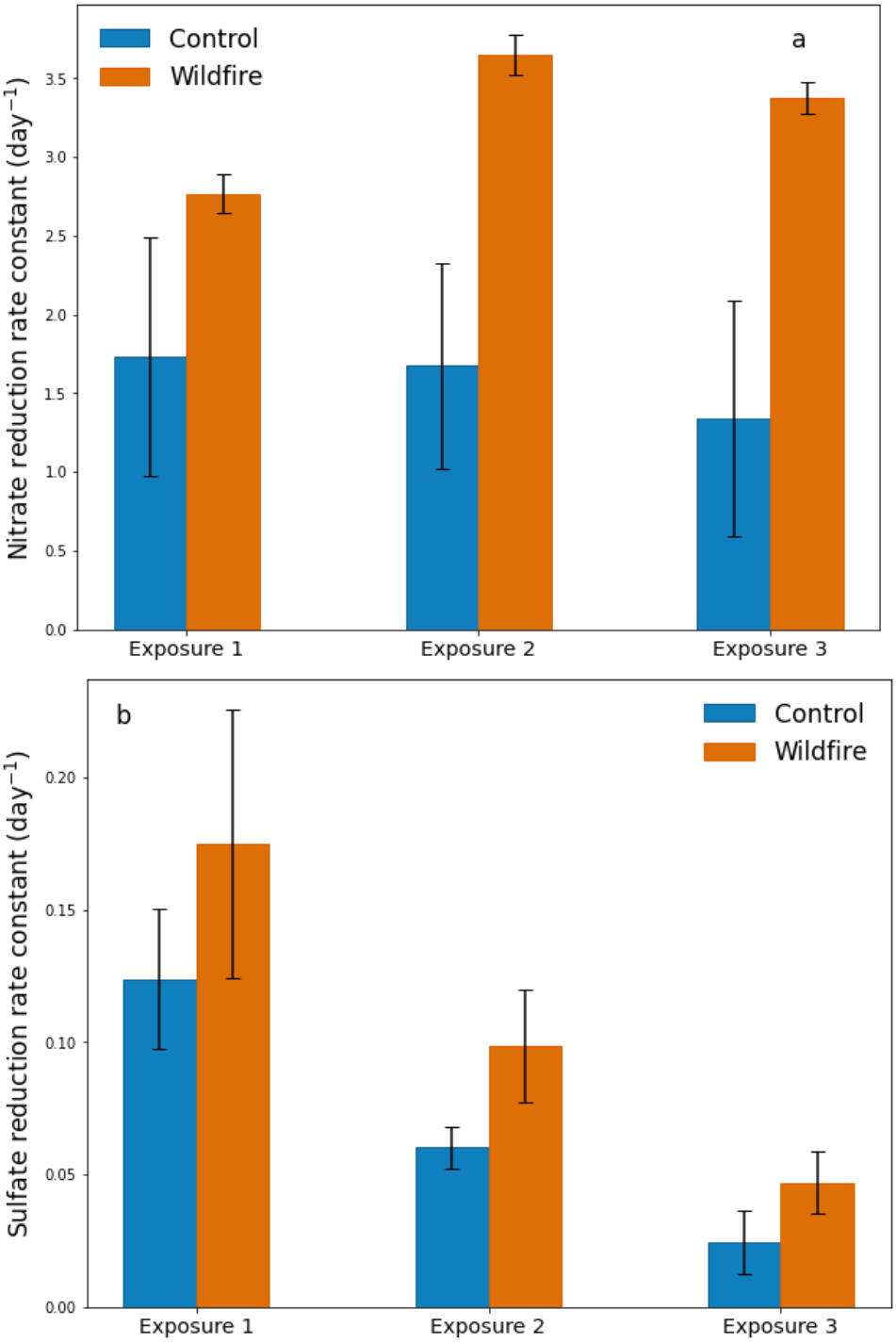


Figure 4: The decay constants of each trial of nitrate (a) and sulfate (b) reduction for each batch as well as error bars representing 1 standard deviation. Despite high standard deviations in the control batches due to some of the batches exhibiting different nitrate reduction rates, the wildfire batches had consistently and significantly higher nitrate reduction rates. Furthermore, it is observed that the k-values remained similar over each spike with no clear pattern of a change in rate following each exposure to nitrate pollution. For sulfate, it is observed that although the

rate of sulfate reduction becomes slower after each spike, wildfire batches consistently have higher k-values than control batches.

