Effects of enhanced monitored natural recovery of conventional heavy crude on biofilm and phytoplankton at the IISD-Experimental Lakes Area, Northwestern Ontario

By

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Abstract

The Freshwater Oil Spill Remediation Study (FOReST) evaluated the effectiveness and environmental impact of enhanced monitored natural recovery following simulated spills of conventional heavy crude oil into shoreline enclosures (5 x 10m) of a boreal lake. Six enclosures, equally divided into treatment and reference groups, were used in this study. Remediation included the flushing of trapped conventional heavy oil and recovery with sorbent pads, and a secondary remediation method referred to as enhanced monitored recovery (eMNR), which includes the addition of nutrients to stimulate microbial and algal oil-degrading activity. Effects were then studied on phytoplankton, biofilm growth and community dynamics over 400 days. Chlorophyll a concentration, ash-free dry mass (AFDM) and algal taxonomy were not significantly different between the treatment and reference enclosures. Therefore, we concluded that the eMNR secondary remediation treatment did not affect the phytoplankton and biofilm community but continued monitoring for eutrophication should be conducted to reduce the risk of environmental degradation.

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CHAPTER 1

1.0 Introduction

The oil and gas industry is the major energy producer in the world far eclipsing other types of power and energy generation (Smil 2003). Oil and gas and their derivatives are used as the primary fuel for power plants and the major power source for vehicles and will remain so until the decarbonization of power generation can occur, potentially in the coming generation (To et al., 2017, Zheng et al., 2019).

Oil plays a pivotal role in Canada's economic landscape, contributing significantly to the country's gross domestic product (GDP) and employment rates (Rooney et al 2012). Alberta's oil sands, one of the largest reserves of oil in the world, have been a major driver of economic growth (Irvine et al 2014). The extraction and processing of oil sands provide direct employment opportunities, stimulate related industries, and generate substantial government revenue through taxes and royalties (Aggarwal et al 2017). Canada relies on its oil resources to meet domestic energy demand (Strigham 2012). This self-sufficiency is crucial for maintaining a stable and resilient energy supply, reducing dependency on foreign sources, and mitigating the impact of global energy market fluctuations (Strigham 2012).

While the economic benefits of Canada's oil industry are clearly significant, it is not without its challenges and concerns. The transportation of oil by rail and pipelines carries the risk of oil spills. In 2020, the Transportation Safety Board of Canada reported that from 2010 to 2019 there were 124 pipeline safety occurrences per year (Government of Canada, Transportation Safety Board of Canada, 2021). An example of a pipeline

accident was the Enbridge line rupture of July 2010, where about 3.2 million litres of diluted bitumen was spilled into Talmadge Creek a tributary of the Kalamazoo River in Michigan which cost over $1.2 billion USD for cleanup (Kalamazoo River Oil Spill | SaBIN Center for Climate Change Law, 2015).

Rail transport is not safer than pipeline transportation of oil “In fact, the Transportation Safety Board of Canada statistics show that rail transport of oil is 4X more likely to spill oil, normalized to distance travelled and oil transported, than pipelines” (Safety in the Transportation of Oil and Gas: Pipelines or Rail? (fraserinstitute.org) with reported accidents such as the Canadian Pacific Railway Company train derailment in Guernsey, Saskatchewan where 20 tank cars released approximately 1.77 million litres of crude oil into the environment on the 9th of December 2019 (Government of Canada, Transportation Safety Board of Canada, 2019). These spills can have serious deleterious effect on the ecological functions and aesthetics of aquatic environments (Kammoun et al., 2020). Because all methods of transportation of crude oil carry a risk of a spill, the importance of oil spill response preparedness is critically important.

1.1 Physical and Chemical Properties of Oil

While there are different types of crude oil products, Canada primarily produces two products conventional heavy crude oil and diluted bitumen (CAPP, 2018). Diluted bitumen (dilbit) is a mixture of bitumen, a heavy viscous product with lighter crude oil or natural gas to produce a less viscous product, that can readily flow through pipelines (EC, 2013). This mixture is primarily transported from the Alberta Athabasca oil sand deposit to

refineries on Canada's east coast, into the United States and across the ocean to overseas refineries (Speight 2009). Dilbit can also be transported in trucks and rail to markets or refineries for further processing. Other types of crude oil, which are less viscous than dilbit are also extracted and transported by various means without the need for dilution and can also lead to increased potential for spills (Zhong et al., 2016).

Conventional heavy crude oil sometimes referred to as "extra heavy oil," is characterized by its high viscosity and density (Meyer et al., 2013). API gravity is a measure used in the oil industry to determine whether a liquid, such as crude oil, is "light" or "heavy" relative to water. Expressed in degrees (°API) on a scale developed by the American Petroleum Institute, it helps identify how easily the oil can flow and how suitable it is for refining. A liquid with an API gravity greater than 10 is lighter than water and will float, while one with an API gravity below 10 is heavier and will sink (USGS 2015). To understand the API gravity scale, water is used as the baseline, which has an API of 10. Light crude oils, such as West Texas Intermediate (WTI), have an API gravity of approximately 40°API. These oils are lightweight and flow quickly, making them ideal for refineries due to their efficiency in creating high-demand products such as petrol and diesel. Medium crude oils, with API gravities ranging from 20°API to 30°API, are heavier but still suitable for processing. Heavy crude oils, such as those from Venezuela, have an API gravity below 20°API and are substantially thicker, making them more difficult and expensive to refine. At the low end of the spectrum, extra-heavy crude or bitumen, such as oil from Canada's oil sands, frequently has an API gravity below10°API (USGS 2015).

Major deposits of heavy crude oil are found in Canada (Alberta's Oil Sands), Venezuela (Orinoco Oil Belt), Mexico, and parts of the Middle East. Of these, the largest

known reserves are in Venezuela and Canada (US Energy Information Administration 2001). Heavy crude oil is typically characterized by a high concentration of heavier hydrocarbons (asphaltenes and resins) and impurities (sulfur, nitrogen, and heavy metals) (Babalola & Susu 2019). Its high sulfur content leads to more greenhouse gas emissions than lighter oils, making it less environmentally friendly (Maroto-Valer 2002).

Crude oil is a mixture of numerous compounds such as alkanes, alkenes, sulphur, nitrogen, oxygen and some metals (Overton et al. 2016) Among aromatics, polycyclic aromatic compounds (PACs) are the primary drivers of toxicity in aquatic environments because of their persistence and propensity to bioaccumulate (Abdel-Shafy & Mansour 2016). When PACs are metabolized in mammalian cells, they produce substances such as epoxides which are both carcinogenic and mutagenic (Pashin & Bakhitova 1979). Aquatic invertebrates are also affected by elevated PAC levels, including narcosis in *Daphnia* sp. (Sverdrup et al., 2001) and increased mortality in *Hyalella* sp. (Gauthier et al., 2015). Benthic community composition can change with an increase in tolerant macroinvertebrate taxa (Culp et al., 2018). In biofilm, chlorophyll levels decline with exposure to hydrocarbons such as PACs in diesel (Nayar et al., 2004).

1.2 Oil Spills

Crude oil spills pose significant challenges across multiple dimensions, including environmental, social, economic, and aesthetic (Babatunde 2020). Oil spills represent a significant environmental hazard with the potential to cause extensive damage to marine, freshwater, and terrestrial ecosystems (Ivshina et al., 2015). Spills, involve the uncontrolled release of crude oil and petroleum products and are a form of pollution that can have both immediate and long-term environmental impacts. Prolonged environmental impacts may encompass the disruption of food webs and enduring damage to sensitive habitats (Das & Chandran, 2011). Simultaneously, the social repercussions of oil spills could be profound. Such incidents can result in the displacement of communities, especially in regions where livelihoods are intertwined with the affected environments, such as fishing communities (Babatunde, 2020). Moreover, health complications can emerge from exposure to the toxic components of crude oil (Aguilera, et al., 2010).

Economically, oil spills can cause significant economic losses. These include the cost of cleanup operations, tourism decline, downturns in local industries such as fishing and agriculture, and potential legal costs associated with the spill (Isidiho et al., 2020). Long-term economic impacts can be substantial, particularly in areas where the local economy is heavily reliant on natural resources (Cohen, 2010). An example is a report that estimated that a potential rupture of Enbridge line 5 in the straits of Mackinac in Michigan would cause $5.6 billion USD in damage to natural resources, tourism, fisheries, coastal property, and municipal water systems (Richardson & Brugnone 2018). The famous Deepwater Horizon oil spill over a 10-year period (2010 – 2020) caused a loss of 25,000 jobs and $2.3 billion in lost industrial economic activities (Court et al., 2020).

Furthermore, crude oil spills can have a considerable aesthetic impact. They can lead to visual pollution of natural landscapes and water bodies, affecting tourism and the general enjoyment of the environment by local communities and visitors (Ritchie 2014). In conclusion, the impact of crude oil spills is multifaceted and far-reaching, affecting various aspects of society and the environment. Specific impacts can vary depending on the scale of the spill, the environment in which it occurs, and the response to the spill.

1.3 Shoreline Cleanup Assessment Technique (SCAT)

Shoreline Cleanup and Assessment Technique (SCAT) is a procedure used to gather data for use in decision-making in shoreline oil cleanup by various stakeholder to coordinate and communicate their efforts in oil spill response and remediation (Environment and Climate Change Canada SCAT Manual 2018). The procedure was developed after the 1989 Exxon Valdez oil spill disaster in Alaska to assess the effect of the spill and to determine the best method to treat the affected shoreline (Shoreline Cleanup and Assessment Technique (SCAT) | response.restoration.noaa.gov, 2023). To initiate the procedure, an SCAT team includes representatives from all government tiers, the company that caused the spill, local agencies, and other stakeholders (Environment and Climate Change Canada SCAT Manual, 2018). The team conducts an affected shoreline survey before cleanup to choose the best method for a particular scenario and then monitors the effectiveness of cleanup to ensure that the results meet the agreed standards without risking further damage to the environment (Environment and Climate Change Canada SCAT Manual 2018). SCAT can also be integrated with other tools and technologies, such as geographic information systems (GIS), remote sensing, and environmental sensitivity mapping to enhance the quality and accessibility of the collected

data (Shoreline Cleanup and Assessment Technique (SCAT) | response.restoration.noaa.gov, 2023).

1.4 Oil Spill Treatment Methods

When a spill occurs, the clean-up method used varies depending on the environment where the oil was spilled, the type of oil, and the volume of oil spilled. Depending on the above factors oil spill cleanup can be subdivided into three different methods: 1) physical oil recovery, 2) chemical remediation, 3) and biological remediation.

1.4.1 Physical/Mechanical Oil Recovery

Physical remediation strategies play a pivotal role in the management of oil spills but are labour-intensive and very expensive requiring the movement and training of cleanup personnel and the use of heavy machinery (Dhaka & Chattopadhyay 2021). These strategies often involve the removal of contaminated materials or the containment of pollutants, aiming to physically remove or isolate the oil from the environment, thereby mitigating its impact (Michel et al., 2013). Examples include the use of manual labour or heavy machinery to excavate contaminated soil and materials such as vegetation on the shoreline to a specialized landfill for oil disposal. Physical removal of oil involves the removal of a substantial part of the shoreline including sediments that are unoiled. The excavation of shoreline material also causes increased turbidity in the littoral zone, and combined with increased human activities could cause a drop in productivity of the shoreline for an extended time (Dicks et al., 2002).

When oil is spilled into water, the physical removal method mostly depends on the environment where the cleanup is performed (Lim et al., 2016). In aquatic environments

booms and floating barriers are used first to contain the spilled oil and to prevent it from spreading to important environmental components and infrastructure (Ventikos et al., 2004). After containment, a few methods can be used to recover the oil. Skimmers are devices designed to recover oil by skimming oil off the water surface (Laforest et al 2021). In-situ burning is where the contained oil is burned. Various types of sorbent materials can be used to absorb oil (Laforest et al 2021). Different materials can be used as sorbents. For example, natural substrates like peat, moss, vermiculate and clay or synthetic materials made from polyurethane, polyethylene and polypropylene plastics come in the form of pads, rolls, booms, and loose particulate (Sorbents| Emergency Response| US EPA 2017).

The effectiveness of these physical remediation methods, however, can generally be influenced by the conditions of the spill, including the type of oil, the state of the aquatic environment, and the weather (response.restoration.noaa.gov, 2022). The effectiveness of oil recovery is also heavily influenced by the “weathering” of oil, which refers to the chemical and physical changes that occur when the oil is exposed to the elements over time (Tarr et al 2016.,). Individually, each method also needs careful consideration before implementation. Skimmers are more effective when the oil is still on the surface of water and in-situ burning is only effective when the oil has not weathered and when a slick thickness > 2mm can be achieved (Naseri and Barabady 2014). Burning is usually carried out on the high seas due to the heavy air pollution immediately after burning. Sorbents are used for small spills in stable environments, such as shorelines (response.restoration.noaa.gov, 2022). Therefore, the selection and implementation of these methods require careful consideration of these factors.

Although physical remediation techniques can be effective in managing oil spills, they are often used in conjunction with other methods, such as chemical and biological remediation, to ensure a more comprehensive cleanup of the affected environment. This underscores the need for a multifaceted approach in oil spill management that leverages various strategies to enhance the effectiveness and scalability of remediation efforts.

1.4.2 Chemical Remediation

Chemical remediation strategies, particularly the application of dispersants play a pivotal role in the management of marine oil spills. Dispersants are primarily used in offshore marine environments and are composed of surfactants and solvents that function to reduce oil into smaller droplets, thereby preventing the oil slick from reaching the shoreline and increasing the surface area of the oil exposed to microorganisms for degradation (Mapelli et al., 2017). Dispersants can be classified into three generations the first set of products, released in the 1960's, were similar to degreasers and industrial cleaning agents but due to their high aquatic toxicity they have been phased out (Elarbaoui et al 2022). The second-generation dispersants (Type 1) were designed for treating oil spills in the sea by spraying from a boat or low-lying aircraft (Chapman et al 2007). They comprise typically 15–25% surfactant and a hydrocarbon solvent with little to no aromatic component (Chapman et al., 2007). They were applied directly from the container because diluting them beforehand with seawater would make them ineffective. Additionally, a high dose rate (dispersant to oil) was needed (i.e.,1:1 or 1:3) (Chapman et al., 2007). While they were better than first-generation dispersants, they were still far more toxic than third-generation compounds. Type I dispersants are no longer in use in many countries (Chapman et al., 2007).

Third-generation dispersants consist of glycol and light petroleum distillate solvents combined with two or three surfactants (Elarbaoui et al 2022). The most often utilized surfactants are anionic (sodium alkyl sulphosuccinate) and non-ionic (fatty acid esters and ethoxylated fatty acid esters) (Tadros 2013). Type II and Type III dispersants belong to the third generation of dispersants. Type II dispersants need a high dosage of 2:1 to 1:5 (dispersant/water mix to oil) to be effective (Chapman et al 2007). They are usually diluted with seawater before usage, usually around 10% dispersant (Chapman et al 2007). Although they can also be employed from vessels, Type III dispersants do not require diluting and are principally designed to enable effective application from aircraft at dosage rates from 1:5 to 1:50 (Chapman et al 2007). The most widely accessible dispersants now are Type III, third-generation dispersants (Chapman et al 2007).

The application of chemical dispersants, such as Corexit® 9500 a type III dispersant, can have detrimental effects on marine ecosystems. For instance, research on the fire coral *Millepora alcicornis* has shown that exposure to Corexit 9500 negatively impacted the host physiology by causing bleaching and a significant change in the host bacterial community (Silva et al 2021). A study also showed that oil-dispersant mixtures cause changes in the community structure of biofilm communities with *Mougeotia* and *Oedogonium* dominating in an experimental mesocosm compared to a control (Scott and Glooschenko 1983).

1.4.3 Biological Remediation

Despite the effectiveness of chemical remediation methods for increasing the bioavailability and surface area of oil, the ultimate degradation of hydrocarbons must be facilitated by microorganisms. Microbial consortia, comprising bacteria, filamentous fungi,

and yeast, can help mitigate the impacts of oil, substantially degrading the polycyclic aromatic and n-alkane fractions and maintaining the physiological integrity of marine organisms (Das & Chandran 2011). Therefore, one of the most promising methods of spill cleanup is bioremediation, a minimally invasive and environmentally sustainable approach that leverages the inherent capabilities of microorganisms to degrade pollutants into less harmful substances (Azubuike et al., 2016).

However, the efficacy of bioremediation is not without its limitations. The success of this approach is heavily influenced by a variety of environmental factors, which can complicate its large-scale application. To address these challenges, researchers have explored two primary strategies to enhance bioremediation: biostimulation and bioaugmentation.

Biostimulation involves the modification of environmental conditions to stimulate the activity of indigenous pollutant-degrading microorganisms. The best-known approach is the addition of nutrients to speed up the growth of petroleum-degrading microorganisms (Adams et al 2020). This method may alter the balance of the ecosystem by increasing the number of microorganisms or other competing organisms over the carrying capacity of the environment (Adams et al 2020). For example, the addition of nutrients to stimulate oil-degrading bacteria could cause eutrophication and subsequent algal blooms.

Bioaugmentation involves the introduction of specific strains of pollutant-degrading bacteria into a contaminated environment to break down crude oil (Adams et al 2020). This method can be used where the local population of organisms cannot break down crude oil on their own. However, this practice introduces a new potentially invasive species into a new environment.

1.5 Biofilms:

Assemblages of bacteria, fungi, algae, and protozoans have been referred to by many names. The term periphytes was proposed by Zobell and Anderson in (1936) to describe them in their study of bacteria in stored seawater. In his book *Fundamentals of Limnology* published in 1953, limnologist Franz Ruttner used the name aufwuchs which referred to surface growth and the mass of organisms we presently call biofilm. (Ruttner, 1953). Biofilms differ only slightly from biofilm but biofilm and biofilm are mostly used interchangeably. Biofilms defined by Wetzel (1983) are assemblages of bacteria, algae, fungi, and protozoa within a protective matrix of extracellular polymeric substances and detritus, which colonize submerged surfaces in lakes and rivers" while he defined biofilm as "an assemblage of freshwater organisms mainly composed of photoautotrophic algae, heterotrophic and chemoautotrophic bacteria, fungi, protozoans, metazoans, and viruses which grow upon a benthic substrate."

Biofilms are also given different names according to the substrate on which they grow because the substrate plays a significant role in determining the composition and structure of the attached community. (Romaní, 2004a). On rocky outcrops, biofilms are called epilithic and tend to have higher algal biomass compared to biofilms found on other substrates. Epilithic biofilm can have a total carbon percentage as low as 60% to as high as 90%. (Romaní, 2010). Episammic and epipelic biofilms refers to microorganisms found in sandy and muddy substrates, respectively. The biofilms in these ecosystems have high fungi and bacteria representation and play a primary role of breaking down organic matter

(Pusch et al., 1998; Romaní and Sabater, 2001). Epiphytic biofilms attach to living plants such as *Typha, Hydrilla* and *Lemna*. Biofilms not only grow on living plants but are also known to form relationships with the plants to which they are attached, a study by DeWolf et al., (2021) found a relationship between water lilies and biofilm where plants got defensive immunity against other microbes in exchange for carbon and oxygen needed for the growth of the biofilm. Epiphytic biofilms can facilitate nutrient cycling and provide organic compounds to the plants. In return, plants provide a substrate and exude compounds from their roots which are taken up by the biofilm (Wetzel, 1993; Heilman and Carlton, 2001).

Even within the same environment, biofilm community composition and diversity can vary depending on the kind of habitat (for example, benthic and hyporheic zone, living or decaying plant materials, suspended particles) (Wilhelm et al 2014). According to Brunke and Gonser (1997) and Romani (2010), different biofilm habitats have different substratum characteristics (organic or inorganic, surface area, stability), hydrologic conditions and temporal and spatial gradients (e.g., light, temperature, nutrients, oxygen). These factors are all likely to affect the structure and function of biofilms.

High microbial diversity frequently makes it impossible to obtain a thorough census of the microbial community in each environment (Logue et al., 2012). Numerous statistical methods have been created and used to evaluate the diversity of microbial communities but due to the differences in analysis methods, among analytical laboratories direct comparisons are not possible. However, it is generally accepted that Cytophaga/Flavobacterium/Bacteroides group make up a substantial part of biofilm communities. (Hall et al., 2012). Cyanobacteria are another prokaryotic group found in

biofilms and are known to be quite common in lake biofilm communities (Ylla et al., 2009). Algae from the eukaryotic kingdom, most frequently Bacillariophyta and Chlorophyta, make up a significant portion of biofilms that are exposed to light (Arnon et al., 2007; Battin et al., 2003; Wilhelm et al., 2014). Fungi, particularly Ascomycota, are significant participants in the degradation of wood and leaf litter and are a prominent structural element of biofilms growing on submerged organic waste (Das et al., 2007; Golladay and Sinsabaugh, 1991). Protists, such as flagellates, ciliates, and amoebas, can modify the form and function of biofilms and regulate their growth (Böhme et al., 2009; Bott and Kaplan, 1989). Biofilms can also be a significant viral reservoir in aquatic ecosystems, which may have an impact on biofilms by organizing community diversity and composition (Jackson and Jackson, 2008).

Assessing biofilm communities can provide valuable information for estimating the relative health of aquatic ecosystems (Minshall 1978). Algal biomass is affected by several factors in the aquatic environment, and some factors can have a positive effect on biomass, including increased nutrient loading, light penetration, and higher water temperatures. Other factors such as sediment resuspension, and toxicants can cause a decrease in algal growth rate and biomass among the population. Benthic algae, important components of biofilm, are especially important for assessing the health of aquatic systems (Stevenson and Smol, 2015). They occupy a unique niche in these environments due to their sensitivity to environmental changes and non-motility (Dam et al. 1994). Initially the use of algae as bioindicators focused on diatoms where richness and abundance matrixes of diatom species were used (Lambert et.al., 2008; Gaiser, 2009), but numerous novel studies have shown that considering other groups of micro

algae in the analysis improve the predictive capacity for assessing environmental health. (Leland and Porter, 2000; Fetscher et al., 2014).

There are several methods for assessing biofilm communities in relation to the environment. Algal biomass can be determined in the laboratory using assays of chlorophyll a, dry mass, ash-free dry mass, algal cell density and algal biovolume (Stevenson & Rollins 2017). Chlorophyll a is typically extracted with a solvent of acetone and methanol. The extracted sample is then analyzed using fluorometry, spectrophotometry or high-performance chromatography (HPLC) (Samways et al., 2015; Thomas et al.,2013). Chlorophyll-a should not be used alone to measure algal biomass due to issues such as degradation over time and blocking from other pigments and because non-photosynthethic algae are not accounted for by chl a measures. The complimentary measure, Dry mass and ash free dry mass are gravimetric measures of algal samples determined after drying and combusting the sample respectively that account for both photosynthetic algae and non-photosynthethic algae (Hamilton et al., 2005). Algal biovolume is determined using microscopic techniques where sizes of different cell types are measured, and the result is multiplied by the number of each cell type. The results are summarized according to the volume for each taxon as well as total biovolume for the community (Wetzel and Likens 1991).

The use of taxonomy is important for assessing stressor conditions in the environment. Differences in taxonomic composition between two different aquatic environments can help to identify different factors and effects operating in these environments. The presence and absence of certain species elucidate the effects of natural and anthropogenic effects in aquatic systems e.g., the presence of species such

as Spirogyra, Mougeotia which occur in low-nutrient systems compared to Cladophora and Oedogonium which are more prominent in high nutrient/eutrophic lakes (Stevenson and Pan 1999). Another taxonomic tool is the use of DNA metabarcoding where DNA is extracted from algal samples to identify the different taxa present in the samples. DNA metabarcoding is still a new technique for algal community analysis, and the development of additional reference libraries that can catalogue all algal species will improve the accuracy of the tool when used for taxonomic identification in aquatic ecosystems (Kress & Erickson).

1.5.1 Factors Affecting Biofilm Growth and Composition

1.5.1.1 Light

Light is one of the most crucial factors affecting the biomass of freshwater biofilm communities. Light is required for photosynthesis, which is a major pathway for energy production in freshwater communities. Without light, most ecosystems are unproductive, which is apparent in freshwater ecosystems where light and primary productivity are directly correlated (Cahoon and Nearhoof, 1999; Hill, 1996). Therefore, light availability determines the proportion of photosynthetic organisms in biofilms (Guash and Sabater 1995), and in some deep water, there is no photosynthetic representation in biofilms. (Lalli & Parsons, 1993). In lentic waters phytoplankton abundance can affect the presence of algae in biofilms by controlling the amount of light reaching the substrate resulting in a decrease in biofilm biomass and community composition with the dominance of low light tolerant species in the biofilm assemblage. (Hill 1996).

In lab conditions where light intensity can be adjusted, it has been observed that biofilms that become more structured and thicker can incorporate more carbon and

nutrients with a more robust EPS (Extracellular Polymeric Substance) than biofilms in darker environments. (Romani et al., 2004b). In these light conditions, microbes will mostly uptake organic matter from the algae in the biofilm assemblage than from the dissolved organic matter and particulate organic matter pool. The attached substrate also determines how much light the algae component can receive. On rocky substrates, light availability is affected by texture due to the increase in surface area in rough, rocky substrates. Light also penetrates less in cohesive sediment limited to the first 2mm of the sediment compared to more loose sediments (Kuhl et al., 1994). While light intensity is important to the growth of biofilms high light environment may lead to a reduction in photosynthetic ability. To adapt to this environment most algae produce carotenoids which help in capturing excess photons of light (Hill, 1996). Light intensity also affects the heterotrophic components found in biofilm communities. Research has shown that light specifically the ultraviolet spectrum limits microbial activities in epixylic biofilms, which grow on wood substrates (Denward et al., 2001).

1.5.1.2 Temperature

Water temperature regulates metabolic and enzyme activities and plays a key factor in biofilm growth and community diversity. It also plays a role in nutrient cycling and primary production (Brunke and Gonser, 1997; Ylla et al., 2014). For biofilms the optimal temperature for growth is between 10 to 30°C. At higher temperatures the biofilm community undergoes stress which reduces growth (DeNicola and Hoagland, 1996). Temperature also affects the richness of biofilm communities with microbes' richness higher closer to the equator compared to higher latitudes (Lear et al., 2009).

1.5.1.3 Nutrient

Nutrients are one of the most important growth components needed by benthic algae for growth. High availability of nutrients in the presence of light leads to algal blooms (Ylla et al., 2007). In biofilms which grow on rocky substrates both primary and secondary producers are affected differently based on nutrient supply. Nutrient levels affect metabolic levels in bacteria while affecting primary production levels in algae (Romani and Sabater 2000).

Biofilms in sediments are affected differently as sediments act as sinks for nutrients and can release them into the water column. They also help to remove excess phosphorous from the water column by temporary sequestration into the sediments (Wetzel,1996). In biofilms growing on dead plant matter high nutrient levels not only speed up biomass production but also aid in organic matter degradation up to a threshold (Woodward et al., 2012).

1.5.1.4 Dissolved Oxygen and pH

Oxygen levels help to modulate biochemical processes in biofilms such aerobic respiration, methane oxidation, anaerobic respiration, and fermentation in anoxic conditions (Brune et al., 2000). Biofilms are known to cause diurnal fluctuations in pH and oxygen in situations of high organic and inorganic pollution in aquatic environments, (Brunke and Gonser, 1997). Biomass and organic matter mineralization are higher in

aerobic compared to anaerobic conditions (Simon et al 1994). In episammic biofilms which grow in loose sediments (sand), oxygen levels decline with sediment depth affecting the biofilm metabolism (Brunke and Gonser, 1997). This causes a change in biofilm communities from aerobic microbes on the surface to anaerobic microbes in anoxic conditions (Brune et al., 2000). In biofilms which are attached to dead plant matter oxygen depletion affects fungal growth and decreases fungal diversity reducing the degradation of dead matter (Madeiros et al., 2009). Lower pH affects biofilms by aiding the removal of metals through precipitation and adsorption (Liehr et al., 1994). In low pH environments, organic matter decomposition is slowed due to the efficiency loss in pH-sensitive enzymes.

1.5.1.5 Waves

Wave action is an important physical factor in lentic waters because it can create turbulence to keep inshore waters from becoming thermally stratified allowing nutrient cycling. (Nybakken and Wallace, 1992). Waves help to control biofilm community diversity because shear stress allows only species such as filamentous algae that are adapted to thrive in turbulent conditions (Howell, 2009).

1.5.1.6 Grazing

One of the key factors modulating growth in biofilms is the presence of primary consumers that feed on biofilms (Thompson et al 2004). Herbivores such as zooplankton impact biofilms not only by feeding on them but also by dislodging biofilm cells from their substrate (Lowe, 1996). Herbivores that feed on biofilms include emergent insects' larvae, molluscs, crustaceans, and fish species. Any effects on these groups of organisms will have a corresponding effect on biofilm biomass (Lamberti 1996). Grazing also helps to

keep microalgal species prominent by reducing their succession by macroalgal species (Steinman, 1996).