3.2 Effect of wildfire residues on organic carbon

SUVA254 analysis revealed that the aromaticity of the dissolved organic carbon in the wildfire batches is higher than in the control batches (Figure 5). In the wildfire and control batches, the aromaticity initially increased during the experiment, implying that aliphatic carbon was depleted at a faster rate.

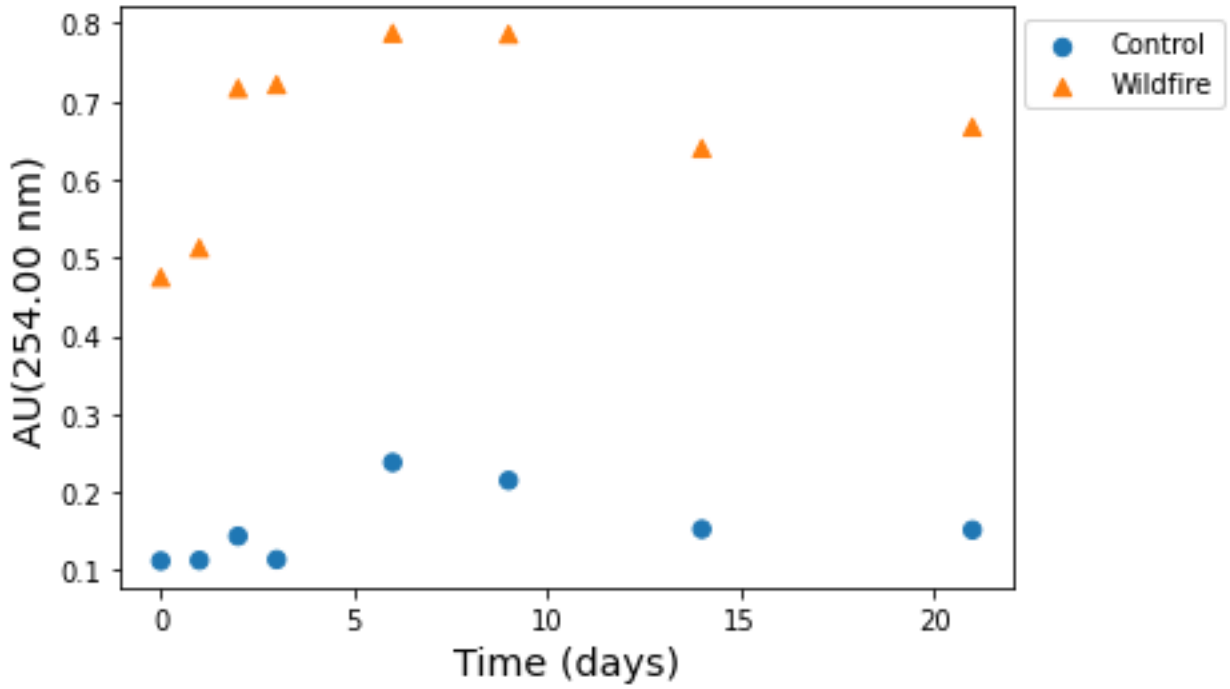
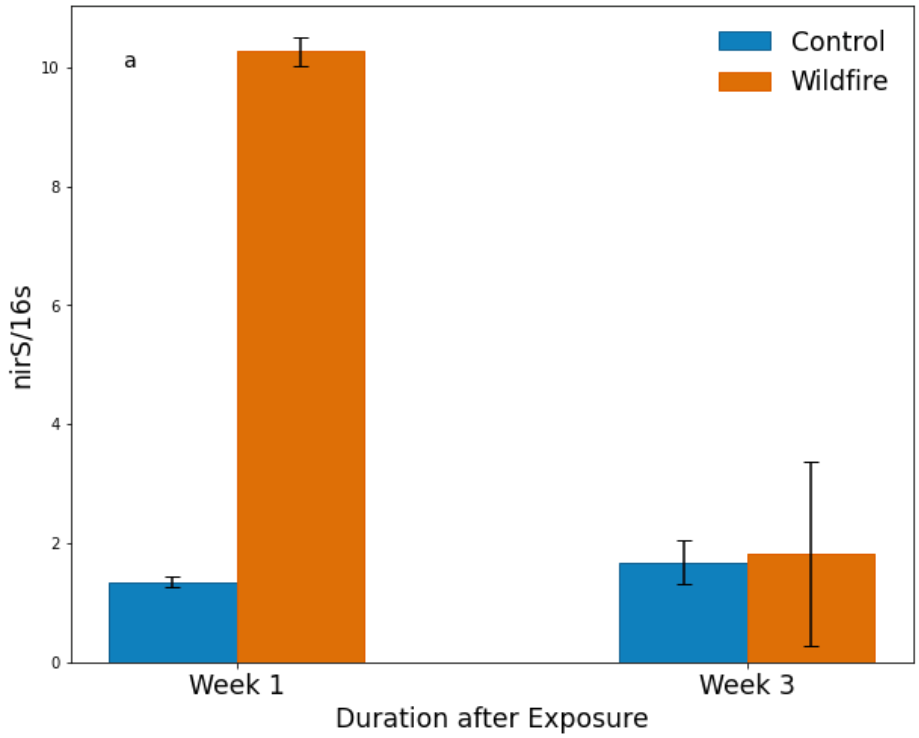


Figure 5: SUVA254 measurements of dissolved organic carbon (DOC) over time in wetland microcosm batches. Higher AU(254.00 nm) indicates a higher aromatic:aliphatic ratio of organic carbon in pore water.

3.3 Effect of wildfire residues on the abundance of functional genes

Wildfire residues affected the relative abundance of denitrifying genes in the field experiments (Figure 6). The first gene tested was *nirS*, which is responsible for the reduction of

nitrite (NO_2^-). Initially, a spike in the relative abundance of *nirS* was observed in the first week of the field experiments. However, at 3 weeks, the relative abundance was no longer significantly different between the two batches with and without wildfire residues (Figure 6a). While the relative abundance of *narG*, which is responsible for the conversion of nitrate to nitrite, spiked at one week, the difference was insignificant by 3 weeks. Therefore, upon initial exposure to wildfire residues, denitrifying gene abundance increased, but as other redox processes begin to take over, the spike in abundance is no longer observed. Due to issues with the *dsrAB* assay, it is unknown whether wildfire residues have a significant effect on the relative abundance of sulfate reducing genes.



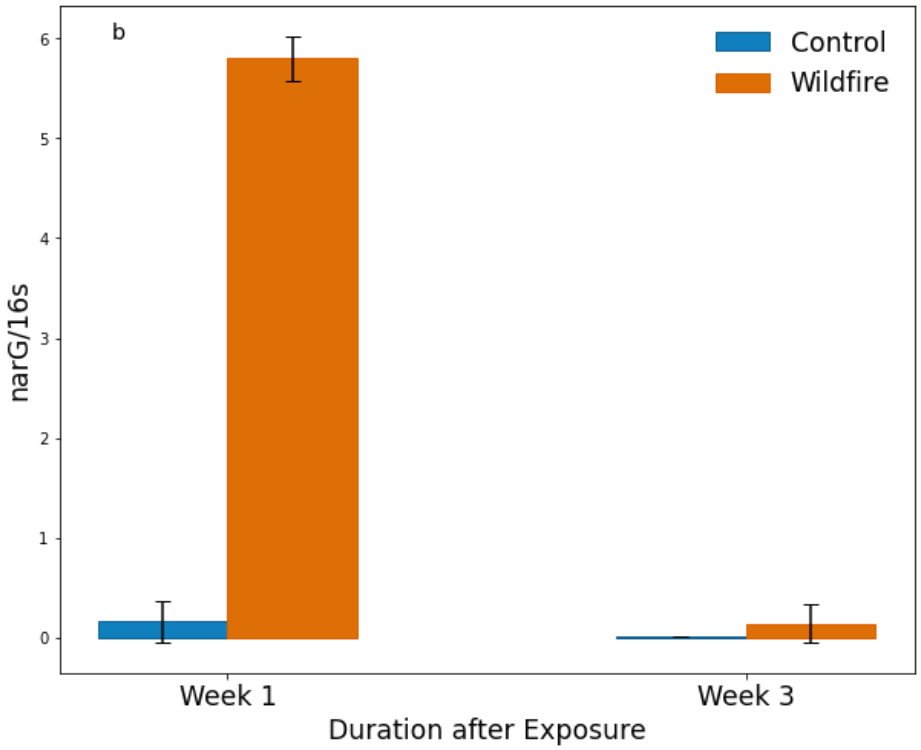


Figure 6: Effect of wildfire residues on the relative abundance of denitrifying genes. Genes were measured using qPCR and a standard curve method and normalized to 16s copy numbers.

In the field, 16s abundance in the presence of wildfire residues initially was lower that the control (Figure 6), implying exposure to wildfire residues could reduce the overall microbial populations initially. However, by 3 weeks, the difference in overall population was insignificant between control and exposed sediments (Figure 7). This evidence suggests that the microbial community may be resilient following 3 weeks of initial exposure to environmental pollution.

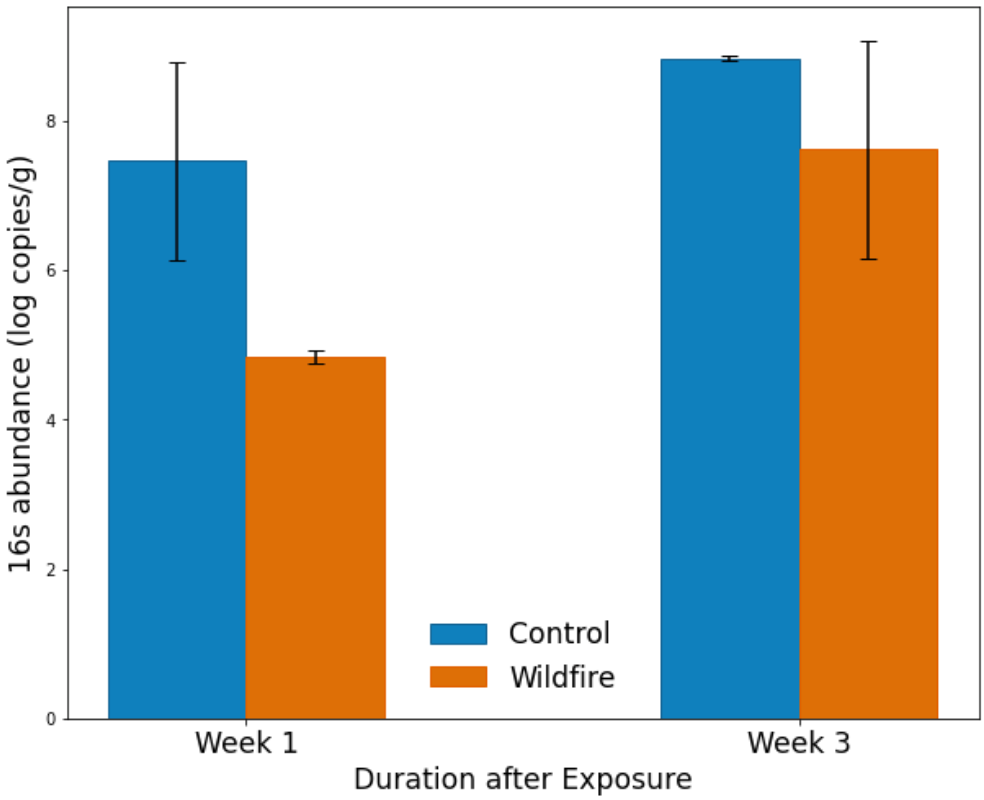


Figure 7: 16s abundance in the field over time. Universal 16s primers were used that target both bacterial and archaeal 16s genes.