1.5.2 Effect of crude oil on Biofilm

Due to industrialization and anthropogenic activities freshwater environments receive an influx of chemicals with the potential to cause effects at multiple trophic levels. One of the challenges is deciding how to assess the effects of pollutants so that regulators and policy decision-makers can act to protect valuable ecosystem components (Hering et al., 2010).

The biological composition of biofilms includes algal, bacterial, fungal, protozoan, and micro-invertebrates which includes many species in each group. Among all these groups algae play a major role in biofilm ecotoxicological studies (Corcoll et al., 2012a). The few existing studies have focused on microbes while other biofilm components have received little to no attention (Proia et al., 2012a).

Ecotoxicological studies of biofilms mostly include the addition of a grazer to model environmental conditions which allows effects to be modeled on both biofilms and grazers and to understand biomagnification of substances through the food web. (Geiszinger et al., 2009). Most of these experiments are performed in laboratory settings to ensure all other environmental factors are controlled (Ledger et al., 2009). This experiment type has been used to study the ecotoxicological effects of pesticides and emerging contaminants (Munoz et al., 2001; Kim et al., 2012).

Crude oil is known to cause toxic effects in biofilms by coating biofilm communities preventing photosynthesis, respiration and enzymatic processes. (Lewis & Pryor, 2013).

Oil is also known to cause declines in dissolved oxygen levels due to microbial activities which could impair the heterotrophic (secondary producers) components of biofilms slowing down their growth and causing changes in the makeup of the biofilm community (Overton et al., 2016).

However, studies have also been recorded specifically on polycyclic aromatic compounds (PACs) which cause the majority of the toxicity of crude oil. Diatom assemblages have been shown to undergo changes to frustule shapes and structure when exposed to the PAC fluoranthene in a microcosm study (Rimet et al., 2004). Pyrene another PAC causes phototoxicity in benthic algae at environmentally measured concentrations, especially in diatoms where silicate uptake is reduced (Petersen et al., 2008). PAC mixtures are also known to affect biochemical endpoints such as chlorophyll-a fluorescence, oxygen production, CO_2 fixation and nitrogen fixation in algae species (Buhari et al., 2023). All of these studies show that crude oil causes toxicity in biofilms affecting growth and community composition.

1.6 The Freshwater Oil Spill Remediation Study (FOReSt)

The Royal Society of Canada (RSC) published a report in 2015 highlighting knowledge gaps and the need for oil spill research in specific areas (Lee et al., 2015). Some of the high-priority gaps highlighted in the report included

- Research is needed to better understand the environmental impact of spilled crude in high-risk and poorly understood areas such as Arctic waters, the deep ocean and shores or inland rivers and wetlands.

- Research to understand the effects of oil spills on aquatic life and wildlife at the population, community and ecosystem levels

- Research to investigate the efficacy of spill responses and to take full advantage of spill of opportunity.

To address the knowledge gaps, the Freshwater Oil Spill Remediation Study (FOReSt) was designed to study the efficacy of minimally invasive oil spill remediation methods applied in freshwater shoreline environments (Palace et al 2021a). FOReSt performed simulated oil spills in a shoreline environment mimicking moderate to heavy oiling, conservative spill response times and clean-up procedures. The study took place in Lake 260 at the IISD-Experimental Lakes Area in Northwestern Ontario from 2017-2023 and comprised model oil spill studies of diluted bitumen (2019) and conventional heavy crude oil (2021), including recovery monitoring of the affected environments for > 400 days.

The project focused on the shoreline due to its sensitivity and high productivity in the aquatic environment. Model oil spills were contained within 5 x 10 m enclosures (Figure 1.0) consisting of floatation collars suspending polypropylene curtain walls that were sealed to the sediments with sandbags to contain shoreline environments and adjacent 20,000- 30,000 L aquatic environments.

Strips of the same material as the containment curtains were suspended inside the enclosures to allow biofilm colonies to establish themselves and so that we could collect biofilm samples periodically throughout the study. In 2021, the enclosures were treated with model oil spills of 1.5 kg CHV. After 72 hours of oil interaction with the shoreline, primary cleanup was carried out using freshwater flushing followed by collection of free-floating products using sorbent pads. After this primary cleanup was completed, secondary remediation consisting of eMNR, a non-invasive method using nutrients to enhance oil-degrading microbes, was carried out in the enclosed freshwater environments. Concentrations of PACs were determined for 73 days after the eMNR was applied.

The overall FOReSt project examined environmental effects at multiple trophic levels including biofilm and phytoplankton biomass and composition, zooplankton community composition, effect on amphibian growth and behaviour and effect on different fish life history stages. My specific project evaluated the potential effects of secondary remediation methods on biofilm growth and community dynamics as they serve as the base of the aquatic community and are also important bioindicators due to their non-motility and sensitivity to xenobiotics (Wetzel 1983).

This thesis, a subproject of the FOReSt project seeks to understand the potential effects of the oil and eMNR treatment on the biofilm communities that serve as an important primary producing community in the shoreline environment. Chlorophyll *a*, ash-free dry mass, abundance of cell density and richness of species were measured as indicators of biofilm community productivity and health and to characterize the effects of the treatment for potential use during real oil spill scenarios. The results of this project will provide critical information to stakeholders such as the Canadian Energy Regulator and spill responders on the efficacy of minimally invasive cleanup methods and their suitability for the freshwater environment.

1.6.1 Objective and Hypotheses

The main objective of this thesis is to evaluate the effects of conventional heavy crude oil spills and a secondary remediation method on phytoplankton and biofilms/biofilm within a freshwater boreal lake ecosystem. The study objectives will be addressed according to the following hypotheses:

H1 There will be a significant increase in biofilm biomass (chlorophyll-*a* and ash-free dry mass) and phytoplankton (chlorophyll-*a* in the eMNR (enhanced monitored natural recovery) treated enclosures in response to the external input of nutrients (Adams et al., 2020).

H2 There will be a significant difference in biofilm community structure with an increase in algal bloom species in the eMNR-treated enclosures.

CHAPTER 2

Abstract

The Freshwater Oil Spill Remediation Study (FOReST) was undertaken to bridge knowledge gaps in freshwater oil spill research. The study evaluated the effectiveness and environmental impact of Enhanced Monitored Natural Recovery following a simulated conventional heavy crude into shoreline enclosures (5 x 10m) of a boreal lake. Six enclosures, equally divided into treatment and reference groups, were used in this study. Remediation included the flushing of trapped conventional heavy oil and recovery with sorbent pads and a secondary remediation method using enhanced monitored natural recovery (nutrient additions to stimulate microbial and algal communities). Our objective was to check the effect of the treatment on biofilm productivity and community composition. Phytoplankton and biofilm chlorophyll *a*, ash-free dry weight, species abundance, species richness and dissimilarity index were measured in treated enclosures and then compared to reference enclosures. All Oiled+eMNR enclosures had no statistically significant change in chlorophyll a, ash free dry weight, algal species richness, algal abundance and dissimilarity index compared to the reference enclosures. These results show that the eMNR remediation could be viable for use in oil spill remediation and cleanup as minimal environmental impact was observed.

2.0 Introduction

The oil and gas industry is an important part of the Canadian economy with Canada producing 6% (5.83 million barrels per day) of the world oil supply (U.S Energy Information Administration 2023). The industry helps to ensure Canada's energy security and self-sufficiency (Strigham 2012, Higginson & Vredenburg 2012). However, there are concerns regarding the environmental effects of oil especially from spills arising from failures during drilling or during transport by rail, tanker and pipelines (Kammoun et al., 2020). When oil is spilled into an aquatic environment, it affects not only the aesthetic of the environment but also major ecosystem processes (Lee et al 2015). Oil spills contain toxic compounds that can affect all aspects of the ecosystem from primary producers to fish and aquatic birds (Lee 2015).

Despite strict rules and guidelines for the transportation of crude oil in Canada, incidents still occur regularly. For example, 124 pipeline safety incidents were recorded between 2010 to 2019 (Government of Canada, Transportation Safety Board of Canada, 2021). Therefore, understanding the ecological risks posed by oil spills, and how best to respond is imperative. One example is the release of 5000m^3 of dilbit by a pipeline owned by Nexen in Fort McMurray, Canada in 2015 (Lee et al., 2015).

Primary producers are an important part of aquatic ecosystems. They account for half of global primary productivity and serve as a direct food source for a significant proportion of higher-level organisms (Falkowski et al., 1998). Primary producers can be impaired by oil in the environment, putting ecosystem function at risk. Among primary producers Biofilm functions as a habitat and sanctuary for microorganisms and diminutive

invertebrates (DeWolf et al., 2021). The intricate structure creates microhabitats that promote biodiversity and serve as foraging areas. Additionally, it aids in the preservation of water quality by filtering pollutants, capturing sediments, and absorbing toxins, functioning as a natural biofilter. By restricting nutrient availability to phytoplankton, periphyton can mitigate hazardous algal blooms and preserve environmental equilibrium.

Besides its ecological roles, biofilm serves as a reliable bioindicator of environmental health (Wetzel 1983). It is acutely responsive to variations in water quality, including pollution, light availability, and temperature fluctuations, rendering it an invaluable instrument for monitoring aquatic ecosystems (Wetzel 1983). Biofilm contributes to carbon sequestration by absorbing carbon dioxide during photosynthesis, thereby influencing carbon cycling and impacting climate change mitigation (Ylla 2007).

Biofilm is essential for ecosystem production, nutrient cycling, habitat development, water quality maintenance, and environmental monitoring (Heilman and Carlton, 2001). In freshwater ecosystems, it can compete with phytoplankton for nutrients, affecting water purity and ecosystem productivity. In coastal regions, it sustains food chains by supplying nourishment for herbivores, which are subsequently consumed by higher trophic levels (Ylla et al., 2009). These functions highlight its ecological significance and its contribution to maintaining the health of aquatic environments. A study by Singh and Gaur (1989) showed that crude oil effluents from a refinery caused a reduction in chlorophyll-a and cell count of a biofilm community. Phytoplankton abundance and chlorophyll-a were also

reduced in mesocosm treated with diluted bitumen (Cedarwall et al., 2020). The reduction in growth of biofilm can cause a release of stored nitrogen and phosphorus into the water column increasing the incidence of pelagic eutrophication in the aquatic environment (Santos et al., 2018) and leading to a breakdown of the functioning of said ecosystem.

In addition to the direct impacts of oil on freshwater ecosystems, oil spill cleanup and remediation methods can also negatively affect productivity. One of the methods used in oil spill cleanup is physical/mechanical recovery where the oil and other oiled materials are excavated and removed from the impacted environment (Michel et al., 2013). Freshwater flushing can also be employed where oil is first washed from the shoreline and surrounding riparian vegetation and then captured from the surface of the water using oil-absorbent media (Laforest et al., 2021). But oil cleaning methods such as flushing can wash off benthic algae and grasses which help in maintaining stability on the shoreline (Pezeshki et al., 1999)

Chemical remediation involves the use of dispersant/shoreline cleaners that break down oil into smaller droplets so that the oil can either be captured or degraded *in situ* more readily (Mcfarlin et al., 2021). This must be used with caution because their method of action causes the oil droplet to dissolve in water increasing contact with aquatic biota (Almeda et al., 2013). Specifically, a study by (Harrison et al., 1986) found that a mixture of oil and dispersants harmed diatom growth while fueling growth in microflagellates such as chrysophytes. In Canada, dispersants are not registered for use in freshwater environments, but there is some debate about whether they can be useful for remediating oil-impacted shorelines (Palace et al., 2021)

The potential adverse impacts of physical and chemical remediation techniques have focused attention on the possibility of stimulating microbial communities to degrade oil in situ using minimally invasive approaches (Das & Chandran 2011). The process of applying biological remediation can be summarized in two major categories: a) biostimulation where nutrients are added to stimulate pre-existing microorganisms in the environment to speed up oil degradation and b) bioaugmentation where microorganisms are added to the environment in the absence of native microorganisms that can break down oil (Adams et al., 2020). However, biological remediation may have limitations. Biostimulation can contribute to eutrophication and subsequent harmful algal blooms (Adams et al., 2020). Bioaugmentation could introduce an invasive microorganism species into an environment sometimes causing deleterious effects to the ecosystem (Adams et al 2020).

To better understand the effect of minimally invasive oil spill remediation methods on abiotic and biotic components of a lacustrine shoreline environment e.g microbes, biofilm, zooplankton, fish, emergent insects, and amphibians (Palace et al., 2021a), we undertook the Freshwater Oil Spill Remediation Study (FOReST) (Palace et al., 2021a). This study examined the impacts of crude oil and remediation practices on the biofilm communities in shoreline enclosures treated with model oil spills. The secondary remediation used was a form of biostimulation called enhanced monitored natural recovery in which fertilizer containing nutrients such as nitrogen and phosphorous to stimulate oil-degrading bacteria. The objective of this study was to characterize the effects of eMNR on biofilm in a freshwater shoreline environment. The objective can then be broken down into these hypotheses:

H1 There will be a significant increase in biofilm biomass (chlorophyll-a and ash-free dry mass) in the eMNR (enhanced monitored natural recovery) treated enclosures in response to the external input of nutrients.

H2 There will be a significant difference in biofilm community structure with an increase in algal bloom species in the eMNR-treated enclosures.

The result of this study supports the advancement of policies and best practices for oil spill preparedness and response, ensuring that stakeholders, including government agencies, industry players, and environmental organizations, are better equipped to handle spill incidents. Finally, it contributes to public awareness and education, highlighting the importance of environmental protection and the need for sustainable practices in the oil and gas industry.

2.1 Methods

2.1.0 Study Location

The Freshwater Oil Spill Remediation Study (FOReST) was an enclosure-based research program conducted at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA) in Northwestern Ontario, Canada (49°40’N, 93°44W). Its purpose was to evaluate and compare methods for remediation of oil spills from affected freshwater shorelines and to examine the potential effects of these methods on biotic components of the aquatic environment. The studies took place on Lake 260,

(Figure 2.1) a small remote boreal lake that is one of the lakes designated for research by the Canadian Fisheries Act, Section 36(5.2) Experimental Lakes Area Research Activities. Lake 260 is an oligotrophic boreal holomictic lake with a surface area of 32.8 hectares, a total volume of 1975.971 m^3 and an average depth of 7.2 metres. The lake is remote and does not have any anthropogenic interactions apart from the research previously conducted on it (see Kidd et al 2007., Ankley et al 2021., Rodriguez-Gil et al 21) and atmospheric inputs. The components of the FOReSt study described here were performed from 29 May 2021 to 16 September 2021.

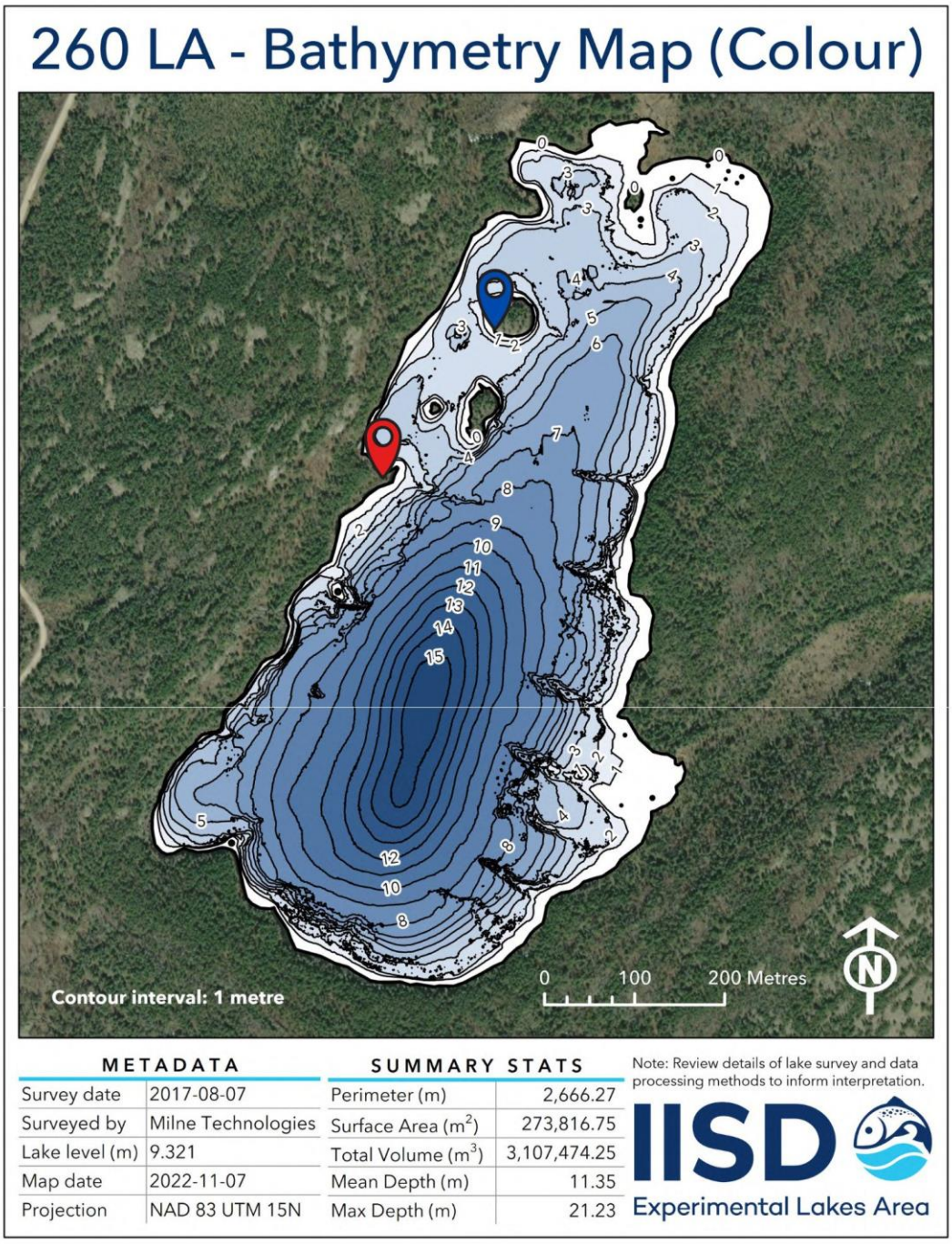


Figure 2.1: A bathymetric and physical attribute map of Lake 260, IISD Experimental Lakes Area (IISD 2023). 5 enclosures were located on an island north of the lake (Blue marker), while the last enclosure is located in a bay west of the lake. Permission for reprinting image provided by IISD-ELA.

2.1.1 Study Design

The study was conducted using rectangular (5 X 10 m) enclosures installed on wetland shorelines with soft organic sediments (Figure 2.2) similar to those described by Palace et al (2021). The enclosures were made by Curry Industries (Winnipeg, Canada) and consisted of a yellow flotation collar (8" x 8") and vertical polypropylene curtains attached to the collars that were sealed to the lake bottom with sandbags to prevent lake water- enclosure exchange. The depth at the deep end of the enclosure was 1.5 – 2 m deep with each enclosure holding a water volume of approximately 27 m^3. The shorelines behind the enclosures had low-lying vegetation with a forested background upslope of the shoreline (Figure 2.3). A total of six enclosures were deployed, three were used as treatment enclosures and the other three were left unoiled as reference enclosures. After oil treatments were applied and primary cleanup was completed enhanced monitored natural recovery (eMNR) was applied as a secondary remediation method. This non-invasive method consists of adding nutrients to stimulate microbes that can break down oil (Ankley et al 2021). The work in my thesis focuses on the response of the biofilm community within the context of the larger FOReSt study.

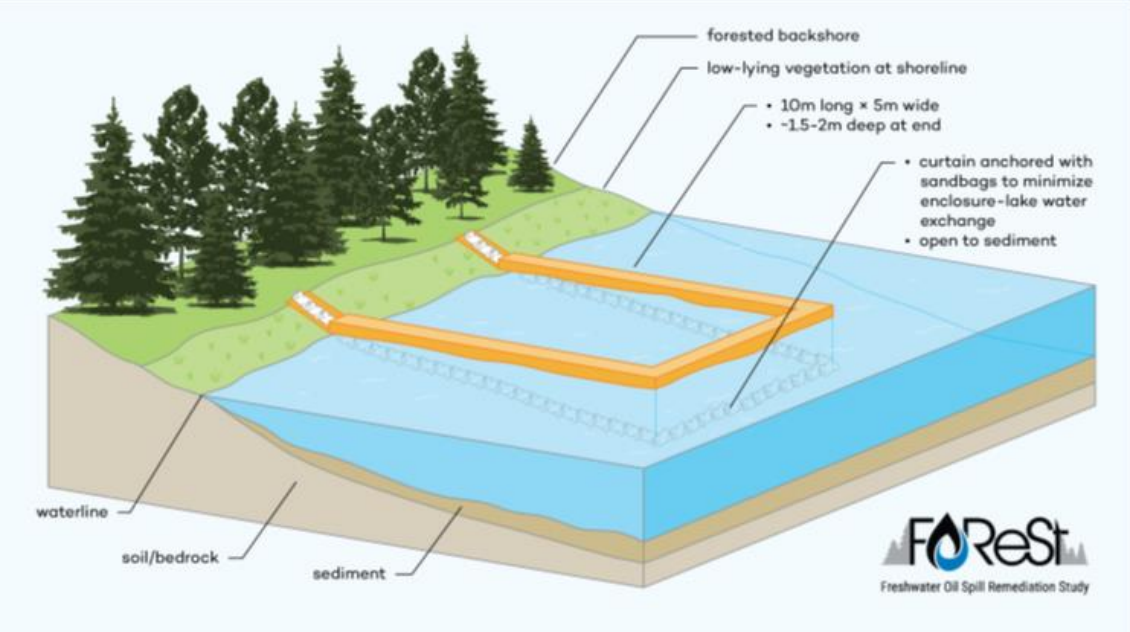


Figure 2.2 Schematic design of the enclosures used for the Freshwater Oil Spill Remediation Study (Reproduced with permission from Palace- 2021a).

Figure 2.3 Drone shot of enclosures used for the 2021 FOReST project (Photo credit: Jose Luis Rodriguez).

2.1.2 Application of treatment

Conventional Heavy Crude oil (CHV) was acquired from the Canadian Association of Petroleum Producers (CAPP) from their pipeline stocks and was weathered prior to its use for model oil spills. To weather the oil, 9 kg of conventional heavy crude (CHV) was placed with approximately 225L of Lake 260 water in a stainless-steel evaporation pan with a diameter of 1.1 m and exposed to the elements (i.e., sunlight and open to the air) for 36 hours. This weathering step was intended to model an aquatic spill in which viscous, weathered oil becomes re-deposited onto a shoreline area. After weathering, CHV was recovered from the surface of the water with slotted stainless-steel spoons, placed in glass jars and transported to Lake 260. All enclosures selected for oil treatment had c. 1.5 kg of weathered oil applied on 25 June 2021, henceforth referred to as study day 0 of the experiment. The oil was applied to the water of each enclosure about 50 cm from the shoreline by pouring directly from the jar to achieve a target oiled thickness of 0.1 cm on the shoreline, based on modelled wave height in the lake (data not shown). The oil spill was intended to model a real-life spill in a Canadian context, which is recorded to deposit oil of about 0.1 to 1.0 cm of oil on lakes with the same morphology with Lake 260 (Michel et al., 2013). During the first hour after oil additions and to simulate natural conditions such as wind and waves, waves were manually imparted into the enclosed area by gently oscillating the distal end of the collar to drive the oil up onto the shoreline. After this the weathered crude was left for 72 hours.

Primary oil spill cleanup began on 28 June 2021 (Day 3), by first flushing oil from the shoreline and vegetation using a low-pressure spray manifold. The manifold (Figure 2.4) delivered streams of water every 10 cm sourced from inside the enclosures and

pumped over the shoreline substrates and vegetation at an average of 1200 litres of enclosure water over a 10-minute period.

Figure 2.4: Shoreline washing (primary cleanup) of oiled enclosure using a low-pressure spray-manifold (Photo credit: Lauren Timlick).

The washed oil was then recovered by using 15 x 19” pre-weighed oleophilic sorbent pads (Spill Ninja Oil Only Pads, MEP Brothers, Winnipeg, MB) which were then hung and left to dry overnight and weighed to estimate the total amount of CHV recovered from the treatment enclosures.

Twenty-four (24) hours after the primary cleanup (Day 5) secondary remediation was implemented. Secondary treatment comprised enhanced Monitored Natural Recovery (eMNR) a treatment method where nutrients are added to stimulate oil degrading microbes found in the lacustrine environment. The eMNR treatment included adding of 10 g of a granular Nitrogen(N): Phosphorous (P) fertilizer (Scott’s Osmocote Classic Slow-Release Pro 19:6 N:P by weight) to treatment enclosures. This was intended to increase N and P to approximate the Redfield Ratio (106:16:1) where carbon from the oil (estimated to be 87% C) was balanced by N and P additions.

After the end of the sampling period in 2021, the enclosures were left in place throughout the remainder of the year, including through the winter. A follow-up study to track the recovery of the enclosures was conducted in 2022. No treatment was imposedto the eMNR enclosures in 2022.

2.1.3 Sample Collection

2.1.3.0 Physico-chemical water parameters

Physico-chemical water parameters including temperature, pH, dissolved oxygen and specific conductivity were measured using a YSI Pro 1020 handheld probe from 16 June 2021 (Day -9) to 21 September 2021 (Day 88) in 2021.

In 2022 the same parameters were measured using the same instrument from 22 June 2022 (Day 362) to 11 August 2022 (Day 412). Note sampling dates for physico-chemical parameters were different from water chemistry sampling dates.

Water samples for additional chemistry analysis were collected bi-weekly starting on 22 June 2021 (Day -3) and ending on 1 September 2021 (Day 68). Samples were analyzed for alkalinity, ammonia (NH_3), calcium (Ca), chloride (Cl), dissolved inorganic carbon (DIC), dissolved organic carbon (DOC), iron (Fe), magnesium (Mg), manganese (Mn), nitrate (NO_3), nitrite (NO_2), particulate carbon (PC), particulate nitrogen (PN), particulate phosphorus (PP), potassium (K), soluble reactive silica (SRSi), sodium (Na), sulfate (SO_4), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), total dissolved solids (TDS), total nitrogen (TN), total phosphorus (TP), total suspended solids (TSS), turbidity, chlorophyll-a, and pH. Water samples were collected using a Spectra Field Pro II- professional grade peristaltic pump with food grade PVC tubing passed into each enclosure. Water was pumped through the tube for about 10 – 15 seconds, to clear the tubing of any water trapped in the tube. The sample bottles are rinsed in triplicate with enclosure water and then is filled. Dissolved inorganic carbon, conductivity, and pH were collected into a 125 mL Nalgene bottle, while the other water quality and nutrient samples were collected into 500 mL Nalgene bottles. All bottles were labelled and placed into

individual Ziploc bags and transported in a cooler with ice packs (transport time < 3h) to the lab for subsequent analysis as described below.

2.1.3.1 Polycyclic Aromatic Hydrocarbons (PAH) Sampling

Surface water samples were collected from the enclosures using 1 L amber bottles, rinsed three times and then filled with water from the enclosures. The samples were transported in a cooler (transport time < 3h) to the lab for subsequent analysis as described below.

2.1.3.2 Phytoplankton and Biofilm Sampling

Samples of the same polypropylene material as the walls of the enclosures were cut into strips (20 cm by 5 cm), hung vertically, and anchored with sandbags from a pole at the deepest end (approximately 1.5m deep) of the enclosures to act as a substrate for biofilm colonization (Figure 2.5). The biofilm samplers were added a week before treatment (16 June 2021) to allow time for colonization and initial sampling before oil addition and cleanup. Phytoplankton chlorophyll-a samples were collected as water grab samples with other water chemistry parameters in 500 mL Nalgene bottles as stated in Section 2.1.3.0

Figure 2.5: The polypropylene strips used for sampling biofilm during the FOReST project. (Photo credit: Madeline Stanley).

Biofilm curtain strips were sampled biweekly from 24th June 2021 (Day -1) through 16th September 2021(Day 83). Each strip was removed from the pole and brought into the boat where separate 5 cm by 5 cm strips were cut for analysis of chlorophyll- *a* and ash-free dry mass. The strips were wrapped in aluminium foil to prevent loss of the sample. Chlorophyll-a samples were preserved in a 30 mL glass Fisher brand screw-top test tube. Ash-free dry mass (AFDM) samples were scraped on to aluminium foil and washed into a 118 mL Whirlpak bag with deionized water, and then placed into a cooler for transportation to the laboratory. Samples were then placed in a conventional freezer (-20° C) until samples could be processed.

New polypropylene strips were added to the enclosures after the commencement of the sampling season. The new strips were added on 16 June 2022 (Day 356) and sampling for biofilm started a week after on 23 June 2022 (Day 363) and ended on 9 August 2022 (Day 410). Sampling was performed following methods from 2021. Phytoplankton chlorophyll-a sampling was also sampled using methods in 2021. Samples were collected from 6 June 2022 (Day 346) to 20 September 2022 (Day 452).

Samples for taxonomy were collected on the 9th of September 2022 (Day 425) using the same techniques as for chlorophyll samples, but samples were fixed with 5 mL Lugol's solution.

2.1.4 Sample Analysis

2.1.4.0 DOC and DIC

The Shimadzu TOC-VCPH + TNM-1 Total Organic Carbon and Total Nitrogen analyzer, equipped with a Gas Purification kit, was used to analyze dissolved organic carbon (DOC) in water samples. After filtering samples through a 0.45 μm membrane to remove particulates, each sample was acidified and sparged to remove inorganic carbon, ensuring only DOC remained. The sample was then injected into a combustion chamber heated to 680°C, where DOC was oxidized to CO_2 in the presence of a catalyst. This CO_2 was detected by a non-dispersive infrared (NDIR) detector, and DOC concentration was calculated based on a calibration curve generated from standard solutions. The Gas Purification kit ensured the carrier gases remained free from contaminants that could interfere with detection, and regular use of blanks and calibration standards maintained accuracy throughout the analysis (Havens et al.,2024). Dissolved inorganic carbon (DIC) was analyzed using the LI-850 Li-Cor CO_2 analyzer by converting DIC in water samples to CO_2 gas for detection. Samples were first filtered through a 0.45 μm membrane to remove particulates, then acidified with phosphoric acid to lower the pH below 4, converting bicarbonate and carbonate ions to CO_2. This CO_2 was purged from the sample with an inert carrier gas (such as nitrogen) and directed into the LI-850, where it was measured via non-dispersive infrared (NDIR) technology. Calibration was performed with CO_2 gas standards to ensure accuracy. (Havens et al., 2024).

2.1.4.2 NH_3, NO_2, and NO_3

Water samples were filtered through a 0.45 µm membrane to remove particulate matter. The filtrate was subsequently analyzed colorimetrically using the SEAL AutoAnalyzer 3HR, following the protocol described by Havens et al. (2024).

2.1.4.3 TDN and TDP

TDN samples were analyzed in the IISD-ELA chemistry laboratory using a short UV irradiator and the SEAL AutoAnalyzer 3 HR according to the method detailed in Havens et al., (2024). TDP samples were analyzed in the IISD-ELA chemistry laboratory using a Short 44 UV irradiator and a Shimadzu 1800 scanning spectrophotometer set to 885 nm according to Havens et al., (2024).

2.1.4.4 Particulate Phosphorus, Carbon, and Nitrogen

An aliquot of 100 mL enclosure water was filtered using a 42.5 GF/C filter with a nominal pore size of 1.2 micrometers, which had been pre-rinsed with ultra pure water. The filter was then folded and placed in a 15 mL glass vial and sent to the University of Alberta Biogeochemical Analytical Service Laboratory for analysis of particulate phosphorus. A GF/C filter prefiltered with ultrapure water was filtered with 200 mL of enclosure water and the filter was then put in a desiccator for 48 hours before being processed for particulate carbon and nitrogen using a Shimadu TOC-L CPH Model Total Organic Carbon Analyzer with an AI-L and TNM-L at the University of Alberta Natural Resources Analytical Laboratory.

2.1.4.5 TSS and Major Ions

Major ions and TSS were analyzed by the University of Alberta Biogeochemical Analytical Service Laboratory using an ICP-OES (Thermo ICAP-6300 ICP-OES) and Ion Chromatography (Dionex DX-600 Ion Chromatograph), respectively. Turbidity samples were analyzed in the IISD-ELA chemistry laboratory using an Orion AQUAfast AQ4500 Turbidimeter. SRSi samples were measured in the IISD-ELA chemistry laboratory using a Shimadzu UV-1800 scanning spectrophotometer set to 820 nm.

2.1.4.6 Polycyclic Aromatic Hydrocarbons

A gas chromatograph mass spectrometer (GC-MS) with a triple quadrupole mass spectrometer was used to detect and estimate polycyclic aromatic hydrocarbon (PAH) levels using methods described by Idowu et al (2017). Within 24 hours of collection 250mL of each water sample was vacuum filtered (1.2 µm, Whatman GF/C filter) and liquid-liquid extraction was completed following methods previously described in Dearnley (2022). Briefly, filtrates were transferred into separatory funnels which received 20 µL of 5 ng/µL recovery internal standard (suite of d8-naphthalene, d8-acenaphthylene, d10-acenaphthene, d10-fluorene, d10-phenanthrene, d10-pyrene, d12-benz(a)anthracene, d12-chrysene, d12-benzo(b)fluoranthene, d12-benzo(k)fluoranthene, d12-benzo(a)pyrene, d12-indeno(1,2,3-c,d)pyrene, d14-dibenzo(a,h)anthracene, and d14-benzo(g,h,i)perylene) and 10 g of sodium chloride (NaCl). Samples were extracted twice with 50 mL dichloromethane (DCM) by gently swirling liquids for 1 minute. DCM was collected into round bottom flasks (total 100 mL DCM) and was reduced to approximately 2 mL using rotary evaporation. Sodium sulphate (Na2SO4) was added to remove any residual water from the extraction

process (Dearnley, 2022) and samples were transferred to 12 mL round bottom borosilicate glass tubes with a Teflon lined screw caps. The remaining sodium sulphate was rinsed three times with Optima ™ grade hexanes (ThermoFisher Scientific), which was then transferred into the glass test tubes. Solvent exchange from DCM to Optima ™ hexanes was conducted using a nitrogen gas evaporator (N-EVAP™111, Organomation Associates Inc., MA, USA and OA-SYS Heating System), until 1 mL of sample remained. Each sample was spiked with 20 µL of an instrument performance internal standard (5 ng/µL d10-anthracene) and was transferred into an amber GC vial with a Teflon cap. During each sampling period, a field blank was collected by opening an amber bottle containing 200 mL Optima ™ water (ThermoFisher Scientific) for the duration of sample collection. PAC extraction of field blanks followed the same protocols described above for experimental samples. Final concentrations were calculated using internal standard recoveries and blank correction.

2.1.4.7 Phytoplankton Chlorophyll-a

Chlorophyll-a analysis was completed by the IISD-ELA chemistry lab. Samples were filtered with a Whatman GF/C 42.5 mm glass fiber filter. Filters were rinsed with 50 mL of MilliQ water before filtering 200 mL of the water chemistry sample. Each filter was then vacuum desiccated and stored in the dark for 3 days then placed in 50 mm petri dish and stored at ≤ -20 °C in the dark until extraction and fluorometric analysis following methods used from the FOReSt project in 2019 (Perry 2021).

Each filter was transferred to a glass extraction test tube using tweezers folded into thirds and pushed to the bottom of the test tube. Extraction solvent (68% methanol, 27% acetone, and 5% ultrapure water) was added to the test tube containing the filter. The

amount of extraction fluid needed (2-8 mL) varied based on how much chlorophyll-a appeared to be on each filter. Test tubes were then inverted three times and stored in the dark at 4°C for 16 hours.

Samples were analyzed for chlorophyll-a content using a benchtop fluorometer by Turner Design, Trilogy Model (minimum detection limit 0.025 µg/L).

2.1.4.8 Biofilm Chlorophyll-a

Biofilm chlorophyll-a samples were stored in a freezer until analysis following standard methods (Arar & Collins 1997), To extract pigments, 15 mL of extraction solvent (68% methanol, 27% acetone, and 5% ultrapure water) was added to each test tube containing the biofilm strip samples, Test tube were agitated to allow complete mixing of the solvent with the strip and then the wrapped in aluminium foil and then stored at 4^{o}C for 16 hours for total chlorophyll-a extraction. Samples were then analyzed using a Turner Designs, Trilogy laboratory fluorometer (minimum detection limit 0.025 µg/L).

2.1.4.9 Biofilm Ash Free Dry Mass

Ash-free dry mass samples were thawed then filtered with a Whatmann GF/C 42.5 mm glass fibre filter that was pre-baked at 400^{o}C for 12 hours, Filters were then dried at 105^{o}C for 12 hours then weighed to determine dry mass. The samples were then burned at 450^{o}C for 24 hours in a muffle furnace and then reweighed. Ash-free dry mass was calculated as the difference in mass between the dry mass and the sample after burning.

2.1.4.10 Biofilm Taxonomy

Biofilm sample taxonomic counting and identification were performed using the book A Species List and Pictoral Reference to Phytoplankton of Central and Northern Canada

Part 1 and 2 by D.L Findlay and H.J Kling. Briefly, a 2 mL sample was gravity settled for 24 hours, and species identification and counting were undertaken using an inverted microscope at 125x and 500x using Uttermohl method (Nauwerck 1963). Only cell count (abundance) was measured.

2.1.5 Data Analyses

2.1.5.1 Physico-chemical parameters

Physico-chemical parameters for 2021 and 2022 were analyzed using a linear mixed effect model from the nlme package version (3.1). Treatment and study day (time) and the interaction between treatment and study day were fixed effects. The random effect was study day nested in each enclosure. Study day was converted into a categorical variable. Our random effect structure was chosen to account for the lack of independence in our samples taken from the enclosures throughout the study. Study day was plotted as a categorical variable to estimate different effects for each individual sampling date. This allowed us to have estimated marginal contrast of all measured parameters on all sample collection dates.

Estimated marginal contrast between reference and treatment enclosures were plotted from the linear mixed effect model on individual sampling days using the “emmeans” package (version 1.10). This allowed us to assess the effect of treatment on individual days of sampling both before oil addition and all sampling dates post oil addition. Estimated mean differences between eMNR and reference enclosures on all days of sampling were estimated from our contrast. All statistical differences were ascertained at the 95% confidence level (i.e. $p < 0.05$). A pooled standard error from the linear mixed model was reported with the mean differences. The pooled standard error

in a mixed-effects model reflects the combined variability from both the fixed effects (e.g., treatment effects) and the random effects (e.g., variation among subjects or sites).

2.1.5.2 Phytoplankton Chlorophyll-a, Biofilm Chlorophyll-a, and AFDM

Phytoplankton chlorophyll-a, Biofilm chlorophyll-a, and Biofilm AFDM was analyzed using the same methods and model structure described in Section 2.1.5.1. AFDM was square root transformed before being analyzed with the linear mixed effect model to ensure a better fit of the model.

2.1.5.3 Biofilm Taxonomy

Richness and abundance of biofilm were compared between treatment and reference enclosures for the single time point (day 425) using a Student's t-test which was ascertained at the 95% confidence level ($p = 0.05$). Diversity indices and Bray-Curtis were calculated using the vegan package from the R programming language.

2.1.5.4 Nutrient Mass Balance

Nutrient mass balance was calculated using nutrient concentrations (PP, PN, TDP, TDN) and enclosure dimensions estimates. The nutrient mass balance was calculated for Day-3 (before oil and nutrient addition), Day 6 (nutrient addition date), Day 11(next sampling date after nutrient addition) Day 68 (last sampling date for year 1 of the FOReSt study) and Day 452(last sampling date for year 2 of the FOReSt study). This gives us a rudimentary understanding of nutrient pathway in the enclosures.

2.2 Results

2.2.1 Oil Exposure

After oil addition and shoreline washing, oleophilic sorbent pads were used in CHV recovery. About 24% of the total oil (355.83g +/- 24.34g) was recovered from the enclosures with the sorbent pads. The eMNR treatment was then applied to remove the residual CHV of 1121g +/- 132g.

The crude oil addition caused an increase in total polycyclic aromatic compound (tPAC) concentrations to levels above 1000 ng/L by Day 4 within the eMNR treatment enclosures followed by a decline to background levels by Day 60. (Figure 2.6). Concentrations of tPAC remained low (< 50 ng/L) in the reference enclosures throughout the study.

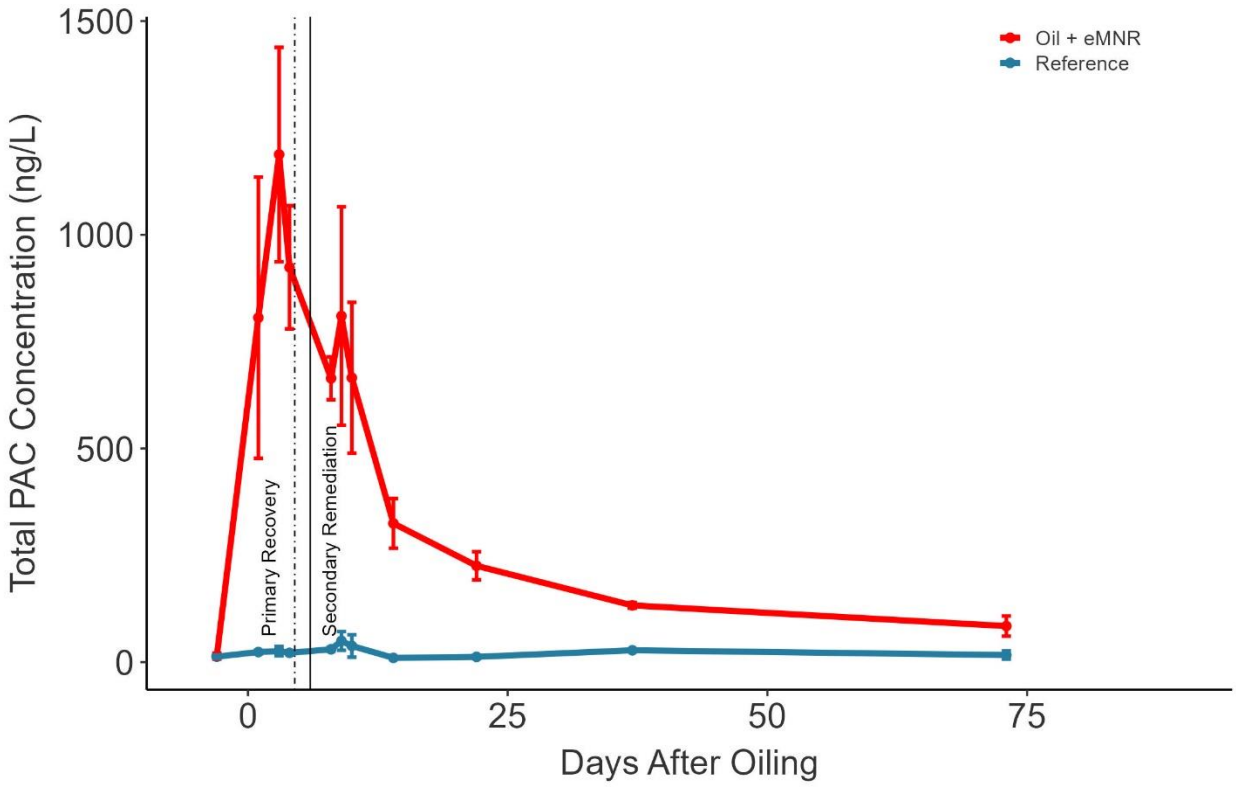


Figure 2.6 Total PAC concentration within reference and treatment enclosures during the FOReST study. Each point is the mean(n=3), and error bars represent the standard deviation.

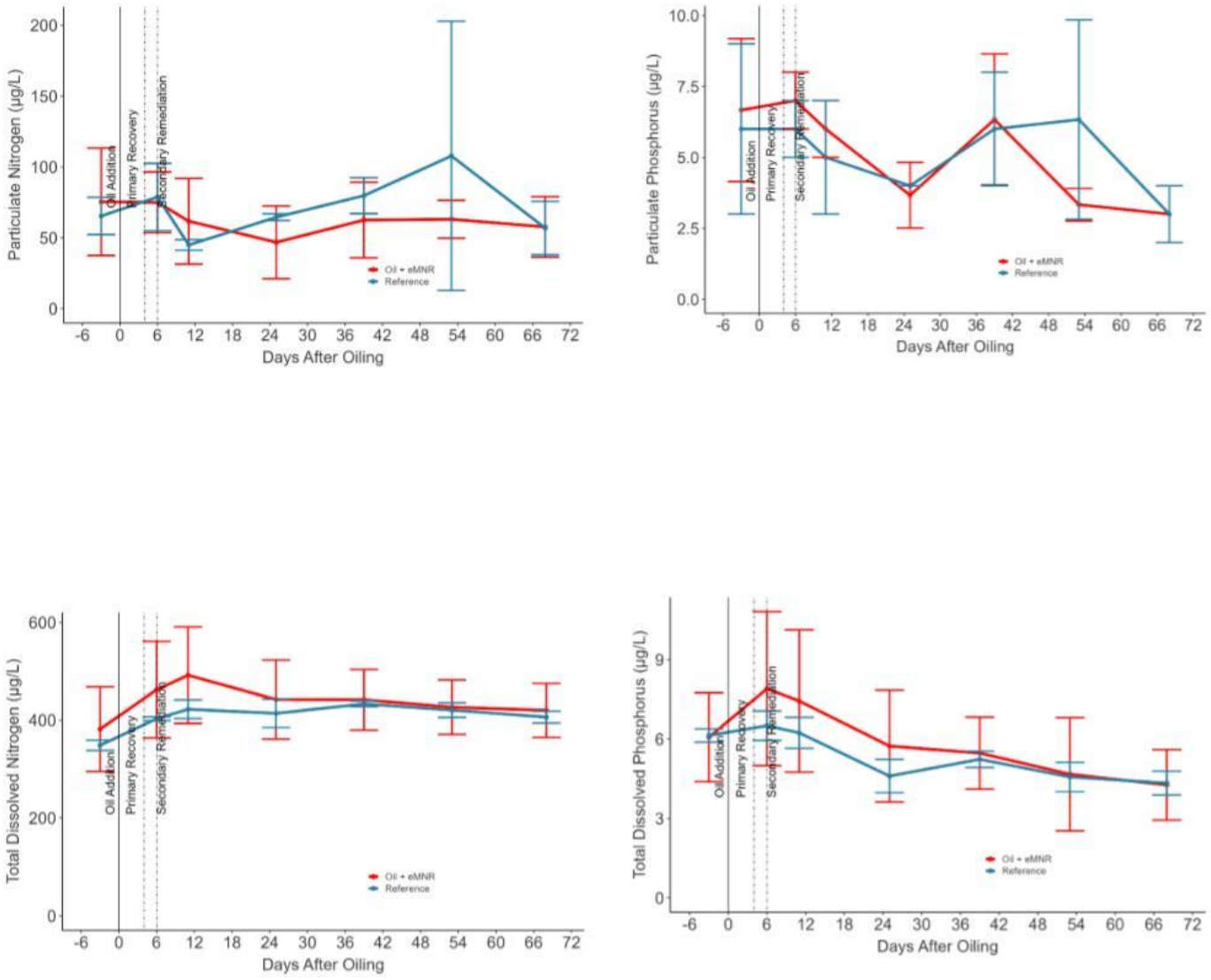
2.2.2 Physico-chemical water parameters

2.2.2.1 Physico-chemical water parameters

Water temperature ranged from 13.5°C to 25.3°C in the enclosures, while conductivity (22.1 to 52.6 µmhos/cm) and pH were generally between (5.9 to 6.93). Water temperature (SI figure 16), conductivity (SI figure 15), pH (SI figure 14), and dissolved oxygen (SI figure 13) were not significantly different in eMNR enclosures compared to the reference enclosures on all sampled dates from Day -2 to Day 88. Turbidity was not significantly different in eMNR enclosures compared to the reference enclosures before or after oil addition from day 6 to day 68.

TDP concentrations had the highest mean difference on day 6 (higher by 1.40 +/- 1.25 μgL^{-1} p = 0.33) of the study in the eMNR enclosure compared to the reference enclosure. While TDN concentrations mean differences were highest on day 11 (higher by 69.67 +/- 46.45 μgL^{-1} p = 0.20) in the eMNR enclosure compared to the reference enclosures. NH_3, (increased by 31.33 +/- 16.01 μgL^{-1}, p = 0.12) and NO_3 (higher by 5.67 +/- 3.37 μgL^{-1}, p = 0.17) mean differences were highest on day 6 in the eMNR compared to the reference enclosures while NO_2 had the largest increase in the eMNR compared to the reference enclosures on day 25 (higher by 0.40 +/- 0.19 μgL^{-1}, p = 0.10). PN lower by 44.7 +/- 26.82 μgL^{-1}, p = 0.17), PC (lower by 414.07 +/- 266.59 μgL^{-1}, p = 0.20), and PP (lower by 3.00 +/- 1.5 μgL^{-1}, p = 0.12) concentrations had the highest mean difference between eMNR and reference enclosures on day 53 of the study. DIC (higher by 86.67 +/- 61.0 $\mu molL^{-1}$, p = 0.23) and DOC (higher by 78.67 +/- 84.03 $\mu molL^{-1}$, p = 0.23) had the highest mean difference between eMNR and reference enclosures on day 6 of the study.

SRSi values ranged from 0.096 to 0.973 mgL^{-1} throughout the study. SRSi was significantly higher on day 6 (higher by 0.52 +/- 0.15 mgL^{-1}, p = 0.025) and 11 (0.51 +/- 0.15 mgL^{-1}, p = 0.027 in the eMNR compared to reference enclosures but was not significant on day -3 (higher by 0.38 +/- 0.15 mgL^{-1}, p = 0.061), 25 (higher by 0.37 +/- 0.15 mgL^{-1}, p = 0.06), 39 (increased by 0.31 +/- 0.15 mgL^{-1}, p = 0.11), 53 (increased by 0.37 +/- 0.15 mgL^{-1}, p = 0.06), and 68 (increased by 0.33 +/- 0.15 mgL^{-1}, p = 0.09). Alkalinity (SI figure 1), chloride, sulfate, calcium, iron, potassium, magnesium, manganese, and sodium were not significantly different between the eMNR enclosures and reference enclosures on all sample collection dates (day -3 to day 68). All estimated marginal contrast differences are presented in SI.



Nutrient concentrations (PN, PP, TDN, TDP) in treatment and reference enclosures during the FOReST study. Day 0 (Oil addition date) and Day 6 (Secondary remediation eMNR) is represented by a dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

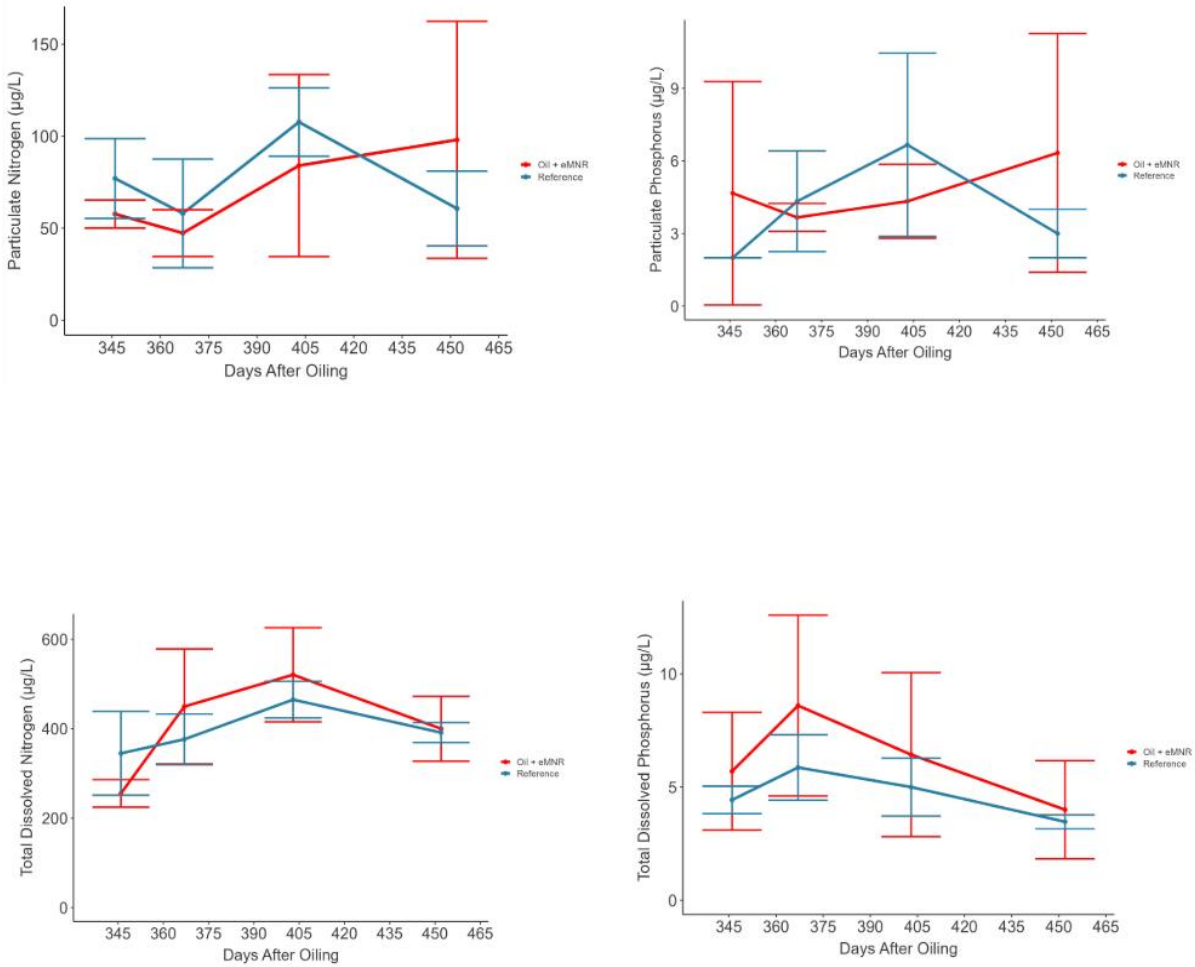
2.2.2.2 Physico-chemical water parameters (2022)

Physico-chemical water parameters measurements continued in 2022. There was no oil exposure in this study year and only recovery monitoring was conducted. Water temperature (SI figure 35), conductivity (SI figure 34), dissolved oxygen (SI figure 32), and pH ($p = 0.98$ SI figure 33) were not significantly different between eMNR and reference enclosures on any of the sampled days (day 362, day 390, and day 412). Turbidity (SI figure 37) was not significantly different between eMNR and reference enclosures on all sampled dates from day 346 to day 452.

TDP (SI figure 31) and TDN (SI figure 30) were not significantly different between eMNR enclosures compared to reference enclosures from day 346 to day 452. NH_3 (SI figure 21) NO_2 (SI figure 25) and NO_3(SI figure 24) were not significantly different between eMNR enclosures compared to reference enclosures from day 346 to day 452. Particulate phosphorus (SI figure 28), particulate nitrogen (SI figure 27), and particulate carbon (SI figure 26) were not significantly different from day 346 to day 452. DOC (SI figure 23) and DIC (SI figure 22) were both not significantly different between eMNR enclosures compared to reference enclosures from day 346 to day 452.

SRSi (SI figure 29) was not significantly different between eMNR enclosures compared to reference enclosures from day 346 to day 452.

All other parameters alkalinity (SI figure 20), chloride, sulfate, iron, potassium, manganese, sodium and calcium were not significantly different between eMNR enclosures compared to reference enclosures (from day 346 to day 452).



Nutrient concentrations (PN, PP, TDN, TDP) in treatment and reference enclosures approximately 1 year after oil addition. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post oil addition.

2.2.3 Nutrient Mass Balance

The mass balance of phosphorus (TP) showed changes over time for both treatment and reference enclosures. On Day -3 before oil and nutrient addition TP levels were comparable between treatment and reference enclosures, with 0.25 +/- 0.022 g in the eMNR enclosures and 0.27 +/- 0.06g in the reference enclosures. By Day 6 the day after nutrients were added to the enclosures TP increased reaching 0.31 +/- 0.038 g in the eMNR enclosures and 0.27 +/- 0.0047 g in the reference enclosures. The reduction continued on Day 68, where TP levels dropped to 0.14 +/- 0.0047g in the eMNR enclosures and 0.15 +/- 0.045 g in the reference enclosure. By Day 452, the eMNR enclosures showed a partial recovery with TP increasing to 0.20 +/- 0.073 gram whereas the reference enclosure's TP levels dropped to 0.13 +/- 0.019 g.

The total nitrogen (TN) mass also exhibited changes over the life of the study. At Day -3, TN levels were 9.26 +/- 1.33 g in the eMNR enclosures and 8.63 +/- 2.35 g in the reference enclosures. By Day 6 a day after eMNR was applied TN increased to 11.01 +/- 2.14 g in the eMNR enclosures and 9.92 +/- 2.75 g in the reference enclosures. TN levels peaked at Day 11, reaching 11.29 +/- 2.15 g in the eMNR enclosures and 9.74 +/- 2.67 in the reference enclosures. By Day 68 TN levels decreased to 9.76 +/- 1.36 g in the eMNR and 9.61 +/- 2.44 g in the reference enclosures. At Day 452, TN levels stabilized at 9.92 +/- 0.30 g in the eMNR enclosures and 9.28 +/- 1.94 g in the reference enclosures.