4. Discussion

4.1 Reasons for increased rates of nitrate reduction in the presence of wildfire residues

Previous studies have shown that deposition of nitrate and nitrite due to wildfire can increase the abundance of denitrifying enzymes, therefore speeding up denitrification (Liao *et. al.* 2013). Our result confirmed the finding. Nitrate reduction was higher in the presence of wildfire residues than the control without wildfire residues. One of the major factors affecting denitrification rates is the concentration and quality of DOC. Wetlands are an ideal environment for nitrate removal due to them having an abundant source of organic carbon (Martinez-Espinosa *et. al.* 2021). Wood ash could have beneficial effects to nitrate reduction, but with the drawback that they can contain toxic metals (Bieser and Thomas 2019). As wetlands are an anoxic environment and already conducive to denitrification (Martinez-Espinosa *et. al.* 2021) and high nitrate concentration was administered through artificial spiking, organic carbon became the limiting substrate. Previous studies have shown that in soils with low DOC concentration, denitrifying enzyme activity may be inhibited (Zhou *et. al.* 2021). SUVA254 analysis revealed that the aromaticity of DOC was higher in the wildfire batches (Figure 5) indicating preferential consumption of aliphatic DOC, which is favorable for denitrification (Sirivedhin *et. al.* 2006).

4.2 Reasons for increased rate of sulfate reduction in the presence of wildfire residues

Sulfate reduction was consistently faster in batches that had received wildfire residues. When DOC is not limiting, and depending on the source of DOC, the bioenergetics of sulfate reduction become more favorable in sediments (Frank et. al. 2015). Over subsequent spikes, although the wildfire batches were consistently faster than the control batches, the rate of sulfate reduction got slower each time (Figure 4b). This could be due to the depletion of organic carbon,

as sulfate reduction becomes more unfavorable over time. If organic carbon is not able to be broken down, it becomes less usable by sulfate reducing microbes (Chen et. al. 2016). Furthermore, as redox conditions change over time in the batches, sulfate reduction may become less favorable as processes further down the redox ladder begin to become prominent. For example, the increasing abundance and activity of methanogens over time may have increased each round of spiking, competing with sulfate reducers and slowing them down. Previous studies have shown that sulfate-reducing bacteria can compete with methanogens for available H_2 which could affect the efficiency of both processes (Zhao and Zhao 2022).

4.3 Microbiome shift following deposition of wildfire residues

The results from qPCR microbial analysis show an elevated abundance of nitrate-reducing gene *narG* and nitrite-reducing gene *nirS* in the wildfire batches in the first week of field experiments. However, at 3 weeks into the field experiments, the relative abundance of both genes is no longer significantly different between control and wildfire batches. As nitrate is the first terminal electron acceptor to be reduced in anoxic environments, it likely ceases to be the dominant process in wetlands (O'Geen et. al. 2010). Batch experiments showed that between 75% to over 99% of nitrate was removed within 48 hours even following spikes of high nitrate concentrations (Figure 3a). A previous study suggests that around the 5th day, sulfate reduction starts to become an important process in the redox tower (Webster 2015). Previous studies have shown that right after a fire event in a wetland, nitrate-reducing enzymes are elevated in the first few days, but return to their original levels within a month of sampling the same site (Liao *et. al.* 2013). Another study tested the effects of copper nanoparticles (CuNP) on denitrification rates in wetlands, and found that after 10 days, denitrification was hindered but after 100 days, it was

similar to wetland samples that had not been exposed to CuNP (Reyes *et. al.* 2018). This implies that either way, the denitrifying community tends to revert to its natural state following exposure to environmental disruptions.