Treatment	**TP (Day -3)**	**TP(Day 6)**	**TP(Day 11)**	**TP(Day 68)**	**TP(Day 452)**
Oil + eMNR	0.25 +/- 0.022 grams	0.31 +/-0.038 grams	0.27 +/- 0.0047 grams	0.14 +/- 0.0047 grams	0.20 +/- 0.073 grams
Reference	0.26 +/- 0.12 grams	0.27 +/- 0.06 grams	0.24 +/- 0.10 grams	0.15 +/- 0.045 grams	0.13 +/- 0.019 grams
	TN(Day-3)	**TN(Day 6)**	**TN(Day 11)**	**TN(Day 68)**	**TN(Day 452)**
Oil + eMNR	9.26 +/- 1.33 grams	11.01 +/- 2.14 grams	11.29 +/- 2.15 grams	9.76 +/- 1.36 grams	9.92 +/- 0.30 grams
Reference	8.63 +/- 2.35 grams	9.92 +/- 2.75 grams	9.74 +/- 2.67 grams	9.61 +/- 2.44 grams	9.28 +/- 1.94 grams

Table 1: Total Phosphorus (TP) and Total Nitrogen (TN) mass balance mean and standard deviation concentrations on Day 3, Day 6, Day 11, Day 68 and Day 452 of the FOReSt study.

2.2.4 Phytoplankton biomass

Phytoplankton chlorophyll-*a* was not significantly different between eMNR and reference enclosures on day -3 (higher by 0.08 +/- 0.51 μgL^{-1} p = 0.88), day 6 (increased by 0.11 +/- 0.51 μgL^{-1} p = 0.84), day 11 (higher by 1.21 +/- 0.51 μgL^{-1}, p = 0.075), day 25 (lower by 0.06 +/- 0.51 μgL^{-1}, p = 0.91), day 39 (lower by 0.27 +/- 0.51 μgL^{-1}, p = 0.63), day 53 (lower by 0.06 +/- 1.25 μgL^{-1}, p = 0.91), and day 68 (lower by 0.23 +/- 0.51 μgL^{-1}, p = 0.67).

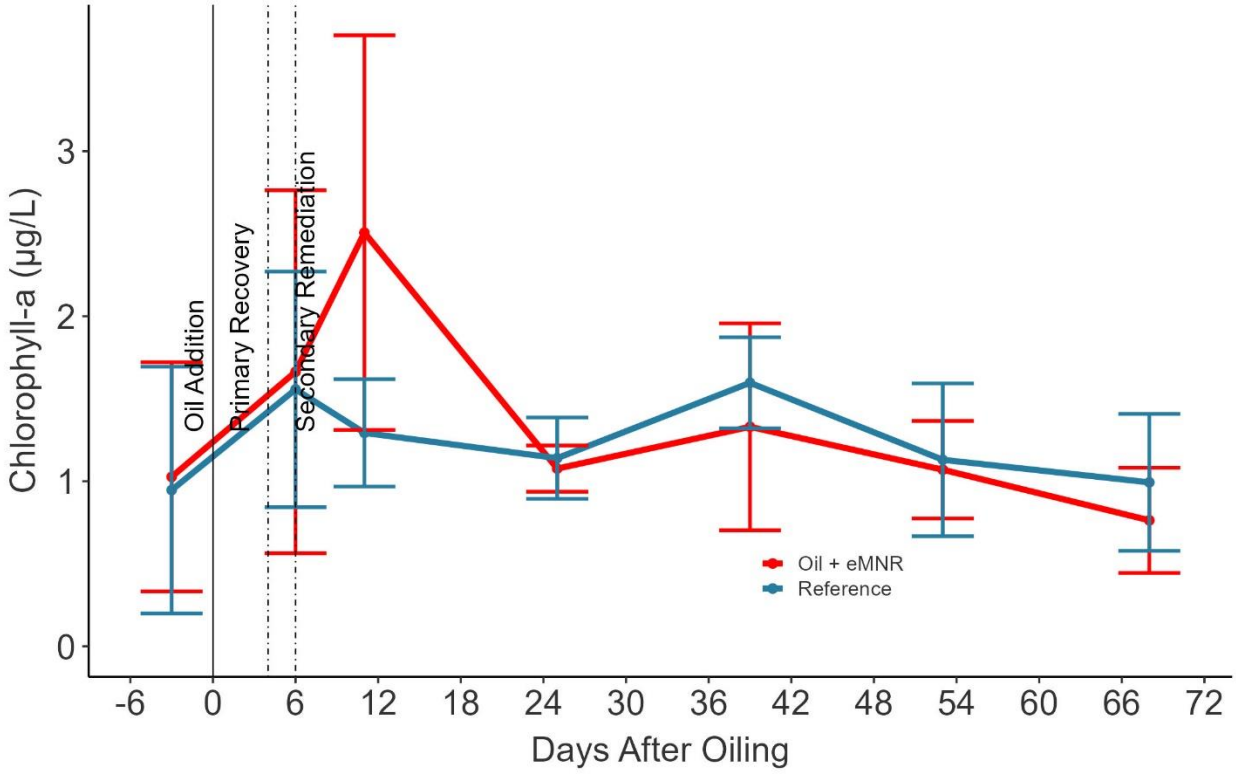


Figure 2.7: Phytoplankton Chlorophyll-*a* concentration in treatment and reference enclosures during the FOReST study. Day 0 (Oil addition date) and Day 6 (Secondary remediation eMNR) is represented by a dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

Phytoplankton biomass measured as chlorophyll-*a* was not significantly different between eMNR and reference enclosures on day 346 (increased by $2.76E^{-16}$ +/- 2.56 μgL^{-1}, p = 1), day 367 (higher by $3.33E^{-03}$ +/- 2.56 μgL^{-1}, p = 0.99), day 403 (lower by $7.27E^{-01}$+/- 2.56 μgL^{-1}, p = 0.79), and day 450 (higher by 4.95 +/- 2.56 μgL^{-1}, p = 0.13) of the second year of study.

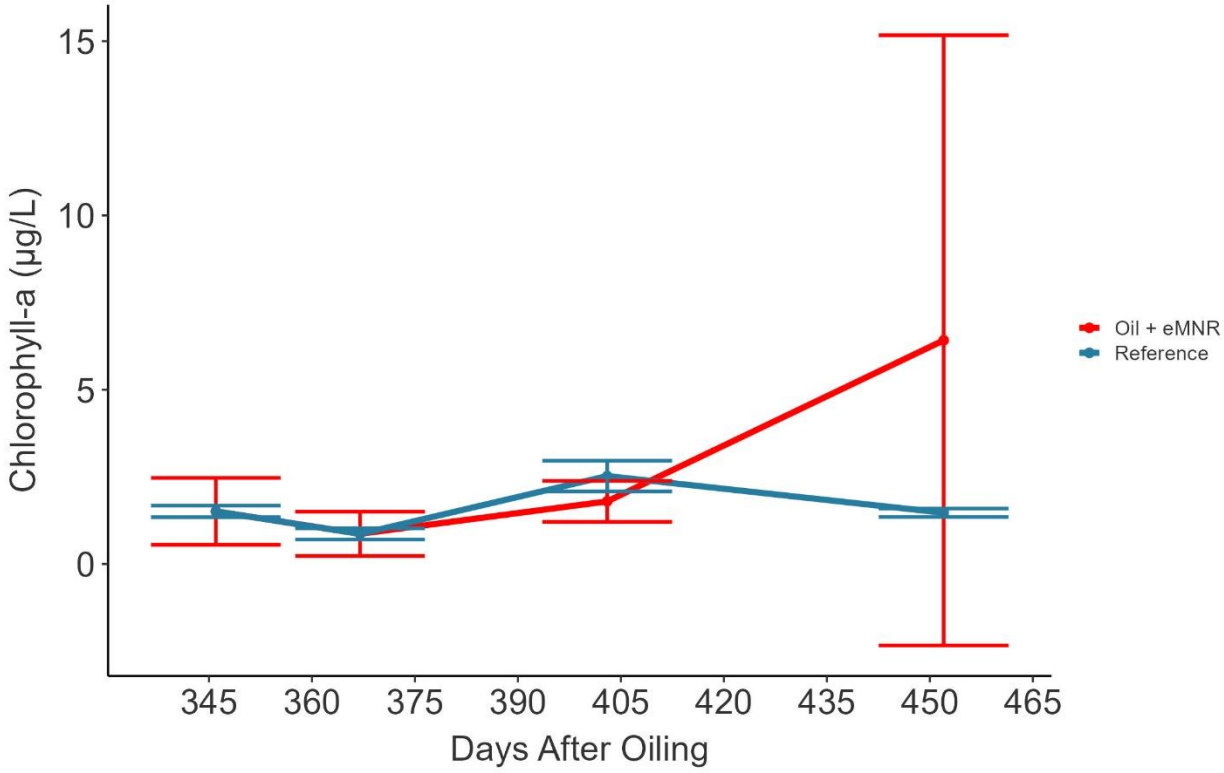


Figure 2.8: Phytoplankton Chlorophyll-*a* concentration in treatment and reference enclosures approximately 1 year after oil addition. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post oil addition.

2.2.4 Biofilm chlorophyll-*a*

Biofilm chlorophyll-*a* concentration was not significantly different between eMNR and reference enclosures on day -1 (lower by 17.40 +/- 104.13 μgL^{-1}, p = 0.87), day 13 (higher by 178.80 +/- 104.13 μgL^{-1}, p = 0.16), day 27 (higher by 99.41 +/- 104.13 μgL^{-1}, p = 0.39), day 41 (higher by 87 +/- 104.31 μgL^{-1}, p = 0.45), day 55 (lower by 156.26 +/- 104.13 μgL^{-1}, p = 0.20), day 69 (lower by 137.43 +/- 104.13 μgL^{-1}, p = 0.25), and day 83 (lower by 0.0013 +/- 104.13 μgL^{-1}, p = 0.99).

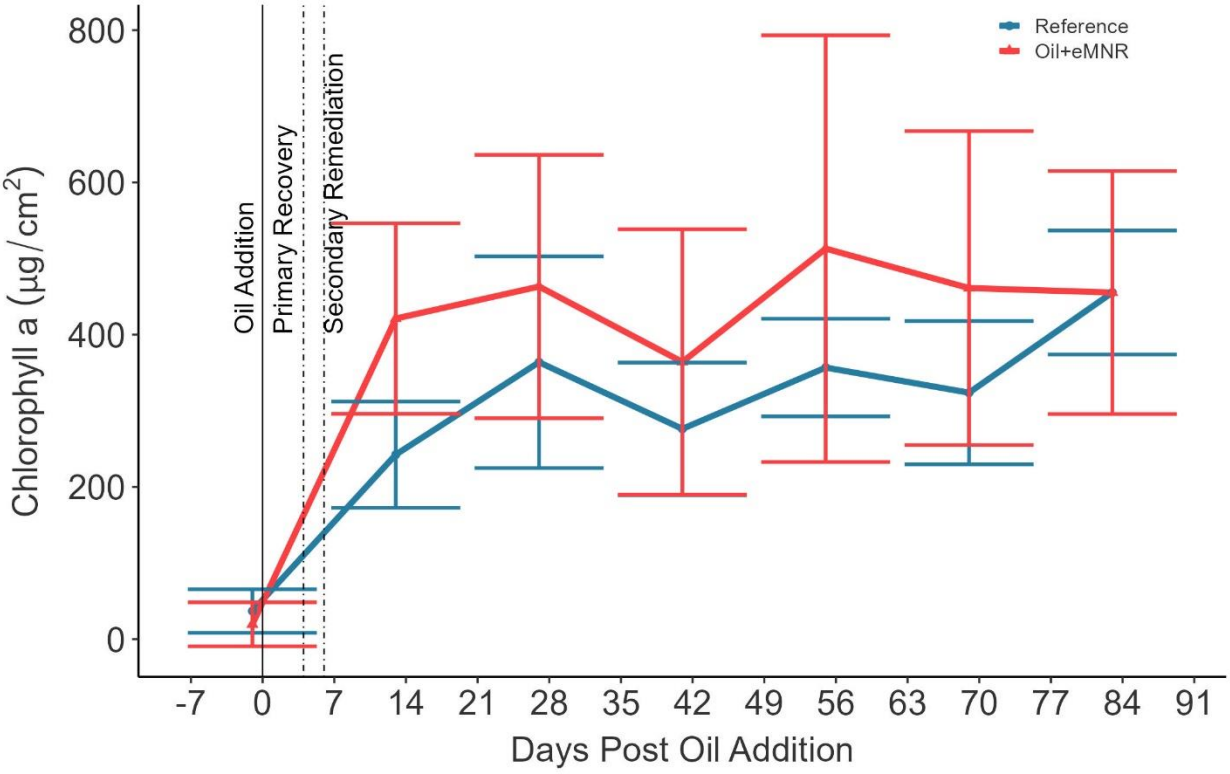


Figure 2.9: Biofilm Chlorophyll -*a* concentration in treatment and reference enclosures during the FOReST study. Day 0 (Oil addition date) and Day 6(Secondary Remediation eMNR) is represented by the dashed line. Each point is the mean (n=3) and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post oil addition.

Biofilm chlorophyll-*a* concentration was not significantly different on day 363 (lower by 27.99 +/- 152.26 μgL^{-1}, p = 0.86), day 391(higher by 235.06 +/- 152.26 μgL^{-1}, p = 0.20), day 410 (higher by 300.83 +/- 152.26 μgL^{-1}, p = 0.12).

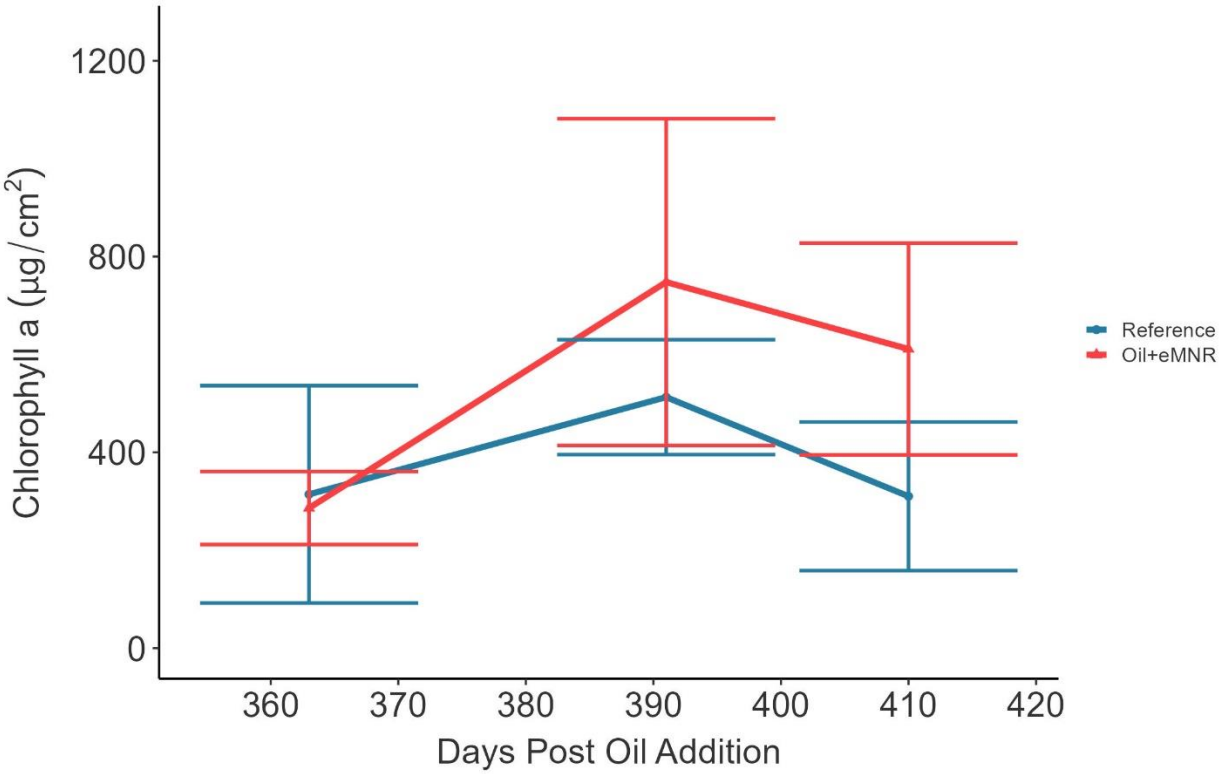


Figure 2.10: Biofilm biomass Chlorophyll-*a* concentration in treatment and reference enclosures approximately 1 year after oil addition. Each point is the mean (n=3) and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

2.2.5 Biofilm AFDM

Peripyton ash-free dry mass (afdm) concentration was not significantly different between eMNR and reference enclosures on day -1 (lower by 5.90 +/- 4.40 μgcm^{-2}, p = 0.25), day 13 (lower by 0.13 +/- 4.40 μgcm^{-2}, p = 0.98), day 27 (higher by 0.34 +/- 4.40 μgcm^{-2}, p = 0.94), day 41 (higher by 1.57 +/- 4.40 μgcm^{-2}, p = 0.74), day 55 (lower by 3.30 +/- 4.40 μgcm^{-2}, p = 0.50), day 69 (lower by 2.42 +/- 4.40 μgcm^{-2}, p = 0.61), and day 83 (lower by 4.49 +/- 4.40 μgcm^{-2}, p = 0.37).

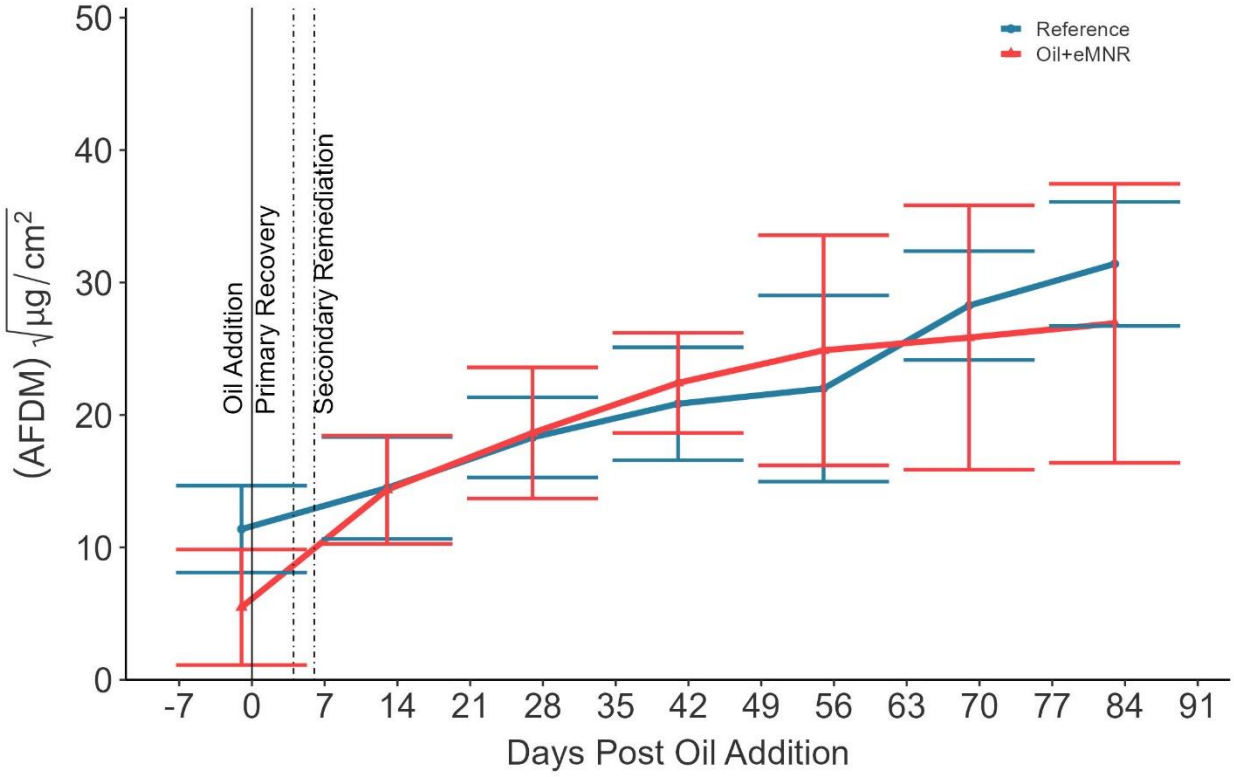


Figure 2.11: Biofilm biomass ash-free dry mass concentration in treatment and reference enclosures during the FOReST study. Day 0 (Oil addition date) and Day 6 (Secondary remediation eMNR) is represented by the dashed line. Each point is the mean (n=3) and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

Peripyton afdm concentration was not significantly different on day 363 (higher by 2.92 +/- 4.54 μgcm^{-2}, p = 0.56), day 391(higher by 0.87 +/- 4.54 μgcm^{-2}, p = 0.86), day 410 (higher by 2.95 +/- 4.54 μgcm^{-2}, p = 0.55)

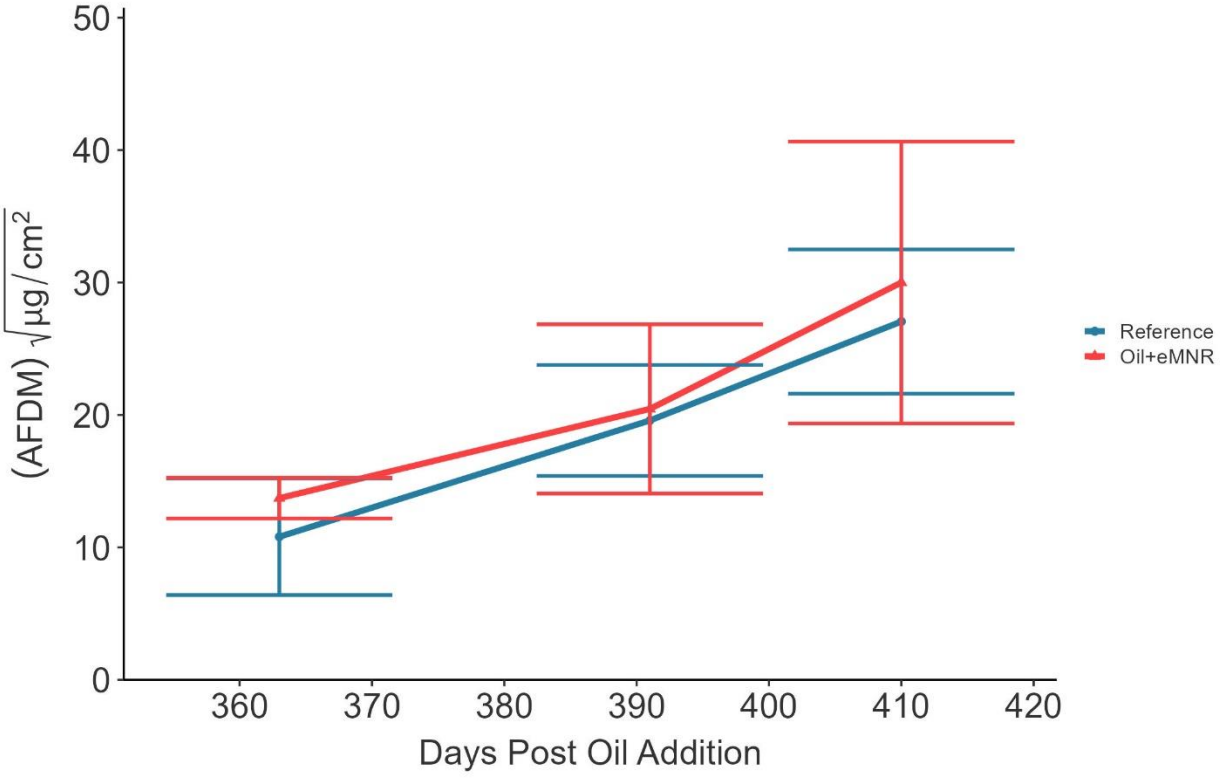


Figure 2.12: Biofilm biomass ash-free dry mass concentration in treatment and reference enclosures approximately 1 year after oil addition. Each point is the mean (n=3) and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post oil addition.

2.2.6 Biofilm Community Dynamics

2.2.6.1 Biofilm Abundance

Biofilm was sampled for abundance on the 24th of August 2022, day 425 of the 2022 FOReST study. Total abundance was not significantly different (p = 0.90) between the eMNR enclosures and reference enclosures. Cyanophyta dominated eMNR1 comprising 50% of the total community while all other enclosures were dominated by Charophyta.

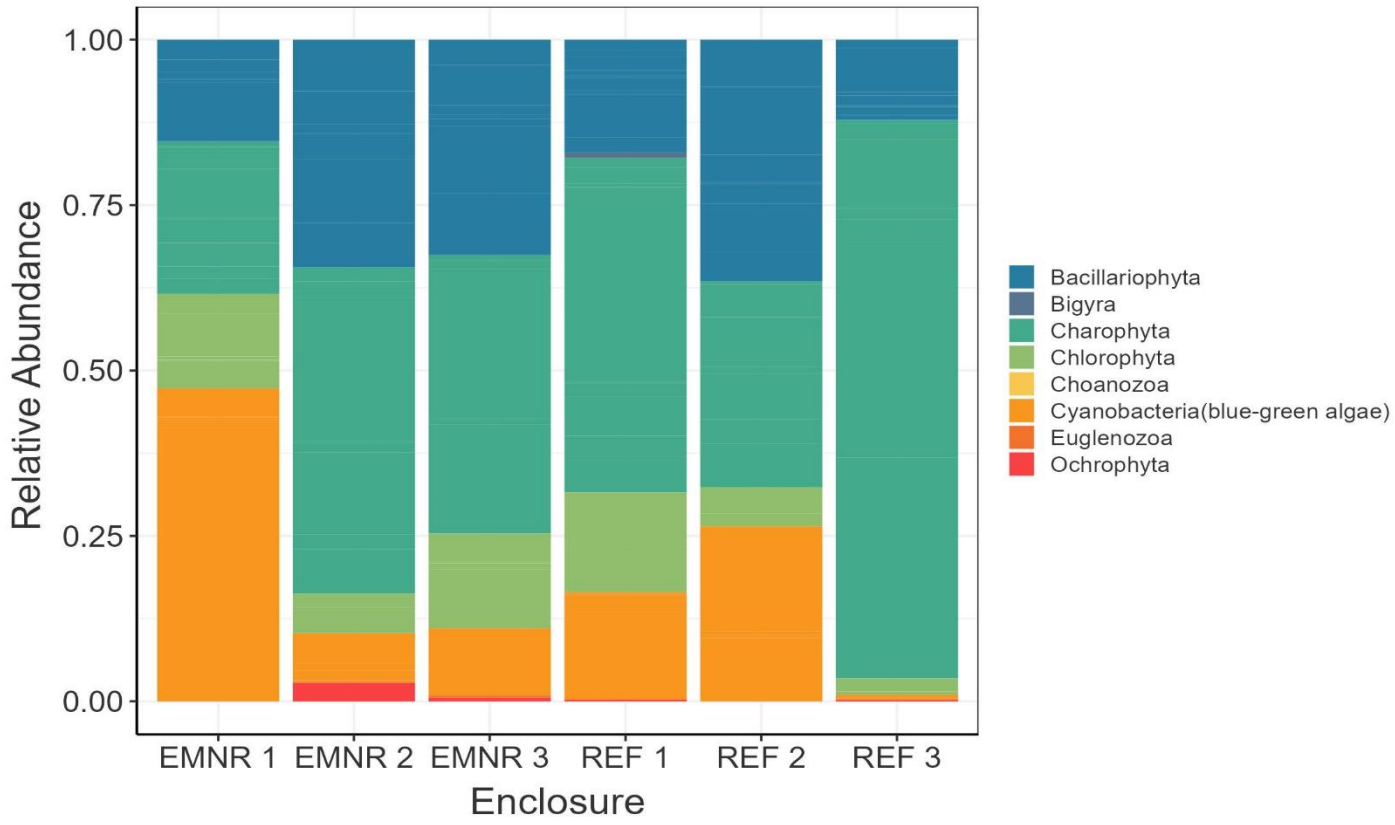


Figure 2.13: Relative abundance of biofilm species composition according to phylum on day 425 of the 2022 FOReST study. There was no statistical difference (Welch t-test) between eMNR and reference enclosure

2.2.6.2 Biofilm richness

Biofilm richness a measure of the number of taxa identified, was not significantly different (p = 0.60) between eMNR and reference enclosures on the sampled date (day 425 of the 2022 FOReST study). The absence of an upper tail is as a result of values clustering near the median.