The qPCR analysis of 16s rRNA revealed that after a week of exposure to wildfire residues, the overall microbial abundance was decreased in the wildfire batches, but by 3 weeks, the wildfire batches had recovered to a similar microbial abundance to the controls. This is in line with a previous study that showed that wildfire residues killed FIB in water, but could also have implications for the microbial community at large, particularly microbes that serve beneficial functions (Valenca *et. al.* 2020). Therefore, even though the relative abundance of denitrifying genes was higher in the wildfire batches, the overall abundance was still lower due to the initial die-off of microbes in the field. However, gene abundance and expression are not necessarily a perfect indicator of enzyme activity, as increased substrate such as bioavailable DOC can increase enzyme efficiency without affecting gene abundance or expression (Glanemann et. al. 2003). Therefore, the denitrifying enzymes in the wildfire batches may have been functioning at a higher rate despite the toxicity to microbes incurred by the wildfire residues.

4.4 Environmental Implications

Increased nitrate removal from aquatic systems is generally beneficial as excess nitrate can drive eutrophication and cause health risks in drinking water. However, incomplete denitrification can lead to emissions of the greenhouse gas N_2O. Following wildfire events, more N_2O is released to the atmosphere and can cause a feedback loop (Niboyet *et. al.* 2011). A combination of global environmental change and wildfires lead to these emissions. Denitrifying

bacteria are responsible, as anoxic conditions, higher nutrient availability, and increased levels of CO_2 are favorable (Niboyet *et. al.* 2011). Therefore, incomplete nitrate reduction may be a concern for climate change following repeated exposure to polluted stormwater flows.

Although sulfate can cause health risks in water, sulfide is a bigger problem as it can cause issues with taste and odor as well as corrode pipes (Chen *et. al.* 2016). Since sulfide is a major product of sulfate reduction, it is essential to ensure that high levels of sulfide do not accumulate in pore water. As such, due to the high levels of sulfate leaching from wildfire residues, sulfide accumulation could be a concern. Carbon-rich plant matter and iron filings in wetlands are key in allowing S to be used as both an electron donor and acceptor and sulfide will not accumulate in pore water (Chen *et. al.* 2016).

5. Conclusions and Recommendations

This study shows that deposition of wildfire residues accelerates the removal of nitrate and sulfate in wetlands. Thus, wetlands can provide a buffer to absorb excess nitrate and sulfate released from wildfire residues or typically present in stormwater runoff. Mechanistically, this likely owes to the fact that wildfire increases the DOC, such that carbon substrate is no longer limiting. The overall microbial abundance is lowered initially by wildfire residues but it recovers in 3 weeks. Denitrification genes are initially in a higher relative abundance in wildfire-affected field studies, but by 3 weeks, the differences between wildfire and control batches are no longer significant. This aligns with the observations that denitrification is accelerated in wildfire batches and that it is no longer an important process in wetlands after about 5 days, when processes lower in the redox tower begin to take over.

Overall, this study shows that nitrate and sulfate reduction are accelerated in wildfire ash-laden runoff affected wetland microcosms, and this effect is associated with increased organic carbon and a higher initial abundance of denitrifying genes. This could have implications for the management and maintenance of natural and constructed wetlands as means to capture and treat stormwater runoff. However, future mechanistic studies could link chemical changes occur after wildfire deposition to the biological response. Future study could be to actively track methylmercury formation in wetlands to examine if elevated concentrations of methylmercury are found in wildfire-affected wetlands. This data could be used to model possible bioaccumulation of methylmercury up the food chain due to deposition of wildfire pollution into watersheds.

It could be of interest to study more biological effects of wildfire residues in wetlands. One study could look into the entire microbial community through phylogenetic analysis to see if the composition, richness, and diversity of the microbial community changes over time. This could have implications for the balance in wetlands maintained by microbial processes.

6. Appendices

List of peer-reviewed journal articles from the work:

1. Indiresan, S., Valenca, R., Raoelison, O.D., Das, T. Mohanty, S.K. et al. (202X). Deposition of wildfire residues in wetlands affects microbial cycling of nitrogen and sulfur. (*In Preparation.*)

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