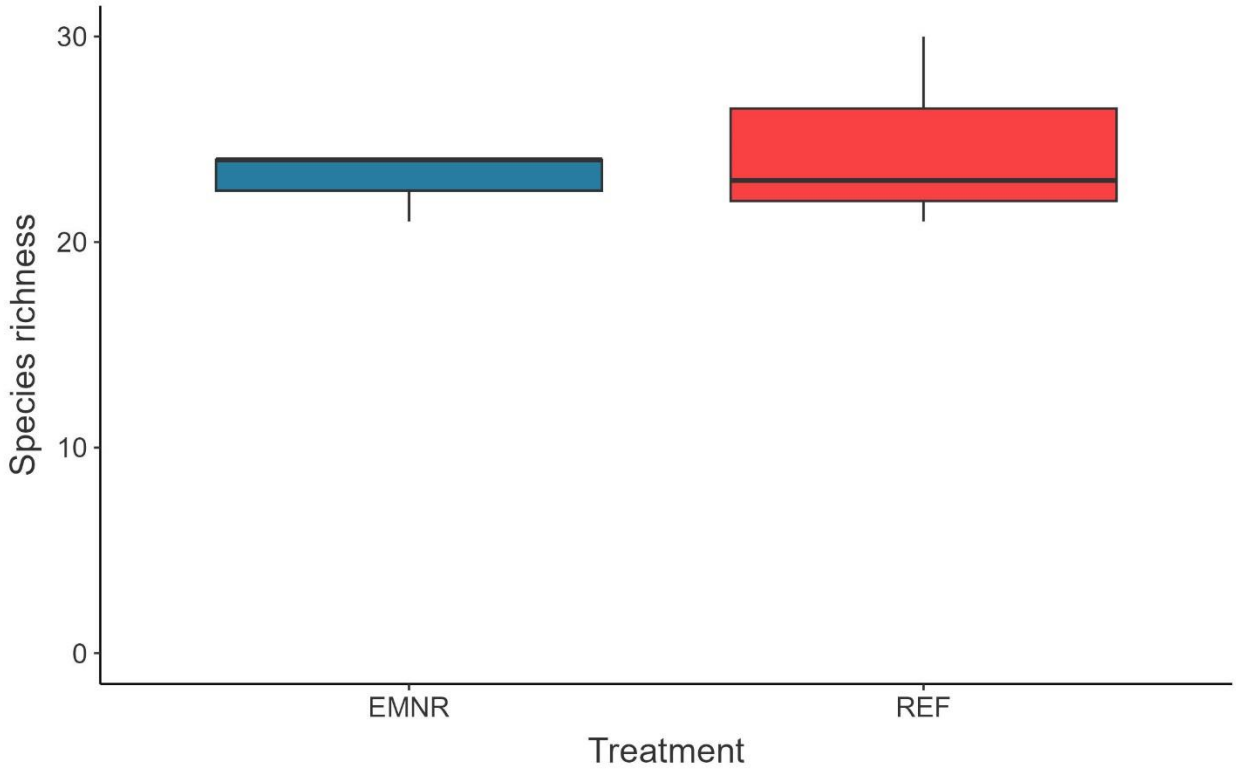


Figure 2.14: Biofilm richness within reference (n = 3) and eMNR enclosures (n = 3) on day 425 of the FOReST study (n = 3). There was no statistical difference (Welch t-test) between eMNR and reference enclosures

2.2.6.3 Biofilm Community Index

Shannon Index, a measure of phytoplankton diversity, was calculated from the community data sampled on the 24th of August 2022 (day 425 of the 2022 FOReST study). There was no significant effect (p = 0.77) in the Shannon Index between eMNR and reference enclosures

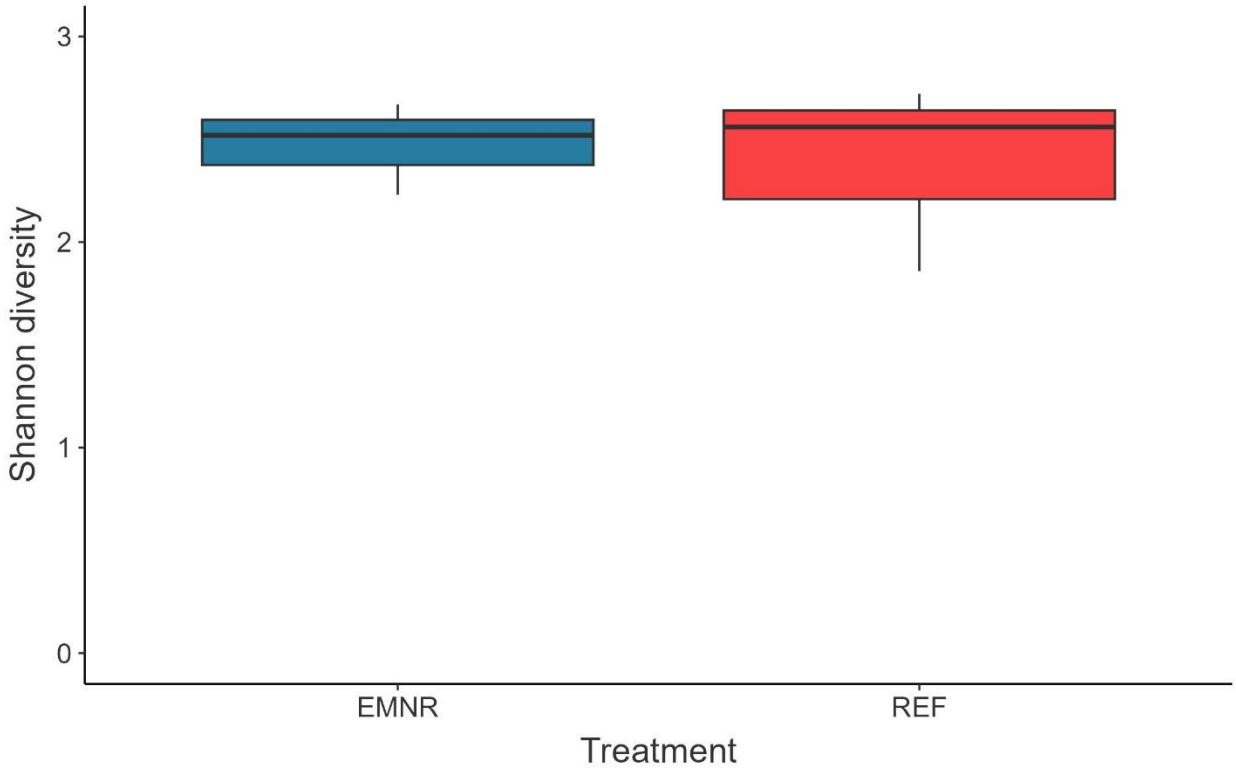


Figure 2.15: Shannon diversity index within reference (n = 3) and eMNR enclosures (n = 3) on day 425 of the FOReST study. There was no statistical difference between eMNR and reference enclosures

2.2.6.4 Bray- Curtis Analysis

The non-metric multidimensional scaling (NMDS) plot represents the differences in biofilm communities based on the eMNR and reference enclosures. The separation in points represents the dissimilarity between individual enclosures. A PERMANOVA was run on the result of the Bray-Curtis dissimilarity with a p-value of 1 showing that the difference in group was not statistically significant.

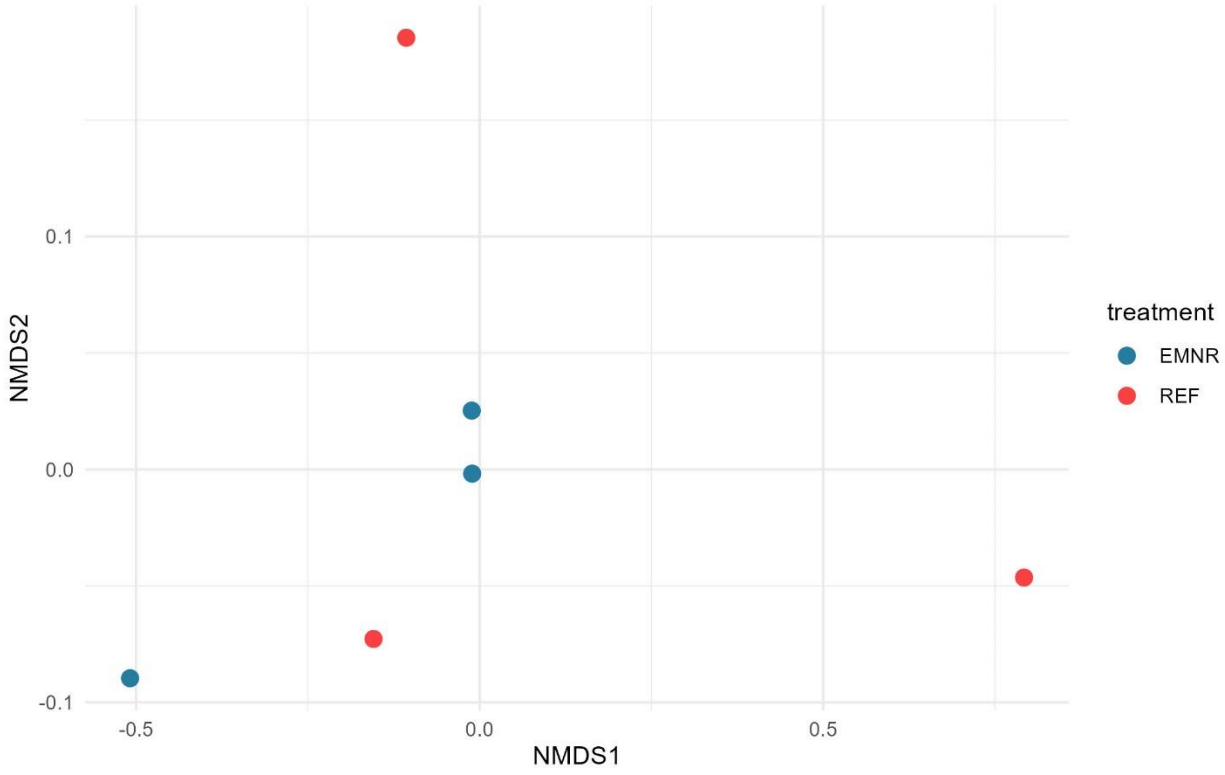


Figure 2.16 NMDS plot of effect of treatment on biofilm community composition on day 92 of the FOReSt study.

2.3 Discussion

Enhanced Monitored Natural Recovery (eMNR) is a remediation strategy that leverages natural processes to restore ecosystems affected by contaminants, such as oil spills (Palace et al., 2021). eMNR relies on the activity of existing microbial communities to degrade oil components, thus restoring affected ecosystems (Palace et al., 2021). The initial study in 2021 was conducted to assess the effect of eMNR on the shoreline ecosystem and a follow-up study in 2022 tracked the recovery of the system compared to reference enclosures. This thesis has explored the impact of eMNR, applied to model oil spills as part of the FOReSt study, on phytoplankton and biofilm biomass and community composition and found a nonsignificant effect on phytoplankton biomass, biofilm biomass and biofilm community composition. But care should be taken when adding nutrients into water bodies as there were days where biologically large chlorophyll-a concentrations were observed in biofilm.

2.3.1 Interpretation of Results

All the results for phytoplankton and biofilm growth should also be interpreted within the context of effect size (mean difference) that measures the difference between the control group and treatment group. Despite the predominance of non-significant results in our measured parameters, differences in estimated marginal means allowed us to estimate the difference in mean response between treatment and reference enclosures from our linear mixed effect model. These values serve as effect sizes for our measured parameters. Some of our effect sizes suggest that, while statistical significance was not achieved, there was biologically meaningful differences in biofilm chlorophyll-*a* and this

should factor into the interpretation of the result. The large effect size of biofilm chlorophyll-a for the in-lake mesocosm is likely a result of higher variability, often encountered in natural environments. The variability of a natural system is due to reasons such as limited control over factors affecting the systems, imbalance of nutrients and starting trajectories.

2.3.1 PAC degradation

The primary goal of applying an eMNR treatment after an oil spill is to reduce concentrations of toxic oil constituents within receiving waterbodies. In this study, the Conventional Heavy Crude (CHV) spill significantly increased PAC levels peaking above background concentrations, peaking above 1000 ng/L on day 3, before dropping back to near baseline levels by day 60. Most of the decline in tPAC levels occurred ~ 10 days after eMNR was applied when tPAC concentrations dropped by about 700 ng/l. while we don't have a positive control. Results show that total PACs degraded by 70% after eMNR treatment. This showed a similar result to the study by Garcia-Blanco et al (2006). In that study 450 g-nitrogen and 135 g-phosphorus were added to 3m X 3m X 3m aquatic mesocosm treated with a total of 8L of Mesa, a light crude oil. About 90% of alkanes and 80% of the aromatics in the enriched plots were degraded after 20 weeks of application. showing the effectiveness of eMNR for degrading toxic petroleum constituents.

2.3.2 Water Chemistry

Physico-chemical parameters measured in the first year were all not statistically significant on all days sampled except for SRSi. However, SRSi concentrations were already high pre-treatment with a p value of 0.06 and therefore it is hard to state that the treatment caused a change in SRSi concentrations as effect size did not vary widely before and after treatment. Similarly in 2022 from day 346 to day 452 all physico-chemical parameters were not statistically significant except for SRSi. This was also caused by the initial high SRSi concentration in the eMNR enclosure see SI Table 1.

Creating favourable conditions for microbial degradation of oil is important for effective treatment, but the addition of nutrients must be carefully considered to avoid eutrophication. The use of a measured quantity of slow-release fertilizer in 2021 was intended to ensure that nitrogen and phosphorus levels (see SI figure 5-9, 11-12) were controlled enough in the eMNR enclosures to avoid significant eutrophication compared to the reference enclosures. Slow-release fertilizer applications for actual oil spills employing eMNR could also reduce the need for repeated nutrient additions. Our results indicated that at the concentrations of nutrients added, while there were no statistically significant effects detected on phytoplankton or biofilm biomass there were days were chlorophyll-a concentration was 80% higher in eMNR enclosures than reference enclosures. For this reason, the quantity of nutrients added for eMNR should be conservatively estimated and should take into account other nutrient sources into water bodies to prevent eutrophication.

Water chemistry analysis was also conducted in 2022 to monitor the recovery of the enclosures treated with crude oil and eMNR in the previous year. By continuing to monitor

water chemistry in 2022 we sought to develop a comprehensive understanding of the recovery process in the eMNR treatment enclosures. The stabilization of basic water chemistry parameters, nutrient levels, and most mineral concentrations to conditions comparable to the reference enclosures indicated substantial recovery post treatment (see SI figures). Similarly in 2022 from day 346 to day 452 all physico-chemical parameters were not statistically significant except for SRSi. This was also caused by the initial high SRSi concentration in the eMNR enclosure. see SI Table 10

2.3.3 Nutrient Balance

The eMNR treatment and the reference mesocosms exhibit distinct nitrogen and phosphorus patterns based on the nutrient mass balance data. On Day 6, slow-release fertilisers were administered, resulting in a notable increase in total nitrogen (TN) and total phosphorus (TP) in the eMNR mesocosms. The observed increases indicate that the additional nutrients were available for utilisation by microbial populations and biofilms, which are essential for the degradation of hydrocarbons in oil-contaminated environments (Atlas & Bartha, 1992; Das & Chandran, 2011).

The temporal data from TN indicate that nitrogen availability increased shortly after fertiliser application and subsequently declined throughout time. The observed decline is most likely attributed to natural processes like denitrification, physical losses, and nutrient absorption by microbial and biofilm communities. The effective utilisation of supplementary nitrogen and the restoration of natural nitrogen cycling mechanisms were evidenced by the stabilisation of TN levels in the eMNR mesocosms at the conclusion of

the study, which mirrored those in the reference systems (Galloway et al., 2003; Seitzinger et al., 2006).

TP levels exhibited an initial increase after the addition of fertilizer in the eMNR treatment, followed by a gradual decline. Similar to nitrogen, phosphorus is essential for microbial and enzymatic processes involved in hydrocarbon degradation. Declining phosphorus levels over time correspond to adsorption to sediments, incorporation into microbial biomass, or removal through chemical precipitation (Wetzel, 2001; Smith et al., 1999). TP levels exhibited partial recovery in the eMNR mesocosms throughout the final stage of the study, likely due to internal system recycling. This recovery demonstrates how effectively managed systems may conserve and recycle nutrients over extended periods, even after initial depletion.

The differences between eMNR and reference treatments underscore the necessity of nutrient enrichment in facilitating microbial activities. Prior bioremediation research (Atlas & Bartha, 1992; Das & Chandran, 2011) demonstrates that the temporary increase in nutrient concentrations likely facilitated the degradation of oil by bacteria. The system's capacity to recuperate from nutrient enrichment is evidenced by the gradual return of nutrient levels to those found in reference mesocosms. This aligns with substantial ecological research indicating that the maintenance of ecosystem stability and function relies on mechanisms for nutrient cycling and retention (Vitousek et al., 1997; Wetzel, 2001).

The results regarding nutrient mass balance substantiate the regulated nutrient input in bioremediation methods for oil spills. While restricting prolonged nutrient excess and thereby reconciling deterioration acceleration with the maintenance of ecosystem restoration

2.3.4 Phytoplankton and Biofilm Biomass

The primary goal of this study was to examine the effect of model oil spills, and remediation using eMNR, on biofilm phytoplankton and biomass. We predicted that there would be an increase in phytoplankton and biofilm biomass (chlorophyll- *a* and AFDM) due to the addition of nutrients in the enclosures treated with oil followed by eMNR remediation. However, while there was a large seasonal increase in chlorophyll-a concentrations during the growing season there were no statistically significant differences in phytoplankton (Figure 2.7) or biofilm chlorophyll-*a* and AFDM (Figure 2.9 and 2.11) between the eMNR and reference enclosures throughout the 2021 season. The lack of statistical response in biomass could indicate that the nutrient addition was not sufficient. This could be due to the kind of fertilizer used (slow release) or quantity used (Schindler et al., 2016).

In 2022 all phytoplankton and biofilm growth parameters (Figures 2.8, 2.10 and 2.12) in the eMNR enclosures generally moved in the same direction as the reference enclosures showing recovery in growth patterns after a year of the eMNR treatment. This

supports the notion that eMNR using low addition rates of N and P reduces the probability of eutrophication in oligotrophic boreal lakes.

Overall, the addition of nutrients had the highest effect on biofilm chlorophyll-a where the largest amount of difference was observed between eMNR and reference enclosures. This agrees with a meta-analysis of 85 biofilm studies by Hillbrand (2002) which discovered that increased nutrient leads to higher biofilm chlorophyll-a concentration especially in the absence of a biofilm grazer e.g. snails or benthic macroinvertebrates. Why we don't have data for macroinvertebrates for the study in 2021 and 2022. Previous iteration of the FOReSt study in 2018 and 2019 shows a drop in benthic invertebrates in oiled enclosures compared to reference enclosures (Palace et al., 2021; Perry 2021). Therefore, a top-down effect should not be discounted when assessing the effect of eMNR treatment on biofilm biomass.

2.3.4 Biofilm Community Composition

The effect of oil and eMNR on biofilm species composition was also assessed as a primary endpoint in this thesis. Regarding biofilm community composition, Charophyta dominated all enclosures, especially reference enclosures. However, in eMNR1 treated enclosures Cyanobacteria dominated Cyanobacteria are known to outcompete other species of algae in the presence of high temperature and nutrient addition (Lurling et al 2017). The difference between eMNR1 and the other enclosures could be a result of the upwelling of oil in eMNR1 enclosures on day 425 likely due to sediment resuspension which has been observed to reintroduce organic carbon and nutrients into the water

column (Liu et al., 2024). A Bray-Curtis dissimilarity analysis was conducted on cell density to assess if treatment influenced community composition. The NMDS-generated plot (Figure 2.7) showed a more homogeneous and closely related biofilm community in eMNR enclosures compared to more diverse reference enclosures. These differences could have resulted from more nutrients availability in the eMNR enclosures relative to the reference environments. Nutrient availability is a driver of phytoplankton and biofilm community composition (Costerton et al., 1995). Despite the difference in composition, a PERMANOVA run on the dissimilarity index showed no statistically significant effect (PERMANOVA p = 1) of treatment on community composition. The non-significance of community composition shows eMNR when applied with proper oil spill management techniques should not significantly change the community composition of biofilms

2.6.5 Conclusion

This study examined the effects of oil spills and minimally invasive secondary remediation techniques on phytoplankton and biofilm communities. The results of our study provide insight into the efficacy of eMNR as a remediation technique for decreasing the presence of crude oil components following a spill, suggesting it is a highly promising approach for cleaning up oil on shorelines.

Our central hypothesis stated that we would have a statistically significant rise in phytoplankton and biofilm biomass from nutrient enrichment in the eMNR enclosures, but we didn't have a statistically significant result in both phytoplankton chlorophyll-*a* and biofilm afdm. Our biofilm chlorophyll-a results despite the absence of statistically significant results, had large effect sizes in both years of the FOReSt study. Signifying

that the majority of our treatment affected mostly algal growth in biofilm especially since we didn't have similar large effect sizes in biofilm afdm. Therefore, when considering the use of eMNR in oil treatment. The larger effect on biofilm should be considered to prevent eutrophication in aquatic bodies.

A PERMANOVA analysis of cell density data revealed no statistically significant effect of treatment on community composition, suggesting that eMNR is a promising approach for oil spill cleanup on oil impacted freshwater shorelines.

The FOReSt study, enabled researchers to manipulate variables such as nutrient levels and oil concentrations, resulting in a precise understanding of the effects caused by these factors. Nevertheless, real-life scenarios involve several unanticipated factors such as fluctuations in weather conditions, dynamics of water flow, and the existence of different pollutants. These characteristics have a substantial impact on the behaviour and outcome of oil spills, as well as the efficacy of remedial measures. Moreover, the complex species assemblages and their interactions in actual ecosystems can be significantly different from mesocosm environments. The study concentrated on biofilm communities in a simplified setting, which may not completely replicate the complex relationships observed in natural ecosystems. The interactions between various species, such as competition, predation, and symbiosis, can affect the results of oil spill restoration operations in real-world situations. Gaining a comprehensive understanding of these interactions is essential for accurately forecasting the operational efficiency of eMNR.

Mesocosm investigations, such as those conducted in the FOReSt project, are characterized by their restricted spatial and temporal scales, allowing for precise monitoring and control. In contrast, oil spills in natural habitats have a significantly larger

spatial impact and can persist for extended periods. Shifting from studying mesocosms to studying field conditions involves dealing with variations in different regions and considering the long-term ecological impacts, which can be challenging to predict based on short-term, small-scale studies. In addition, the distribution of nutrients in mesocosms is relatively homogenous and closely monitored to ensure consistent and uniform input. In contrast, in natural environments, the distribution of nutrients and other restorative chemicals might be uneven because of factors such as water currents, sediment composition, and varying accessibility of different places. To achieve successful implementation in the field, it is imperative to utilize strategies that efficiently manage the existing variability, ensuring the delivery of nutrients to the intended areas and stimulating microbial activity. To improve our study, a spill of opportunity treated with eMNR should be studied to ensure that results from the enclosure studies show degradation of oil without eutrophication carries over to natural systems such as lakes and other freshwater systems.

Chapter 3: Synthesis and Next Steps

3.0 Summary

Crude oil exploration and transportation are expected to continue due to the world's energy needs. Therefore, having solutions to spills that inadvertently occur is important to preserve and protect the natural environment. The study of real-world spills is not convenient because they are incidents that occur sparingly and often in remote areas where research responses are not feasible in the immediate hours and days following release. There is also a gap in oil spill research in freshwater environments as with most studies focus on marine environments. Laboratory studies tend not to include the ecological complexity of natural systems where these spills occur and so there is uncertainty in extrapolating these responses to the field. Lake mesocosm experiments are meant to bridge the gap between real spill studies and laboratory studies bringing the best of both worlds with a semi-controlled environment and real-world ecosystem interaction. This allows biotic and abiotic effects to be included in results and a fuller picture of the ecological risk to be determined. To this end, the FOReSt study was undertaken at the IISD-ELA. Remediation experts in both the private and public sectors can use the FOReSt study as a guide in responding to freshwater spills.

The objective was to characterize the effect of CHV and eMNR on phytoplankton and biofilm communities in in-lake enclosures in a boreal lake. This thesis provides results that help to understand whether CHV+eMNR poses an effect on phytoplankton and

biofilm communities in an ecological context. We found that CHV+eMNR does not have a statistically significant effect on phytoplankton and biofilm growth and community composition under our experimental conditions. Although our findings did not show any statistically significant impacts of enhanced monitored natural recovery treatments, the use of eMNR should consider potential negative ecosystem effects associated with nutrient enrichment such as eutrophication and anoxia to help safeguard aquatic ecosystems.

Finally, additional freshwater spill studies are needed for other treatment types such as oil burning and bioaugmentation and a combination approach of all these secondary treatment methods and in other environment types. An example is a test in lotic systems where different factors operate such as water flow which could necessitate a different application process for the eMNR treatment.

3.1 Ecosystem Effect of CHV+eMNR Treatment

The effect of the eMNR treatment on ecosystem dynamics and especially primary productivity was hard to quantify because there was a sharp increase in primary productivity in both phytoplankton and biofilm immediately after the eMNR treatment. However, the increase was insufficient to cause a statistically significant difference between the eMNR and reference enclosures. The results of the phytoplankton and biofilm chlorophyll-a were similar to the previous FOReSt field study in 2019 that used model spills of dilbit. The eMNR treatment in both studies had an increase in chlorophyll-a which was not significant. (Perry 2021). Phosphorus and nitrogen were not statistically

significant between eMNR and reference enclosures, which was also the case in the 2021 dilbit study (Perry 2021).

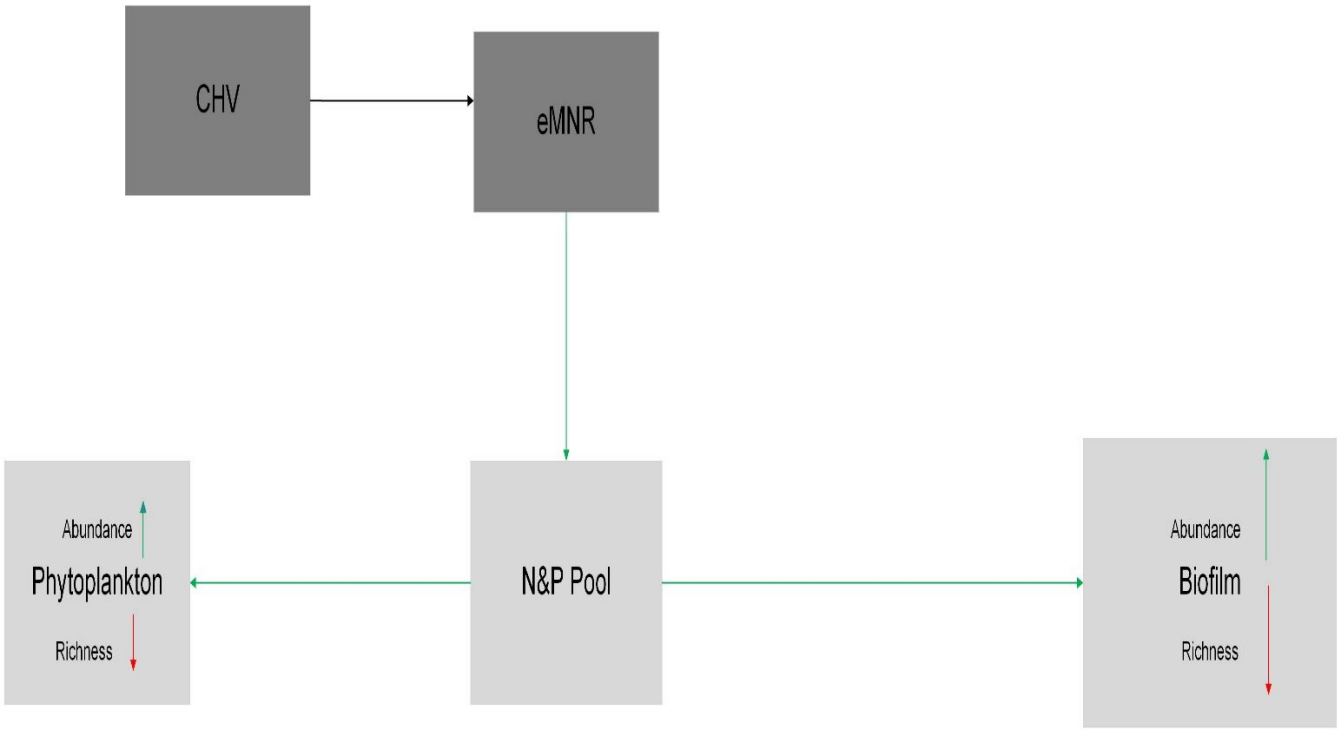


Figure 3.0: Conceptual model of eMNR treatment of CHV as part of the FOReSt study 2021 at the IISD-ELA. Green arrows represent positive effects, while red arrows represent negative effect.

3.2 Limitations, Challenges and Future Work

There are some limitations in terms of the scope of the study. Taxonomy samples were only obtained once at the end of the study. While the information gathered from this one-time sample adds to the study, sampling for taxonomy throughout the study would have given us a more complete picture regarding the effects of the CHV+eMNR treatment on community composition. For example, if there was a short-term change, and then recovery, or if there was no significant change throughout our understanding of the impacts on community composition would have been more thorough. Due to financial and time constraints, some parameters were not measured. In terms of study design a positive control should have been included which only had the primary treatment of pressure washing and sorbent pads to pick up oil. This would allow us to model a clear difference between the secondary eMNR treatment and the primary treatment on the measured parameters in the FOReSt study especially in degradation of tPAC and all other organism responses.

Because mesocosms require an enormous amount of effort to install, there is a limitation of statistical power resulting from few replicates. Lake enclosure studies often include a lot of variability due to the ecological complexity of natural systems and confounding effects compared to laboratory studies where all factors that can cause high variability are controlled. Therefore, the higher risk of having a type II error should be considered when interpreting results. But the reason why lake mesocosm studies have

low power is also why we use them as the uncontrolled biotic and abiotic effects make the results far more relevant to making decisions in natural aquatic systems.

There are still several studies that can be undertaken in the field of freshwater oil research that will improve our understanding of the best practices for responding to an oil spill. Among them is research on other methods of oil cleanup and the effects they have on freshwater ecosystems. An example is oil burning which has been proposed as a method of getting rid of crude oil spills in marine environments or bioaugmentation the supplementing of oil-degrading microbes in aquatic systems. An in-depth study should also be undertaken on the effect of different oil spill cleanup strategies on phytoplankton and biofilm communities that track changes in community composition throughout the life of the treatment.

3.3 Conclusions

This thesis determined that eMNR treatment of oil spills, when carefully applied and monitored does not pose a risk of eutrophication and community change in biofilms and phytoplankton in boreal lake ecosystems under the conditions tested. The findings of this thesis will help remediation experts in choosing relevant treatment methods for containing and remediating oil spills.

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Supplemental information

SI Table 1: Estimated mean difference, confidence interval, standard error, df, t statistic of measured parameters between eMNR and reference enclosures in the FOReSt study

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Alkalinity	(Oil + eMNR)	Reference	-3	39	-88.942	166.942	46.08123	4	0.846332	0.445029
Alkalinity	(Oil + eMNR)	Reference	11	42.86667	-85.0754	170.8087	46.08123	4	0.930241	0.404904
Alkalinity	(Oil + eMNR)	Reference	25	48.06667	-79.8754	176.0087	46.08123	4	1.043086	0.355799
Alkalinity	(Oil + eMNR)	Reference	39	49.4	-78.542	177.342	46.08123	4	1.07202	0.344083
Alkalinity	(Oil + eMNR)	Reference	53	44.73333	-83.2087	172.6753	46.08123	4	0.97075	0.386643
Alkalinity	(Oil + eMNR)	Reference	6	46.33333	-81.6087	174.2753	46.08123	4	1.005471	0.371559
Alkalinity	(Oil + eMNR)	Reference	68	40	-87.942	167.942	46.08123	4	0.868032	0.434352
Alkalinity	(Oil + eMNR)	Reference	346	36.26667	-36.8487	109.382	26.33415	4	1.377173	0.240504
Alkalinity	(Oil + eMNR)	Reference	367	32.4	-40.7153	105.5153	26.33415	4	1.230342	0.285977
Alkalinity	(Oil + eMNR)	Reference	403	14.84	-58.2753	87.95532	26.33415	4	0.563527	0.603168
Alkalinity	(Oil + eMNR)	Reference	452	15.92	-57.1953	89.03532	26.33415	4	0.604538	0.578102

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Ammonia	(Oil + eMNR)	Reference	-3	24	-20.4617	68.46167	16.01388	4	1.4987	0.20832
Ammonia	(Oil + eMNR)	Reference	11	19	-25.4617	63.46167	16.01388	4	1.186471	0.301091
Ammonia	(Oil + eMNR)	Reference	25	6.333333	-38.1283	50.795	16.01388	4	0.39549	0.712667
Ammonia	(Oil + eMNR)	Reference	39	0.666667	-43.795	45.12833	16.01388	4	0.041631	0.968788
Ammonia	(Oil + eMNR)	Reference	53	1.333333	-43.1283	45.795	16.01388	4	0.083261	0.937644
Ammonia	(Oil + eMNR)	Reference	6	31.3333333	-13.1283	75.795	16.01388	4	1.956636	0.122025
Ammonia	(Oil + eMNR)	Reference	68	0.333333	-44.1283	44.795	16.01388	4	0.020815	0.98439
Ammonia	(Oil + eMNR)	Reference	346	1.00E+00	-6.57539	8.57539	2.728449	4	3.67E-01	0.732549
Ammonia	(Oil + eMNR)	Reference	367	3.33E-01	-7.24206	7.908724	2.728449	4	1.22E-01	0.908657
Ammonia	(Oil + eMNR)	Reference	403	3.33E-01	-7.24206	7.908724	2.728449	4	1.22E-01	0.908657
Ammonia	(Oil + eMNR)	Reference	452	-2.66E-15	-7.57539	7.57539	2.728449	4	-9.77E-16	1
Calcium	(Oil + eMNR)	Reference	-3	0.623333	-1.06055	2.30722	0.60649	4	1.027772	0.362143
Calcium	(Oil + eMNR)	Reference	11	0.63	-1.05389	2.313886	0.60649	4	1.038764	0.357579
Calcium	(Oil + eMNR)	Reference	25	0.9	-0.78389	2.583886	0.60649	4	1.483949	0.211983

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Calcium	(Oil + eMNR)	Reference	39	0.8	-0.88389	2.483886	0.60649	4	1.319065	0.257591
Calcium	(Oil + eMNR)	Reference	53	0.66	-1.02389	2.343886	0.60649	4	1.088229	0.337672
Calcium	(Oil + eMNR)	Reference	6	0.6533333	-1.03055	2.33722	0.60649	4	1.077237	0.342008
Calcium	(Oil + eMNR)	Reference	68	0.6033333	-1.08055	2.28722	0.60649	4	0.994795	0.376141
Calcium	(Oil + eMNR)	Reference	346	0.57	-0.49465	1.634646	0.383457	4	1.486479	0.21135
Calcium	(Oil + eMNR)	Reference	367	0.49	-0.57465	1.554646	0.383457	4	1.27785	0.270423
Calcium	(Oil + eMNR)	Reference	403	0.4333333	-0.63131	1.497979	0.383457	4	1.130072	0.321619
Calcium	(Oil + eMNR)	Reference	452	0.4033333	-0.66131	1.467979	0.383457	4	1.051836	0.352219
Chlorine	(Oil + eMNR)	Reference	-3	0.00666667	-0.16651	0.179844	0.062374	4	0.106883	0.920028
Chlorine	(Oil + eMNR)	Reference	11	-0.07333333	-0.24651	0.099844	0.062374	4	-1.17571	0.304911
Chlorine	(Oil + eMNR)	Reference	25	-0.06333333	-0.23651	0.109844	0.062374	4	-1.01539	0.367346
Chlorine	(Oil + eMNR)	Reference	39	-0.05666667	-0.22984	0.11651	0.062374	4	-0.9085	0.415
Chlorine	(Oil + eMNR)	Reference	53	-0.04333333	-0.21651	0.129844	0.062374	4	-0.69474	0.525463
Chlorine	(Oil + eMNR)	Reference	6	-0.02666667	-0.19984	0.14651	0.062374	4	-0.42753	0.691004

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Chlorine	(Oil + eMNR)	Reference	68	-0.033 33333	-0.20651	0.139844	0.062374	4	-0.53441	0.621377
Chlorine	(Oil + eMNR)	Reference	346	0.003 33333	-0.0685	0.07517	0.025874	4	0.128831	0.903709
Chlorine	(Oil + eMNR)	Reference	367	-0.013 33333	-0.08517	0.058503	0.025874	4	-0.51533	0.633497
Chlorine	(Oil + eMNR)	Reference	403	0.01	-0.06184	0.081837	0.025874	4	0.386494	0.71881
Chlorine	(Oil + eMNR)	Reference	452	0.003 33333	-0.0685	0.07517	0.025874	4	0.128831	0.903709
DIC	(Oil + eMNR)	Reference	-3	54	-115.375	223.3747	61.00416	4	0.885186	0.426061
DIC	(Oil + eMNR)	Reference	11	60.33 333	-109.041	229.708	61.00416	4	0.989004	0.378648
DIC	(Oil + eMNR)	Reference	25	65.66 667	-103.708	235.0414	61.00416	4	1.076429	0.342328
DIC	(Oil + eMNR)	Reference	39	81.33 333	-88.0414	250.708	61.00416	4	1.333242	0.253315
DIC	(Oil + eMNR)	Reference	53	71	-98.3747	240.3747	61.00416	4	1.163855	0.309172
DIC	(Oil + eMNR)	Reference	6	86.66 667	-82.708	256.0414	61.00416	4	1.420668	0.22845
DIC	(Oil + eMNR)	Reference	68	50.33 333	-119.041	219.708	61.00416	4	0.82508	0.455688
DIC	(Oil + eMNR)	Reference	346	44.33 333	-47.6311	136.2978	33.12309	4	1.338442	0.251765
DIC	(Oil + eMNR)	Reference	367	2	-89.9644	93.96443	33.12309	4	0.060381	0.954749

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
DIC	(Oil + eMNR)	Reference	403	25.66667	-66.2978	117.6311	33.12309	4	0.774888	0.481664
DIC	(Oil + eMNR)	Reference	452	15.66667	-76.2978	107.6311	33.12309	4	0.472983	0.66088
DOC	(Oil + eMNR)	Reference	-3	10.33333	-222.968	243.6345	84.02872	4	0.122974	0.908059
DOC	(Oil + eMNR)	Reference	11	63	-170.301	296.3011	84.02872	4	0.749744	0.495098
DOC	(Oil + eMNR)	Reference	25	77	-156.301	310.3011	84.02872	4	0.916353	0.41133
DOC	(Oil + eMNR)	Reference	39	45	-188.301	278.3011	84.02872	4	0.535531	0.620672
DOC	(Oil + eMNR)	Reference	53	57	-176.301	290.3011	84.02872	4	0.67834	0.534772
DOC	(Oil + eMNR)	Reference	6	78.66667	-154.635	311.9678	84.02872	4	0.936188	0.402178
DOC	(Oil + eMNR)	Reference	68	78.33333	-154.968	311.6345	84.02872	4	0.932221	0.403995
DOC	(Oil + eMNR)	Reference	346	151.33333	-454.41	757.0761	218.1721	4	0.693642	0.526081
DOC	(Oil + eMNR)	Reference	367	208.33333	-397.41	814.0761	218.1721	4	0.954904	0.393701
DOC	(Oil + eMNR)	Reference	403	209.66667	-396.076	815.4095	218.1721	4	0.961015	0.390966
DOC	(Oil + eMNR)	Reference	452	82.33333	-523.41	688.0761	218.1721	4	0.377378	0.725062
Dissolved Oxygen	(Oil+eMNR)	Reference	-2	-0.666666667	-0.85409	2.187422	0.547735	4	-1.21713	0.29045

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Dissolved Oxygen	(Oil+eMNR)	Reference	11	-0.49333333	-1.02742	2.014089	0.547735	4	-0.90068	0.418684
Dissolved Oxygen	(Oil+eMNR)	Reference	18	0.43333333	-1.95409	1.087422	0.547735	4	0.791137	0.473131
Dissolved Oxygen	(Oil+eMNR)	Reference	25	-0.51666667	-1.00409	2.037422	0.547735	4	-0.94328	0.398948
Dissolved Oxygen	(Oil+eMNR)	Reference	32	-0.28666667	-1.23409	1.807422	0.547735	4	-0.52337	0.628373
Dissolved Oxygen	(Oil+eMNR)	Reference	39	-0.35666667	-1.16409	1.877422	0.547735	4	-0.65117	0.550455
Dissolved Oxygen	(Oil+eMNR)	Reference	46	-0.51333333	-1.00742	2.034089	0.547735	4	-0.93719	0.401719
Dissolved Oxygen	(Oil+eMNR)	Reference	53	-0.70628732	-0.76902	2.18159	0.531364	4	-1.3292	0.254528
Dissolved Oxygen	(Oil+eMNR)	Reference	60	-0.15	-1.37076	1.670756	0.547735	4	-0.27386	0.797756
Dissolved Oxygen	(Oil+eMNR)	Reference	67	-0.53333333	-2.05409	0.987422	0.547735	4	-0.97371	0.385338
Dissolved Oxygen	(Oil+eMNR)	Reference	74	-0.13706257	-1.43784	1.171968	0.567238	4	-0.24163	0.820947
Dissolved Oxygen	(Oil+eMNR)	Reference	81	-0.42666667	-1.09409	1.947422	0.547735	4	-0.77897	0.479511
Dissolved Oxygen	(Oil+eMNR)	Reference	88	-0.41666667	-1.10409	1.937422	0.547735	4	-0.76071	0.489205
Dissolved Oxygen	(Oil+eMNR)	Reference	362	-0.80666667	-2.61903	1.005695	0.652763	4	-1.23577	0.284156
Dissolved Oxygen	(Oil+eMNR)	Reference	390	0.72	-2.53236	1.092361	0.652763	4	1.103003	0.331922

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Dissolved Oxygen	(Oil+eMNR)	Reference	412	0.08	-1.732236	1.892361	0.652763	4	0.122556	0.90837
Iron	(Oil + eMNR)	Reference	-3	0.00333333	-0.0673	0.0684	0.023435	4	0.142237	0.89377
Iron	(Oil + eMNR)	Reference	11	0.04	-0.02507	0.105066	0.023435	4	1.706838	0.163044
Iron	(Oil + eMNR)	Reference	25	0.03	-0.03507	0.095066	0.023435	4	1.280128	0.269698
Iron	(Oil + eMNR)	Reference	39	0.02	-0.04507	0.085066	0.023435	4	0.853419	0.441519
Iron	(Oil + eMNR)	Reference	53	0.02	-0.04507	0.085066	0.023435	4	0.853419	0.441519
Iron	(Oil + eMNR)	Reference	6	0.04333333	-0.02173	0.1084	0.023435	4	1.849074	0.138138
Iron	(Oil + eMNR)	Reference	68	0.00666667	-0.0584	0.071733	0.023435	4	0.284473	0.790168
Iron	(Oil + eMNR)	Reference	346	0.04	-0.08013	0.160135	0.043269	4	0.924446	0.407575
Iron	(Oil + eMNR)	Reference	367	0.05666667	-0.06347	0.176801	0.043269	4	1.309631	0.260475
Iron	(Oil + eMNR)	Reference	403	0.04666667	-0.07347	0.166801	0.043269	4	1.07852	0.341499
Iron	(Oil + eMNR)	Reference	452	0.01333333	-0.1068	0.133468	0.043269	4	0.308149	0.773349
Magnesium	(Oil + eMNR)	Reference	-3	-0.24666667	-0.85907	0.365732	0.220569	4	-1.11832	0.326056
Magnesium	(Oil + eMNR)	Reference	11	0.09666667	-0.51573	0.709065	0.220569	4	0.43826	0.683827

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Magnesium	(Oil + eMNR)	Reference	25	0.11	-0.5024	0.722398	0.220569	4	0.49871	0.644162
Magnesium	(Oil + eMNR)	Reference	39	0.09333333	-0.51907	0.705732	0.220569	4	0.423148	0.693947
Magnesium	(Oil + eMNR)	Reference	53	-0.24333333	-0.85573	0.369065	0.220569	4	-1.10321	0.331844
Magnesium	(Oil + eMNR)	Reference	6	0.14	-0.4724	0.752398	0.220569	4	0.634721	0.5601
Magnesium	(Oil + eMNR)	Reference	68	0.10333333	-0.50907	0.715732	0.220569	4	0.468485	0.663828
Magnesium	(Oil + eMNR)	Reference	346	0.12	-0.17211	0.412114	0.105211	4	1.140561	0.317706
Magnesium	(Oil + eMNR)	Reference	367	0.1	-0.19211	0.392114	0.105211	4	0.950467	0.395697
Magnesium	(Oil + eMNR)	Reference	403	0.05	-0.24211	0.342114	0.105211	4	0.475234	0.659408
Magnesium	(Oil + eMNR)	Reference	452	0.06333333	-0.22878	0.355447	0.105211	4	0.601963	0.579655
Manganese	(Oil + eMNR)	Reference	-3	2.30E-02	-0.11265	0.158717	0.048869	4	4.71E-01	0.661967
Manganese	(Oil + eMNR)	Reference	11	2.10E-02	-0.11472	0.15665	0.048869	4	4.29E-01	0.689996
Manganese	(Oil + eMNR)	Reference	25	1.91E-02	-0.11655	0.154817	0.048869	4	3.92E-01	0.715375
Manganese	(Oil + eMNR)	Reference	39	5.27E-02	-0.08302	0.18835	0.048869	4	1.08E+00	0.341824
Manganese	(Oil + eMNR)	Reference	53	1.04E-17	-0.13568	0.135683	0.048869	4	2.13E-16	1

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Manganese	(Oil + eMNR)	Reference	6	4.91E-02	-0.08655	0.184817	0.048869	4	1.01E+00	0.371589
Manganese	(Oil + eMNR)	Reference	68	7.20E-03	-0.12848	0.142883	0.048869	4	1.47E-01	0.889998
Manganese	(Oil + eMNR)	Reference	346	0.12	-0.17211	0.412114	0.105211	4	1.140561	0.317706
Manganese	(Oil + eMNR)	Reference	367	0.1	-0.19211	0.392114	0.105211	4	0.950467	0.395697
Manganese	(Oil + eMNR)	Reference	403	0.05	-0.24211	0.342114	0.105211	4	0.475234	0.659408
Manganese	(Oil + eMNR)	Reference	452	0.06333333	-0.22878	0.355447	0.105211	4	0.601963	0.579655
Nitrate	(Oil + eMNR)	Reference	-3	4.3333333	-5.02666	13.69332	3.371213	4	1.285393	0.268029
Nitrate	(Oil + eMNR)	Reference	11	5.3333333	-4.02666	14.69332	3.371213	4	1.582022	0.188807
Nitrate	(Oil + eMNR)	Reference	25	-0.3333333	-9.69332	9.026655	3.371213	4	-0.09888	0.925993
Nitrate	(Oil + eMNR)	Reference	39	-1	-10.36	8.359989	3.371213	4	-0.29663	0.781514
Nitrate	(Oil + eMNR)	Reference	53	0.3333333	-9.02666	9.693322	3.371213	4	0.098876	0.925993
Nitrate	(Oil + eMNR)	Reference	6	5.6666667	-3.69332	15.02666	3.371213	4	1.680898	0.168078
Nitrate	(Oil + eMNR)	Reference	68	0.6666667	-8.69332	10.02666	3.371213	4	0.197753	0.852882
Nitrate	(Oil + eMNR)	Reference	346	0.6666667	-4.46538	5.798711	1.848423	4	0.360668	0.736588

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Nitrate	(Oil + eMNR)	Reference	367	-4	-9.13204	1.132044	1.848423	4	-2.16401	0.096455
Nitrate	(Oil + eMNR)	Reference	403	-2.6666667	-7.79871	2.465378	1.848423	4	-1.44267	0.222583
Nitrate	(Oil + eMNR)	Reference	452	-1.3333333	-6.46538	3.798711	1.848423	4	-0.72134	0.510613
Nitrite	(Oil + eMNR)	Reference	-3	0.26666667	-0.2592	0.79253	0.189402	4	1.407942	0.231913
Nitrite	(Oil + eMNR)	Reference	11	0.26666667	-0.2592	0.79253	0.189402	4	1.407942	0.231913
Nitrite	(Oil + eMNR)	Reference	25	0.4	-0.12586	0.925864	0.189402	4	2.111913	0.10227
Nitrite	(Oil + eMNR)	Reference	39	0.06666667	-0.4592	0.59253	0.189402	4	0.351986	0.74261
Nitrite	(Oil + eMNR)	Reference	53	0.06666667	-0.4592	0.59253	0.189402	4	0.351986	0.74261
Nitrite	(Oil + eMNR)	Reference	6	0.3	-0.22586	0.825864	0.189402	4	1.583935	0.188382
Nitrite	(Oil + eMNR)	Reference	68	-0.23333333	-0.7592	0.29253	0.189402	4	-1.23195	0.285437
Nitrite	(Oil + eMNR)	Reference	346	0.16666667	-0.9687	1.302033	0.408928	4	0.40757	0.70446
Nitrite	(Oil + eMNR)	Reference	367	0.43333333	-0.70203	1.5687	0.408928	4	1.059681	0.349037
Nitrite	(Oil + eMNR)	Reference	403	0.26666667	-0.8687	1.402033	0.408928	4	0.652111	0.549904
Nitrite	(Oil + eMNR)	Reference	452	0.06666667	-1.0687	1.202033	0.408928	4	0.163028	0.878402

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Particulate Carbon	(Oil + eMNR)	Reference	-3	64.3	-675.869	804.4685	266.5886	4	0.241196	0.821263
Particulate Carbon	(Oil + eMNR)	Reference	11	157.53333	-582.635	897.7019	266.5886	4	0.590923	0.586347
Particulate Carbon	(Oil + eMNR)	Reference	25	-10.6	-750.769	729.5685	266.5886	4	-0.03976	0.970189
Particulate Carbon	(Oil + eMNR)	Reference	39	-57.7	-797.869	682.4685	266.5886	4	-0.21644	0.839236
Particulate Carbon	(Oil + eMNR)	Reference	53	-414.06667	-1154.24	326.1019	266.5886	4	-1.5532	0.195334
Particulate Carbon	(Oil + eMNR)	Reference	6	-32.03333	-772.202	708.1352	266.5886	4	-0.12016	0.91015
Particulate Carbon	(Oil + eMNR)	Reference	68	-22.5	-762.669	717.6685	266.5886	4	-0.0844	0.936794
Particulate Carbon	(Oil + eMNR)	Reference	346	-57.33333	-838.097	723.4301	281.2098	4	-0.20388	0.848399
Particulate Carbon	(Oil + eMNR)	Reference	367	-59.66667	-840.43	721.0968	281.2098	4	-0.21218	0.842341
Particulate Carbon	(Oil + eMNR)	Reference	403	22	-758.764	802.7635	281.2098	4	0.078233	0.9414
Particulate Carbon	(Oil + eMNR)	Reference	452	368.33333	-412.43	1149.097	281.2098	4	1.309817	0.260418
Particulate Nitrogen	(Oil + eMNR)	Reference	-3	9.9666667	-64.4866	84.41997	26.81606	4	0.371668	0.728991
Particulate Nitrogen	(Oil + eMNR)	Reference	11	16.7666667	-57.6866	91.21997	26.81606	4	0.625247	0.565709
Particulate Nitrogen	(Oil + eMNR)	Reference	25	-17.7333333	-92.1866	56.71997	26.81606	4	-0.6613	0.544572

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Particulate Nitrogen	(Oil + eMNR)	Reference	39	-17.2333333	-91.6866	57.21997	26.81606	4	-0.64265	0.555435
Particulate Nitrogen	(Oil + eMNR)	Reference	53	-44.7	-119.153	29.75331	26.81606	4	-1.66691	0.17086
Particulate Nitrogen	(Oil + eMNR)	Reference	6	-3.7666667	-78.22	70.68664	26.81606	4	-0.14046	0.895084
Particulate Nitrogen	(Oil + eMNR)	Reference	68	0.7666667	-73.6866	75.21997	26.81606	4	0.02859	0.978561
Particulate Nitrogen	(Oil + eMNR)	Reference	346	-19.33333	-94.9557	56.28904	27.23712	4	-0.70982	0.517007
Particulate Nitrogen	(Oil + eMNR)	Reference	367	-10.66667	-86.289	64.95571	27.23712	4	-0.39162	0.715305
Particulate Nitrogen	(Oil + eMNR)	Reference	403	-23.66667	-99.289	51.95571	27.23712	4	-0.86891	0.433924
Particulate Nitrogen	(Oil + eMNR)	Reference	452	37.33333	-38.289	112.9557	27.23712	4	1.370678	0.242357
Particulate Phosphorus	(Oil + eMNR)	Reference	-3	0.6666667	-3.51632	4.849657	1.506599	4	0.442498	0.681004
Particulate Phosphorus	(Oil + eMNR)	Reference	11	1	-3.18299	5.18299	1.506599	4	0.663747	0.543155
Particulate Phosphorus	(Oil + eMNR)	Reference	25	-0.3333333	-4.51632	3.849657	1.506599	4	-0.22125	0.835734
Particulate Phosphorus	(Oil + eMNR)	Reference	39	0.3333333	-3.84966	4.516323	1.506599	4	0.221249	0.835734
Particulate	(Oil + eMNR)	Reference	53	-3	-7.18299	1.18299	1.506599	4	-1.99124	0.117284

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Phosphorus										
Particulate Phosphorus	(Oil + eMNR)	Reference	6	1	-3.18299	5.18299	1.506599	4	0.663747	0.543155
Particulate Phosphorus	(Oil + eMNR)	Reference	68	0	-4.18299	4.18299	1.506599	4	0	1
Particulate Phosphorus	(Oil + eMNR)	Reference	346	2.6666667	-3.9426	9.275928	2.380476	4	1.120224	0.325333
Particulate Phosphorus	(Oil + eMNR)	Reference	367	-0.6666667	-7.27593	5.942595	2.380476	4	-0.28006	0.793321
Particulate Phosphorus	(Oil + eMNR)	Reference	403	-2.3333333	-8.9426	4.275928	2.380476	4	-0.9802	0.382488
Particulate Phosphorus	(Oil + eMNR)	Reference	452	3.3333333	-3.27593	9.942595	2.380476	4	1.40028	0.234023
pH	(Oil+eMNR)	Reference	-2	0.06	-0.36737	0.247372	0.110707	4	0.54197	0.616618
pH	(Oil+eMNR)	Reference	11	-0.19666667	-0.11071	0.504039	0.110707	4	-1.77646	0.150304
pH	(Oil+eMNR)	Reference	18	0.26666667	-0.57404	0.040706	0.110707	4	2.408757	0.073654
pH	(Oil+eMNR)	Reference	25	0.13666667	-0.44404	0.170706	0.110707	4	1.234488	0.284586
pH	(Oil+eMNR)	Reference	32	-0.02	-0.28737	0.327372	0.110707	4	-0.18066	0.865421

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
pH	(Oil+eMNR)	Reference	39	-0.04666667	-0.26071	0.354039	0.110707	4	-0.42153	0.695033
pH	(Oil+eMNR)	Reference	46	0.08333333	-0.39071	0.224039	0.110707	4	0.752736	0.493484
pH	(Oil+eMNR)	Reference	53	0.0325	-0.32002	0.255021	0.103557	4	0.313837	0.769331
pH	(Oil+eMNR)	Reference	60	0.13333333	-0.44071	0.174039	0.110707	4	1.204378	0.294833
pH	(Oil+eMNR)	Reference	67	0.11	-0.41737	0.197372	0.110707	4	0.993612	0.376652
pH	(Oil+eMNR)	Reference	74	0.14	-0.46602	0.186018	0.117423	4	1.192274	0.299049
pH	(Oil+eMNR)	Reference	81	0.29	-0.01737	0.597372	0.110707	4	2.619523	0.058829
pH	(Oil+eMNR)	Reference	88	-0.27333333	-0.03404	0.580706	0.110707	4	-2.46898	0.069023
pH	(Oil+eMNR)	Reference	362	0.11666667	-0.12544	0.358771	0.087199	4	1.337932	0.251916
pH	(Oil+eMNR)	Reference	390	-0.10666667	-0.34877	0.135437	0.087199	4	-1.22325	0.288369
pH	(Oil+eMNR)	Reference	412	-0.02666667	-0.26877	0.215437	0.087199	4	-0.30581	0.775002
Potassium	(Oil + eMNR)	Reference	-3	0.03333333	-0.23848	0.305143	0.097899	4	0.340489	0.750619
Potassium	(Oil + eMNR)	Reference	11	-0.00666667	-0.27848	0.265143	0.097899	4	-0.0681	0.948976
Potassium	(Oil + eMNR)	Reference	25	0.08333333	-0.18848	0.355143	0.097899	4	0.851221	0.442605

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Potassium	(Oil + eMNR)	Reference	39	0.15	-0.12181	0.42181	0.097899	4	1.532198	0.200238
Potassium	(Oil + eMNR)	Reference	53	0.013333333	-0.25848	0.285143	0.097899	4	0.136195	0.898246
Potassium	(Oil + eMNR)	Reference	6	0.05	-0.22181	0.32181	0.097899	4	0.510733	0.636434
Potassium	(Oil + eMNR)	Reference	68	0.01	-0.26181	0.28181	0.097899	4	0.102147	0.923556
Potassium	(Oil + eMNR)	Reference	346	0.033333333	-0.11496	0.181627	0.053411	4	0.624087	0.566399
Potassium	(Oil + eMNR)	Reference	367	0.006666667	-0.14163	0.15496	0.053411	4	0.124817	0.90669
Potassium	(Oil + eMNR)	Reference	403	-0.013333333	-0.16163	0.13496	0.053411	4	-0.24963	0.815166
Potassium	(Oil + eMNR)	Reference	452	-0.073333333	-0.22163	0.07496	0.053411	4	-1.37299	0.241695
Sodium	(Oil + eMNR)	Reference	-3	0.003333333	-0.23947	0.246135	0.087451	4	0.038117	0.971421
Sodium	(Oil + eMNR)	Reference	11	-0.03	-0.2728	0.212802	0.087451	4	-0.34305	0.748831
Sodium	(Oil + eMNR)	Reference	25	-0.123333333	-0.36614	0.119469	0.087451	4	-1.41032	0.231262
Sodium	(Oil + eMNR)	Reference	39	-0.076666667	-0.31947	0.166135	0.087451	4	-0.87668	0.430154
Sodium	(Oil + eMNR)	Reference	53	0.01	-0.2328	0.252802	0.087451	4	0.11435	0.91447
Sodium	(Oil + eMNR)	Reference	6	-0.013333333	-0.25614	0.229469	0.087451	4	-0.15247	0.8862

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Sodium	(Oil + eMNR)	Reference	68	0.03666667	-0.20614	0.279469	0.087451	4	0.419284	0.696547
Sodium	(Oil + eMNR)	Reference	346	1.67E-02	-0.32265	0.355985	0.122213	4	1.36E-01	0.898114
Sodium	(Oil + eMNR)	Reference	367	1.67E-02	-0.32265	0.355985	0.122213	4	1.36E-01	0.898114
Sodium	(Oil + eMNR)	Reference	403	-4.67E-02	-0.38599	0.292652	0.122213	4	-3.82E-01	0.721995
Sodium	(Oil + eMNR)	Reference	452	-3.47E-18	-0.33932	0.339319	0.122213	4	-2.84E-17	1
Specific conductivity	(Oil+eMNR)	Reference	-2	3.8	-18.9568	11.35684	5.45908	4	0.696088	0.524701
Specific conductivity	(Oil+eMNR)	Reference	11	3.7	-18.8568	11.45684	5.45908	4	0.67777	0.535097
Specific conductivity	(Oil+eMNR)	Reference	18	5.566666667	-20.7235	9.59017	5.45908	4	1.019708	0.365523
Specific conductivity	(Oil+eMNR)	Reference	25	4.366666667	-19.5235	10.79017	5.45908	4	0.799891	0.468584
Specific conductivity	(Oil+eMNR)	Reference	32	5.4	-20.5568	9.756837	5.45908	4	0.989178	0.378572
Specific conductivity	(Oil+eMNR)	Reference	39	4.766666667	-19.9235	10.39017	5.45908	4	0.873163	0.431859
Specific conductivity	(Oil+eMNR)	Reference	46	3.966666667	-19.1235	11.19017	5.45908	4	0.726618	0.507701
Specific conductivity	(Oil+eMNR)	Reference	53	4.627131763	-19.5202	10.26593	5.364077	4	0.862615	0.436998
Specific conductivity	(Oil+eMNR)	Reference	60	2.766666667	-17.9235	12.39017	5.45908	4	0.506801	0.638955

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Specific conductivity	(Oil+eMNR)	Reference	67	3.866 66667	-19.0235	11.29017	5.45908	4	0.7083	0.517852
Specific conductivity	(Oil+eMNR)	Reference	74	6.753 92324	-22.2319	8.724052	5.574746	4	1.211521	0.292371
Specific conductivity	(Oil+eMNR)	Reference	81	2.633 33333	-17.7902	12.5235	5.45908	4	0.482377	0.654747
Specific conductivity	(Oil+eMNR)	Reference	88	1.333 33333	-16.4902	13.8235	5.45908	4	0.244241	0.81906
SRSi	(Oil + eMNR)	Reference	-3	0.384 6667	-0.02986	0.799193	0.149301	4	2.576451	0.061558
SRSi	(Oil + eMNR)	Reference	11	0.51	0.095474	0.924526	0.149301	4	3.415918	0.026881
SRSi	(Oil + eMNR)	Reference	25	0.374	-0.04053	0.788526	0.149301	4	2.505007	0.06641
SRSi	(Oil + eMNR)	Reference	39	0.310 3333	-0.10419	0.724859	0.149301	4	2.078575	0.106194
SRSi	(Oil + eMNR)	Reference	53	0.380 6667	-0.03386	0.795193	0.149301	4	2.549659	0.063328
SRSi	(Oil + eMNR)	Reference	6	0.518	0.103474	0.932526	0.149301	4	3.469501	0.025595
SRSi	(Oil + eMNR)	Reference	68	0.328	-0.08653	0.742526	0.149301	4	2.196904	0.092973
SRSi	(Oil + eMNR)	Reference	346	-0.026	-0.198	0.146003	0.061951	4	-0.41969	0.696275
SRSi	(Oil + eMNR)	Reference	367	0.012 66667	-0.15934	0.18467	0.061951	4	0.204463	0.847974
SRSi	(Oil + eMNR)	Reference	403	0.169	-0.003	0.341003	0.061951	4	2.727966	0.052553

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
SRSi	(Oil + eMNR)	Reference	452	0.195	0.022997	0.367003	0.061951	4	3.147653	0.034592
Sulfate	(Oil + eMNR)	Reference	-3	-0.1433333	-0.70245	0.415788	0.20138	4	-0.71176	0.515926
Sulfate	(Oil + eMNR)	Reference	11	-0.18	-0.73912	0.379121	0.20138	4	-0.89383	0.421931
Sulfate	(Oil + eMNR)	Reference	25	-0.27	-0.82912	0.289121	0.20138	4	-1.34075	0.25108
Sulfate	(Oil + eMNR)	Reference	39	-0.3033333	-0.86245	0.255788	0.20138	4	-1.50627	0.206464
Sulfate	(Oil + eMNR)	Reference	53	-0.14	-0.69912	0.419121	0.20138	4	-0.6952	0.525201
Sulfate	(Oil + eMNR)	Reference	6	-0.2466667	-0.80579	0.312454	0.20138	4	-1.22488	0.287818
Sulfate	(Oil + eMNR)	Reference	68	-0.2733333	-0.83245	0.285788	0.20138	4	-1.3573	0.246218
Sulfate	(Oil + eMNR)	Reference	346	-0.2166667	-0.73056	0.297226	0.18509	4	-1.1706	0.30674
Sulfate	(Oil + eMNR)	Reference	367	-0.1933333	-0.70723	0.320559	0.18509	4	-1.04454	0.355204
Sulfate	(Oil + eMNR)	Reference	403	-0.08	-0.59389	0.433892	0.18509	4	-0.43222	0.687861
Sulfate	(Oil + eMNR)	Reference	452	-0.11	-0.62389	0.403892	0.18509	4	-0.59431	0.584291
Water temperature	(Oil+eMNR)	Reference	-2	-1.23E-16	-0.91571	0.915714	0.329815	4	-3.74E-16	1
Water temperature	(Oil+eMNR)	Reference	11	0.6	-1.51571	0.315714	0.329815	4	1.819199	0.14301

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
Water temperature	(Oil+eMNR)	Reference	18	0.23333333	-1.14905	0.682381	0.329815	4	0.707466	0.518318
Water temperature	(Oil+eMNR)	Reference	25	0.3	-1.21571	0.615714	0.329815	4	0.9096	0.414485
Water temperature	(Oil+eMNR)	Reference	32	-1.73E-16	-0.91571	0.915714	0.329815	4	-5.23E-16	1
Water temperature	(Oil+eMNR)	Reference	39	0.1	-1.01571	0.815714	0.329815	4	0.3032	0.776853
Water temperature	(Oil+eMNR)	Reference	46	0.13333333	-1.04905	0.782381	0.329815	4	0.404266	0.706699
Water temperature	(Oil+eMNR)	Reference	53	0.07524332	-0.97041	0.819919	0.322413	4	0.233375	0.826926
Water temperature	(Oil+eMNR)	Reference	60	-2.55E-15	-0.91571	0.915714	0.329815	4	-7.73E-15	1
Water temperature	(Oil+eMNR)	Reference	67	0.23333333	-1.14905	0.682381	0.329815	4	0.707466	0.518318
Water temperature	(Oil+eMNR)	Reference	74	-0.16555533	-0.77498	1.106086	0.338753	4	-0.48872	0.650625
Water temperature	(Oil+eMNR)	Reference	81	0.1	-1.01571	0.815714	0.329815	4	0.3032	0.776853
Water temperature	(Oil+eMNR)	Reference	88	0.3	-1.21571	0.615714	0.329815	4	0.9096	0.414485
Water temperature	(Oil+eMNR)	Reference	362	0.03333333	-0.90288	0.96955	0.3372	4	0.098853	0.926011
Water temperature	(Oil+eMNR)	Reference	390	0.06666667	-0.86955	1.002883	0.3372	4	0.197707	0.852915
Water temperature	(Oil+eMNR)	Reference	412	0.23333333	-0.70288	1.16955	0.3372	4	0.691974	0.527024

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
TDN	(Oil + eMNR)	Reference	-3	33	-95.9697	161.9697	46.45137	4	0.71042	0.51667
TDN	(Oil + eMNR)	Reference	11	69.666667	-59.303	198.6364	46.45137	4	1.499776	0.208055
TDN	(Oil + eMNR)	Reference	25	28.333333	-100.636	157.303	46.45137	4	0.609957	0.574842
TDN	(Oil + eMNR)	Reference	39	8.333333	-120.636	137.303	46.45137	4	0.179399	0.866345
TDN	(Oil + eMNR)	Reference	53	6	-122.97	134.9697	46.45137	4	0.129167	0.90346
TDN	(Oil + eMNR)	Reference	6	59.333333	-69.6364	188.303	46.45137	4	1.277321	0.270591
TDN	(Oil + eMNR)	Reference	68	13.666667	-115.303	142.6364	46.45137	4	0.294215	0.78323
TDN	(Oil + eMNR)	Reference	346	-89.666667	-265.268	85.93419	63.24665	4	-1.41773	0.229245
TDN	(Oil + eMNR)	Reference	367	73.333333	-102.268	248.9342	63.24665	4	1.159482	0.310758
TDN	(Oil + eMNR)	Reference	403	55.666667	-119.934	231.2675	63.24665	4	0.880152	0.428481
TDN	(Oil + eMNR)	Reference	452	8.666667	-166.934	184.2675	63.24665	4	0.13703	0.897628
TDP	(Oil + eMNR)	Reference	-3	-0.06666667	-3.53956	3.406225	1.250841	4	-0.0533	0.960051
TDP	(Oil + eMNR)	Reference	11	1.2	-2.27289	4.672891	1.250841	4	0.959355	0.391708
TDP	(Oil + eMNR)	Reference	25	1.133333333	-2.33956	4.606225	1.250841	4	0.906057	0.416149

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
TDP	(Oil + eMNR)	Reference	39	0.23333333	-3.23956	3.706225	1.250841	4	0.186541	0.861099
TDP	(Oil + eMNR)	Reference	53	0.1	-3.37289	3.572891	1.250841	4	0.079946	0.94012
TDP	(Oil + eMNR)	Reference	6	1.4	-2.07289	4.872891	1.250841	4	1.119247	0.325704
TDP	(Oil + eMNR)	Reference	68	-0.06666667	-3.53956	3.406225	1.250841	4	-0.0533	0.960051
TDP	(Oil + eMNR)	Reference	346	1.2666667	-4.09454	6.627874	1.930961	4	0.655977	0.547655
TDP	(Oil + eMNR)	Reference	367	2.7333333	-2.62787	8.094541	1.930961	4	1.41553	0.229842
TDP	(Oil + eMNR)	Reference	403	1.4333333	-3.92787	6.794541	1.930961	4	0.74229	0.499134
TDP	(Oil + eMNR)	Reference	452	0.5333333	-4.82787	5.894541	1.930961	4	0.276201	0.796077
TDS	(Oil + eMNR)	Reference	-3	-21.333333	-172.425	129.7585	54.41915	4	-0.39202	0.715034
TDS	(Oil + eMNR)	Reference	11	28.333333	-122.758	179.4251	54.41915	4	0.52065	0.630102
TDS	(Oil + eMNR)	Reference	25	25	-126.092	176.0918	54.41915	4	0.459397	0.669807
TDS	(Oil + eMNR)	Reference	39	-140	-291.092	11.09178	54.41915	4	-2.57262	0.061807
TDS	(Oil + eMNR)	Reference	53	-7	-158.092	144.0918	54.41915	4	-0.12863	0.903858
TDS	(Oil + eMNR)	Reference	6	-19.333333	-170.425	131.7585	54.41915	4	-0.35527	0.740331

Parameter	Level 1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t	p
TDS	(Oil + eMNR)	Reference	68	-4.666667	-155.758	146.4251	54.41915	4	-0.08575	0.935783
Turbidity	(Oil + eMNR)	Reference	-3	-0.05666667	-0.93994	0.826602	0.31813	4	-0.17812	0.867282
Turbidity	(Oil + eMNR)	Reference	11	0.16	-0.72327	1.043269	0.31813	4	0.50294	0.641437
Turbidity	(Oil + eMNR)	Reference	25	0.03	-0.85327	0.913269	0.31813	4	0.094301	0.929405
Turbidity	(Oil + eMNR)	Reference	39	0.08	-0.80327	0.963269	0.31813	4	0.25147	0.813842
Turbidity	(Oil + eMNR)	Reference	53	-0.58	-1.46327	0.303269	0.31813	4	-1.82316	0.142354
Turbidity	(Oil + eMNR)	Reference	6	0.02333333	-0.85994	0.906602	0.31813	4	0.073345	0.945053
Turbidity	(Oil + eMNR)	Reference	68	-0.09666667	-0.97994	0.786602	0.31813	4	-0.30386	0.776385

SI Table 1: Estimated mean difference, confidence interval, standard error, df, t statistic of phytoplankton and biofilm between eMNR and reference enclosures in the FOReSt study

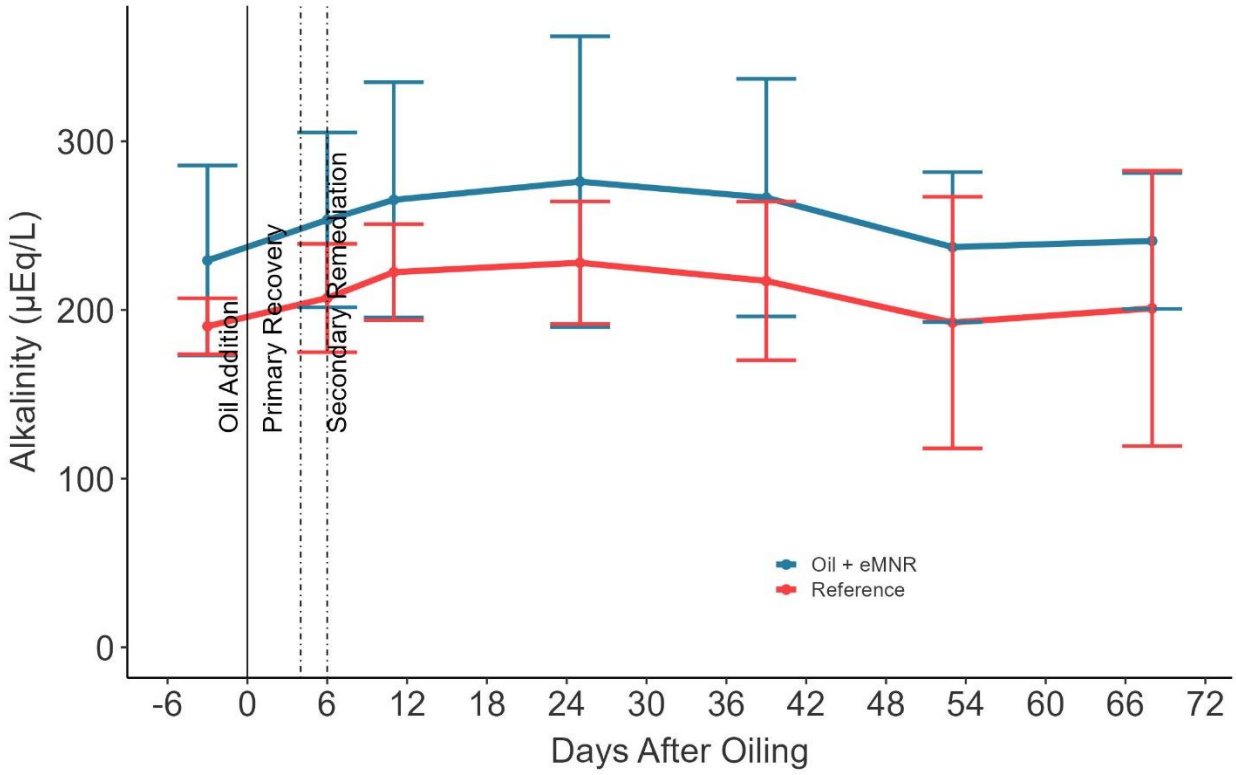
Parameter	Level1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t-statistic	p
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	-3	0.08	-1.33073	1.490732	0.508107	4	0.157447	0.882521
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	11	1.213333	-0.1974	2.624065	0.508107	4	2.387947	0.075336
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	25	-0.06333	-1.47407	1.347399	0.508107	4	-0.12465	0.906817
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	39	-0.26667	-1.6774	1.144065	0.508107	4	-0.52482	0.627449
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	53	-0.06	-1.47073	1.350732	0.508107	4	-0.11809	0.911692
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	6	0.106667	-1.30407	1.517399	0.508107	4	0.209929	0.843982
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	68	-0.23	-1.64073	1.180732	0.508107	4	-0.45266	0.674258
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	346	2.76E-16	-7.104	7.104	2.558667	4	1.08E-16	1
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	367	3.33E-03	-7.10067	7.107333	2.558667	4	1.30E-03	0.999023

Parameter	Level1	Level2	study day	Difference	CI_low	CI_high	SE	df	t-statistic	p
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	403	-7.27E-01	-7.83067	6.377333	2.558667	4	-2.84E-01	0.790504
Phytoplankton Chlorophyll-a	(Oil + eMNR)	Reference	452	4.95E+00	-2.15733	12.05067	2.558667	4	1.93E+00	0.125339
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	-1	-17.4049	-271.711	306.5205	104.1316	4	-0.16714	0.875367
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	13	178.8034	-467.919	110.3122	104.1316	4	1.717091	0.161098
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	27	99.41809	-388.534	189.6975	104.1316	4	0.954735	0.393777
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	41	87.90139	-377.017	201.2142	104.1316	4	0.844138	0.44612
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	55	156.2628	-445.378	132.8528	104.1316	4	1.500628	0.207846
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	69	137.4306	-426.546	151.685	104.1316	4	1.319778	0.257374
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	83	0.001292	-289.117	289.1143	104.1316	4	1.24E-05	0.999991
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	363	-27.9877	-394.763	450.7386	152.2634	4	0.183811	0.863104
Biofilm Chlorophyll-a	(Oil +	Reference	391	235.0558	-657.807	187.6951	152.2634	4	1.543745	0.197527

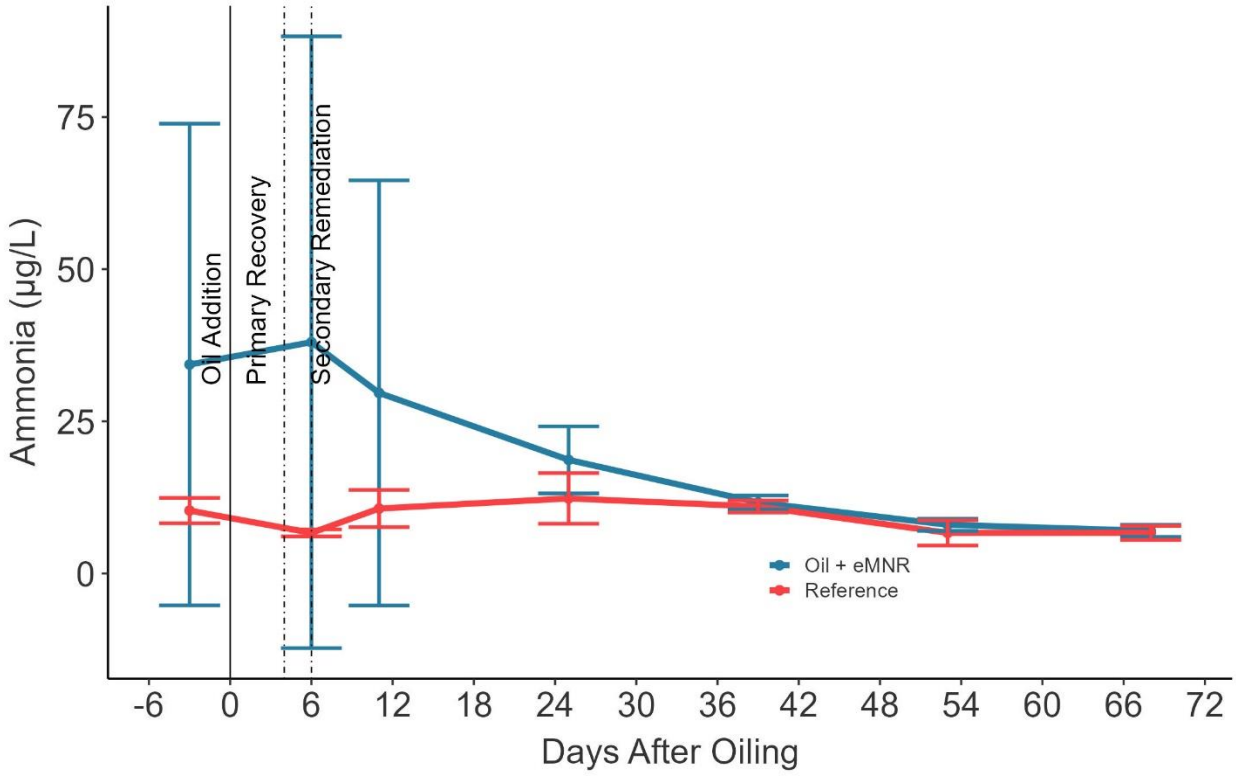
Parameter	Level1	Level2	study day	Difference	CI_low	CI_high	SE	df	t-statistic	p
	eMNR)									
Biofilm Chlorophyll-a	(Oil + eMNR)	Reference	410	300.8331	-723.584	121.9178	152.2634	4	1.975742	0.119382
Biofilm AFDM	(Oil + eMNR)	Reference	-1	-5.90056	-6.31859	18.1197	4.401003	4	-1.34073	0.251085
Biofilm AFDM	(Oil + eMNR)	Reference	13	-0.13356	-12.0856	12.3527	4.401003	4	-0.03035	0.977244
Biofilm AFDM	(Oil + eMNR)	Reference	27	0.341638	-12.5608	11.87751	4.401003	4	0.077627	0.941852
Biofilm AFDM	(Oil + eMNR)	Reference	41	1.572466	-13.7916	10.64668	4.401003	4	0.357297	0.738923
Biofilm AFDM	(Oil + eMNR)	Reference	55	3.296593	-15.5388	8.945564	4.409292	4	0.747647	0.496231
Biofilm AFDM	(Oil + eMNR)	Reference	69	-2.41743	-9.80172	14.63657	4.401003	4	-0.54929	0.612031
Biofilm AFDM	(Oil + eMNR)	Reference	83	-4.48552	-7.73362	16.70467	4.401003	4	-1.0192	0.365735
Biofilm AFDM	(Oil + eMNR)	Reference	363	2.916294	-15.5234	9.690805	4.540734	4	0.642252	0.555669
Biofilm AFDM	(Oil + eMNR)	Reference	391	0.870835	-13.4779	11.73626	4.540734	4	0.191783	0.857255

Parameter	Level1	Level 2	study day	Difference	CI_low	CI_high	SE	df	t-statistic	p
Biofilm AFDM	(Oil + eMNR)	Reference	410	2.945534	-15.5526	9.661565	4.540734	4	0.648691	0.551899

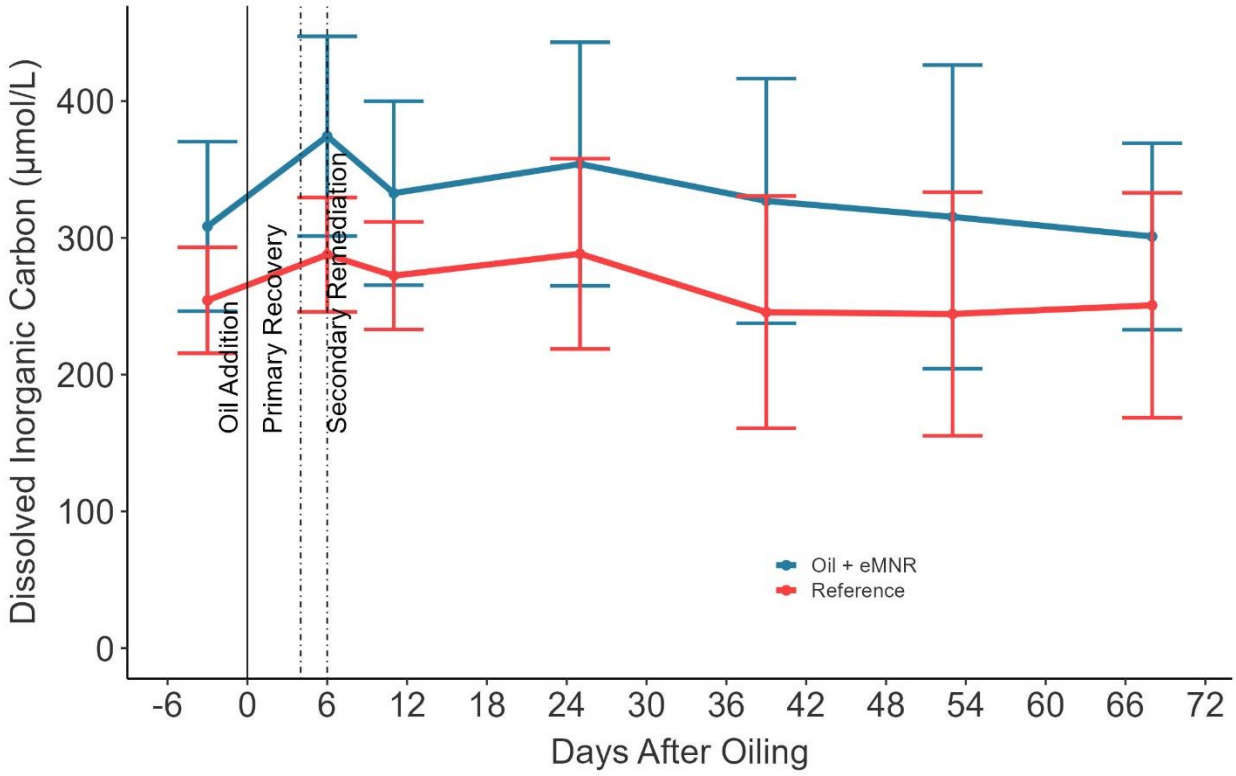
2021 Water quality parameter figures



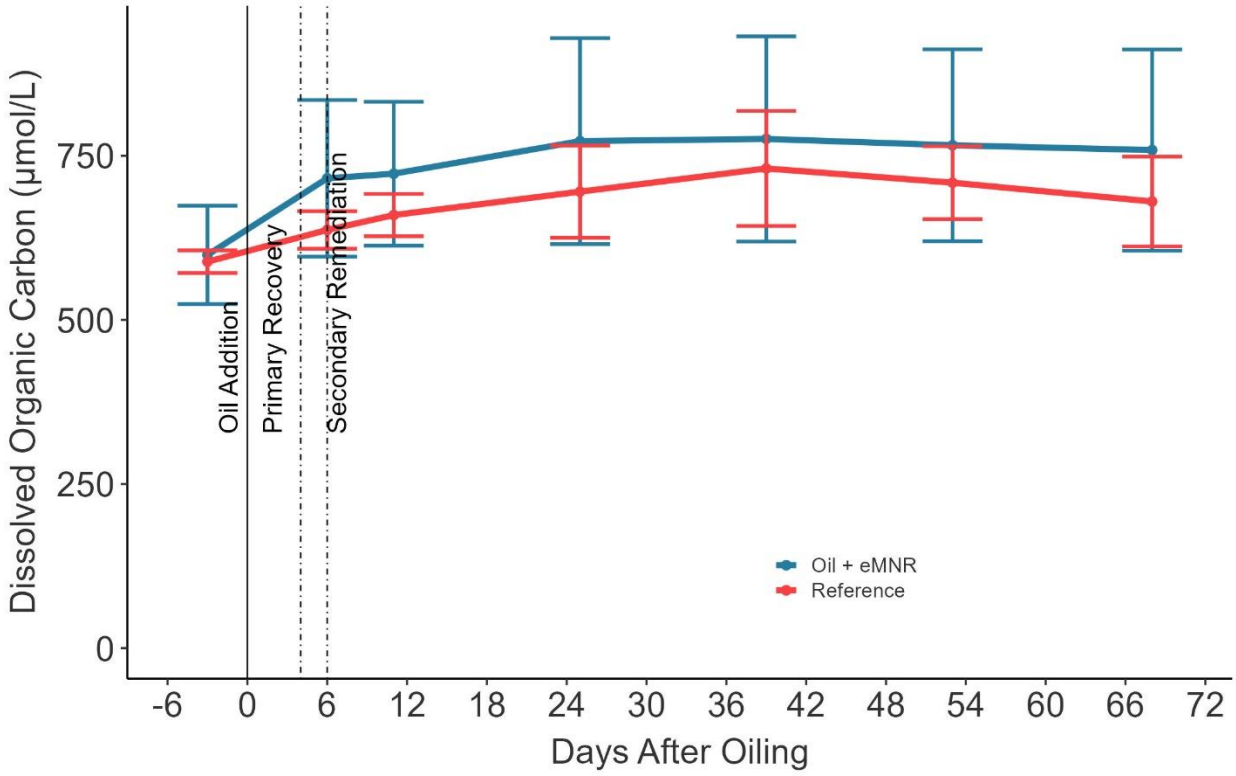
SI Figure 1: Alkalinity concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



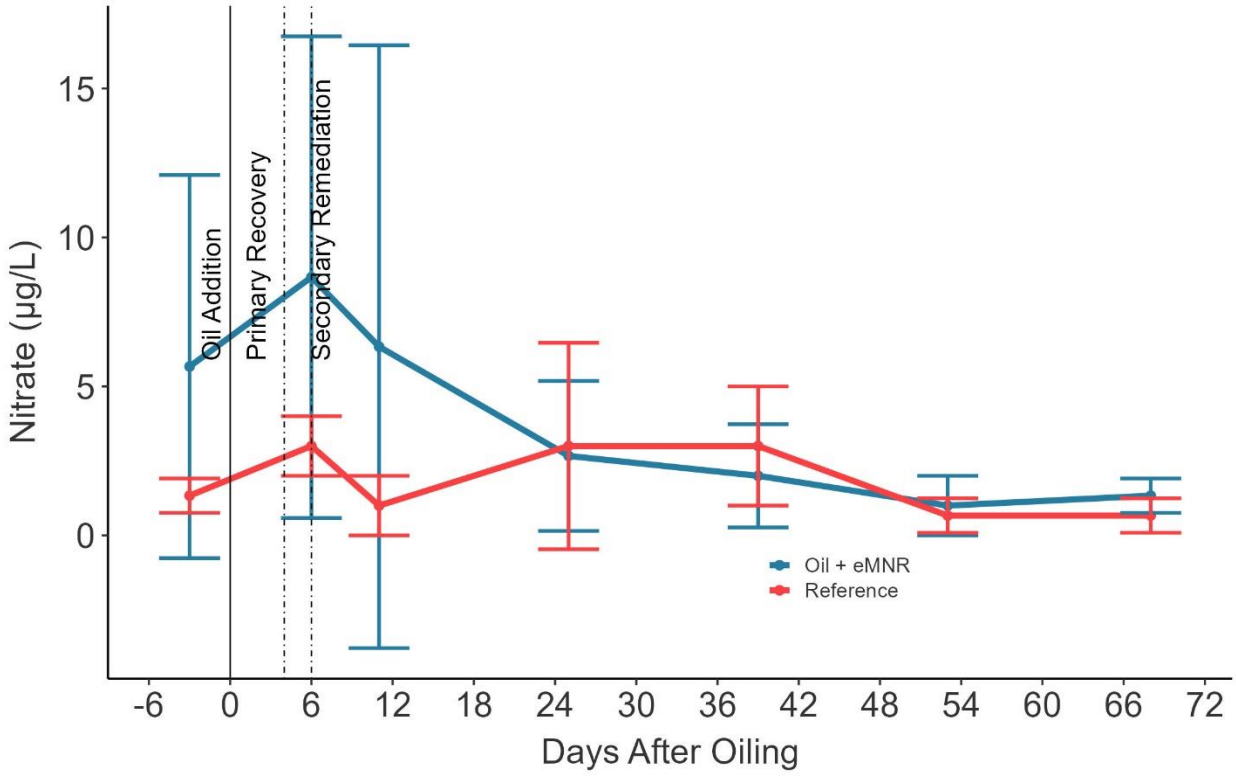
SI Figure 2: Ammonia concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



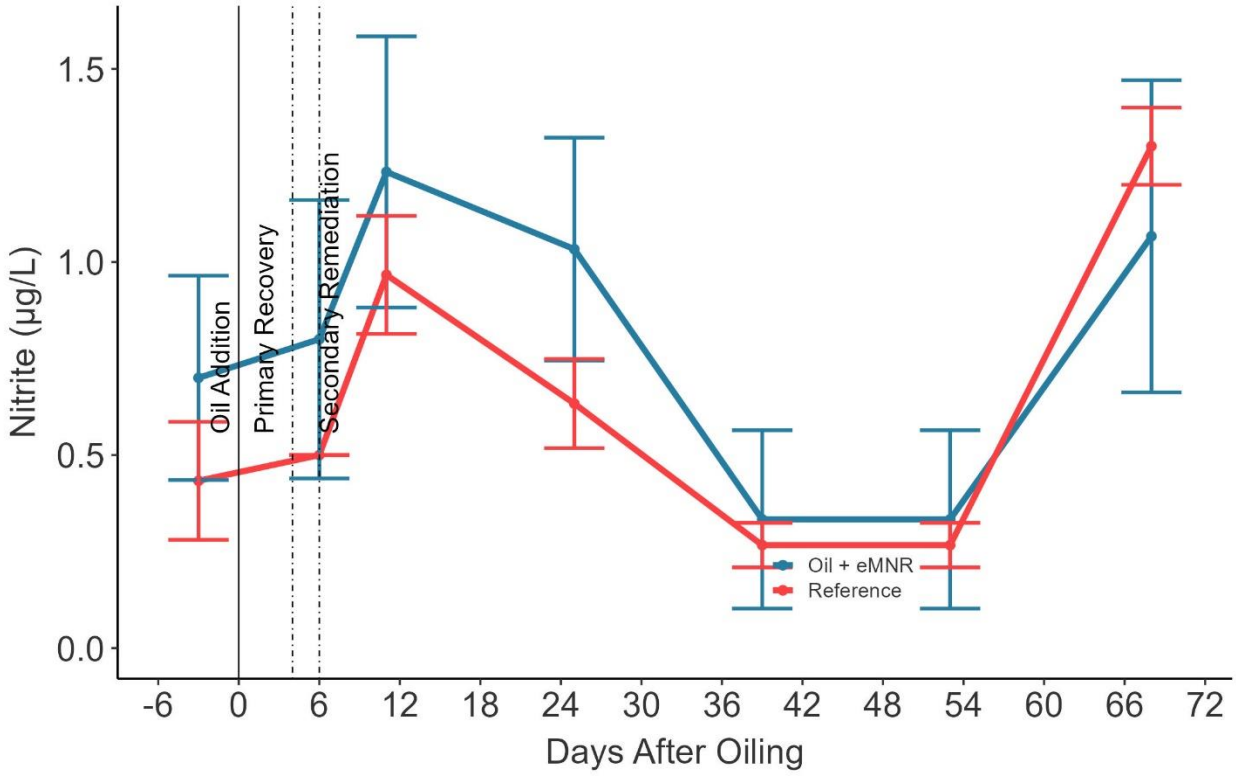
SI Figure 3: Dissolved Inorganic Carbon (DIC) concentration within reference and treatment enclosures during 2021 the FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



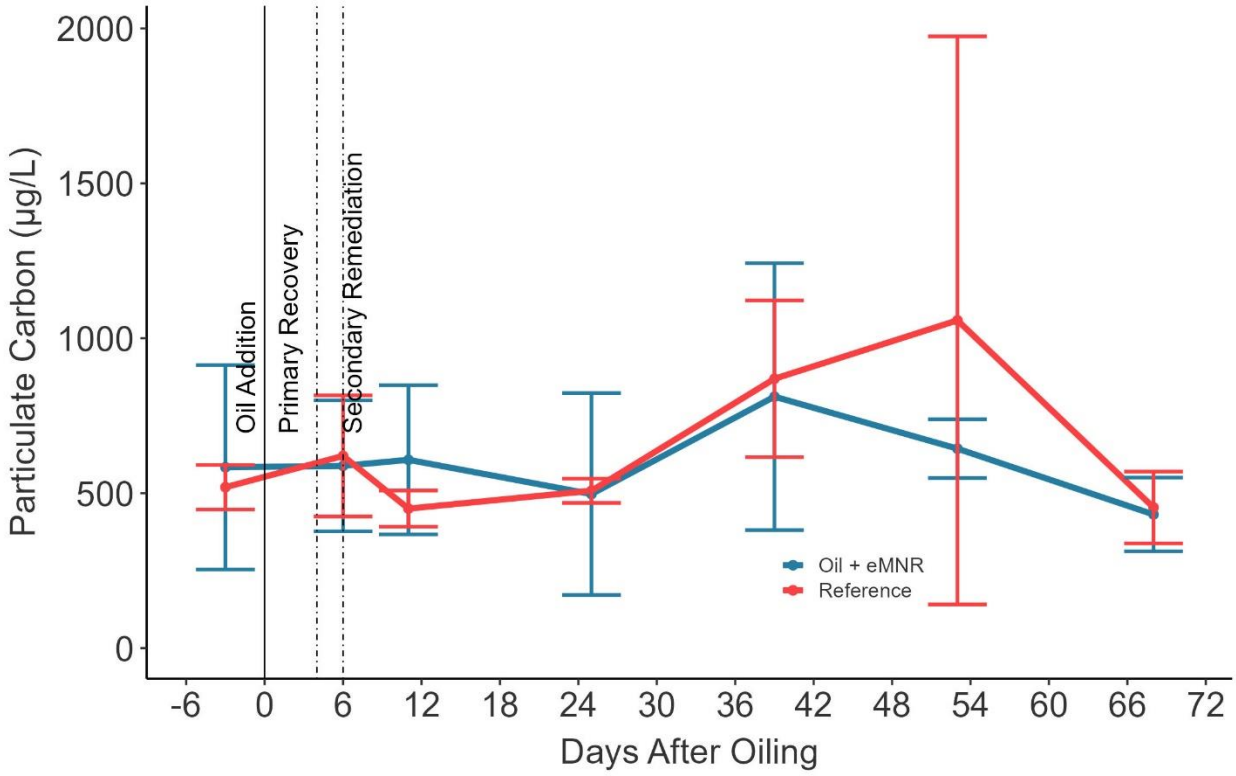
SI Figure 4: Dissolved Organic Carbon (DOC) concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



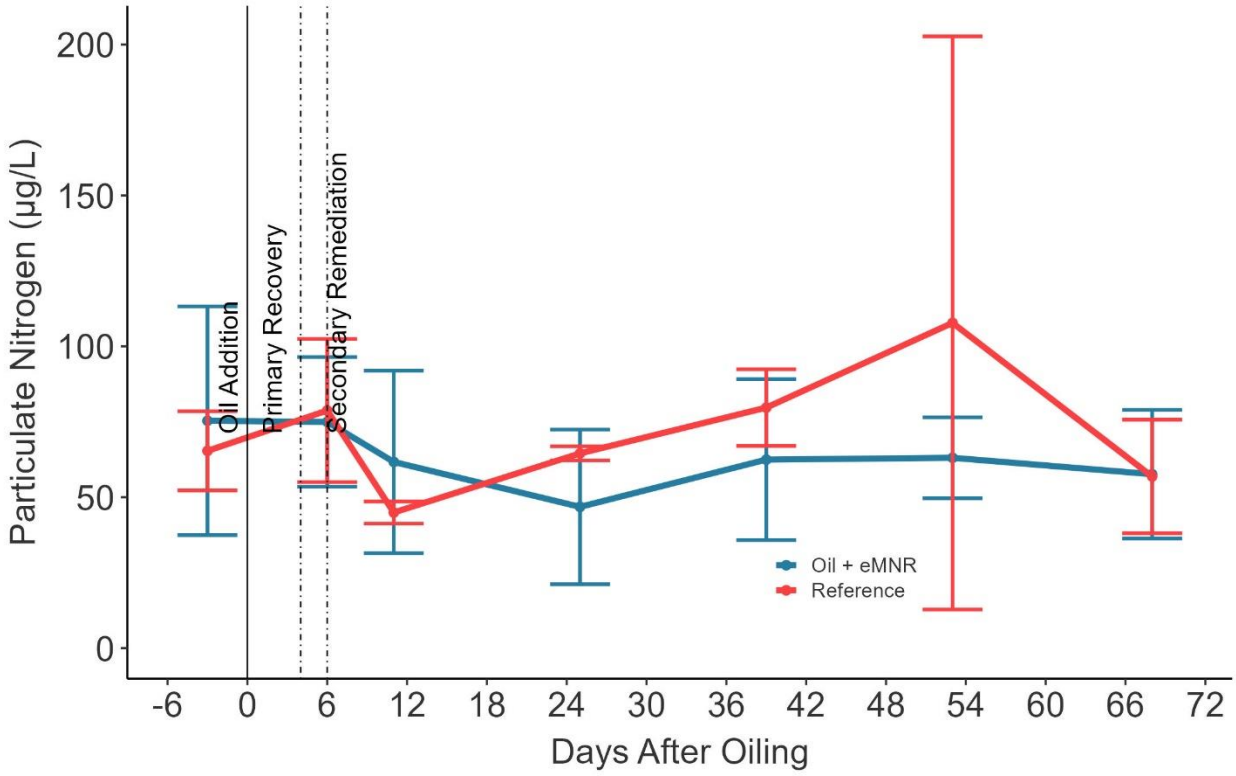
SI Figure 5: Nitrate concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



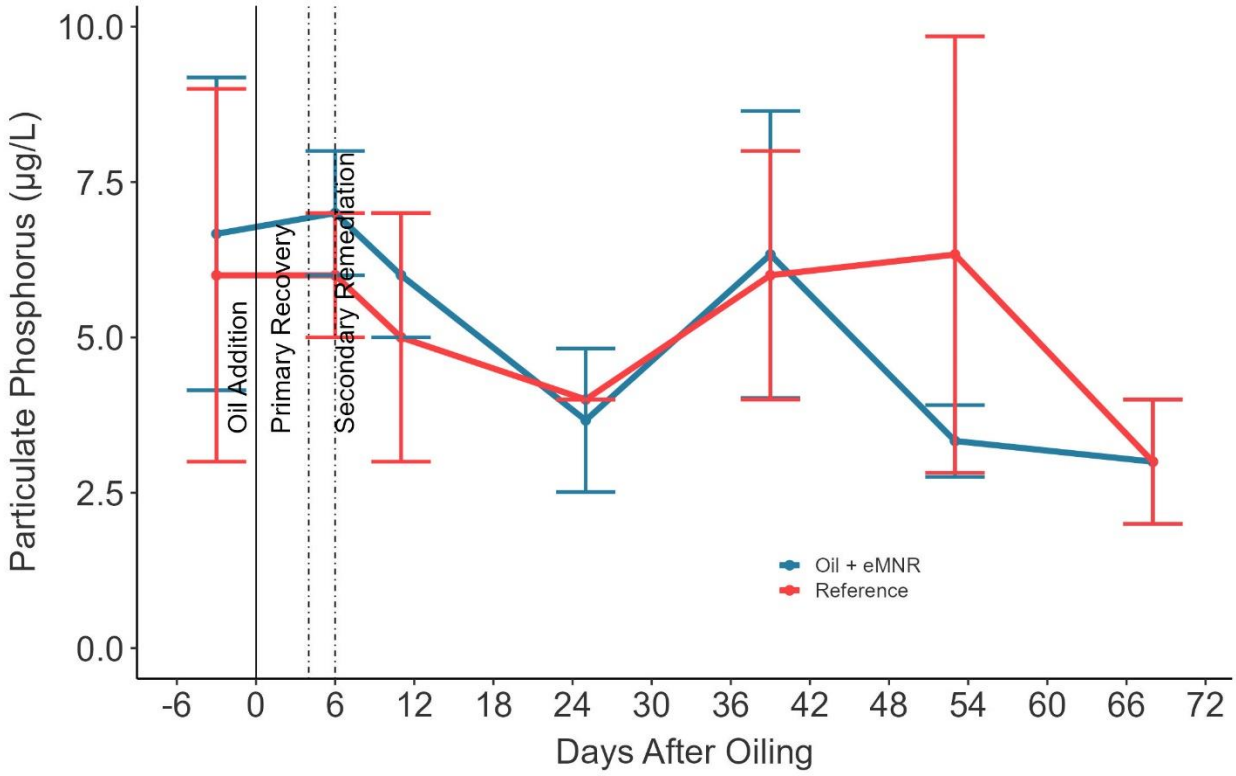
SI Figure 6: Nitrite concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



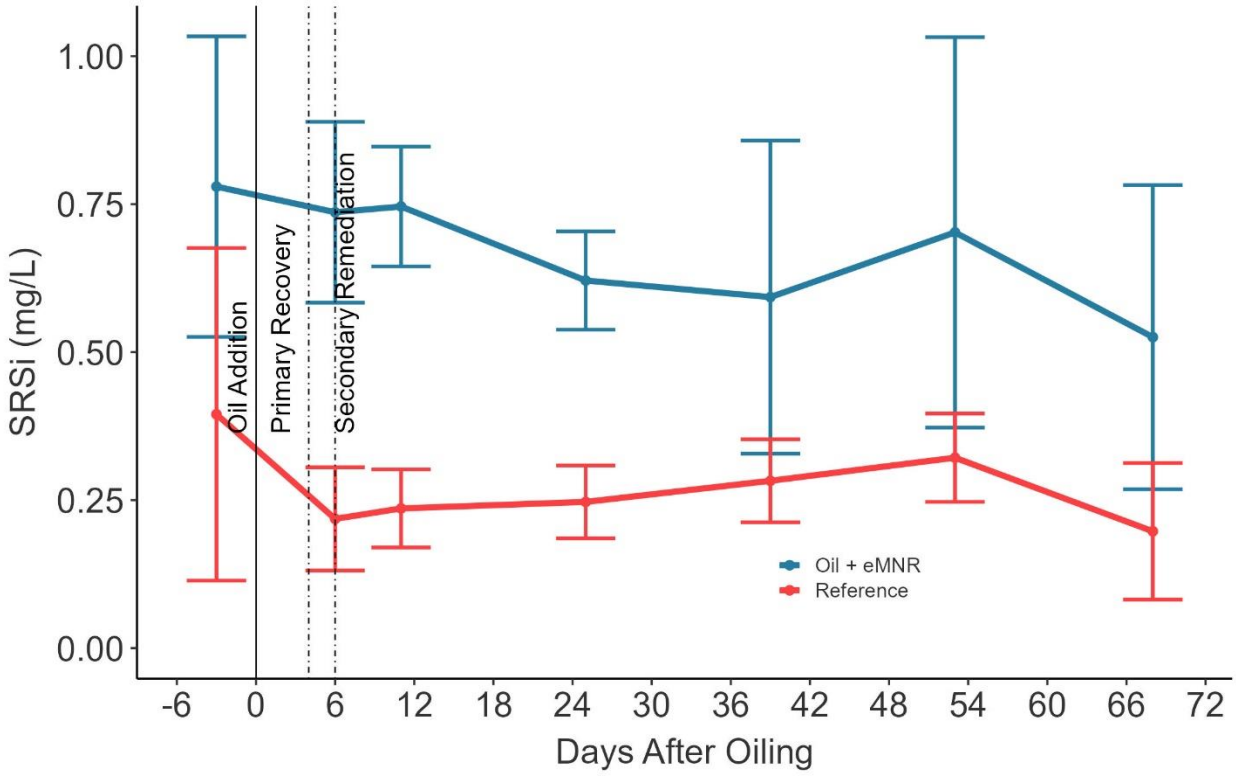
SI Figure 7: Particulate carbon (PC) concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



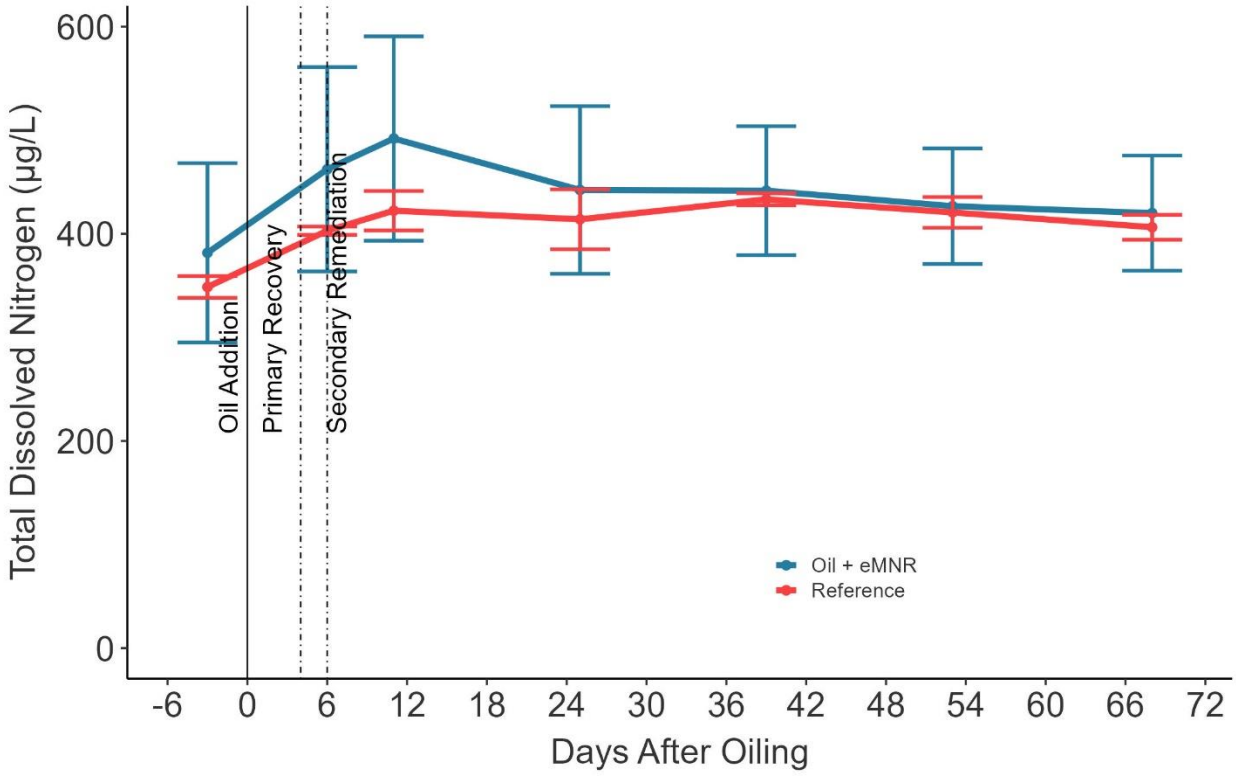
SI Figure 8: Particulate nitrogen (PN) concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



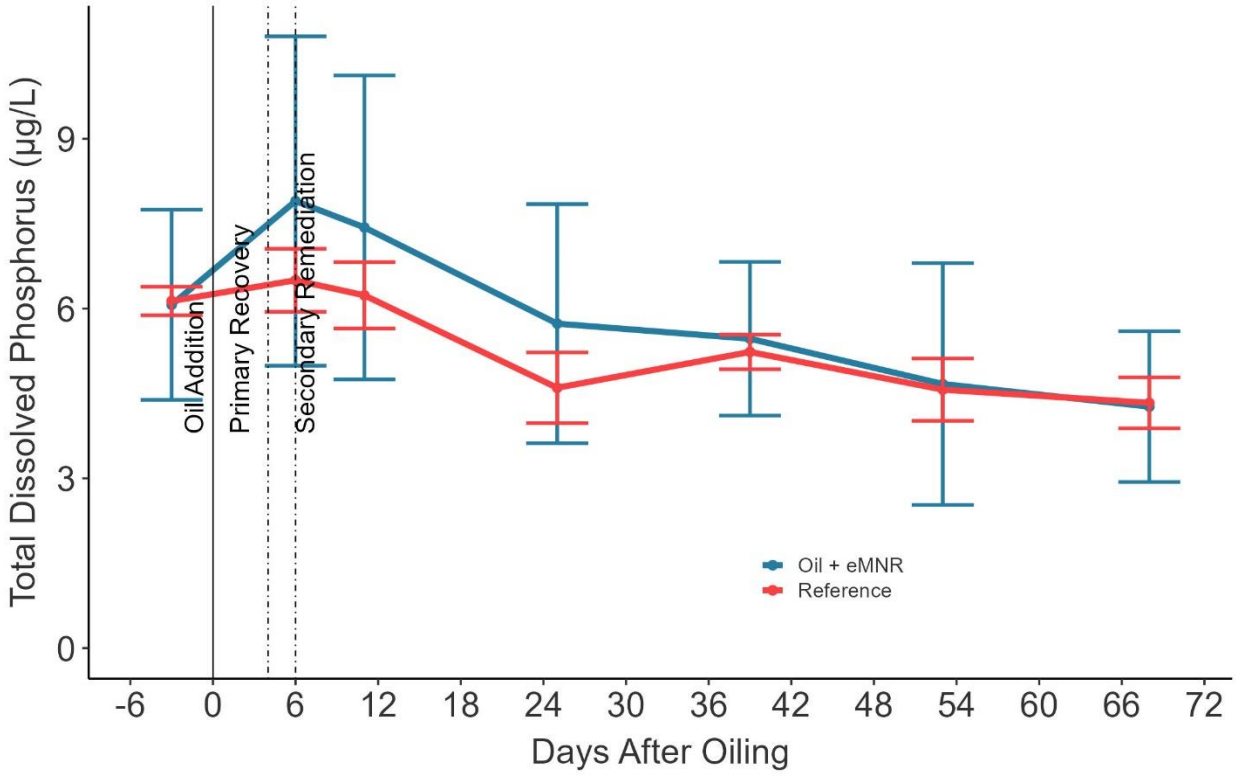
SI Figure 9: Particulate phosphorus (PP) concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



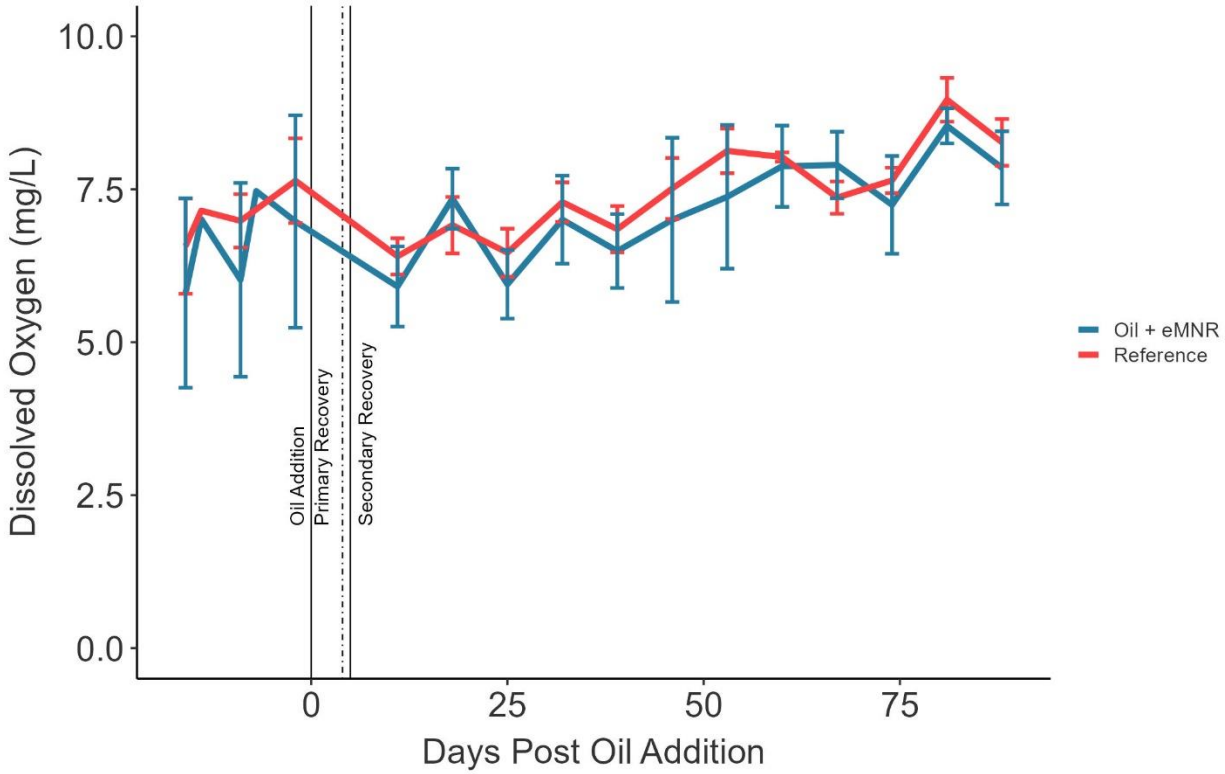
SI Figure 10: SRSi concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



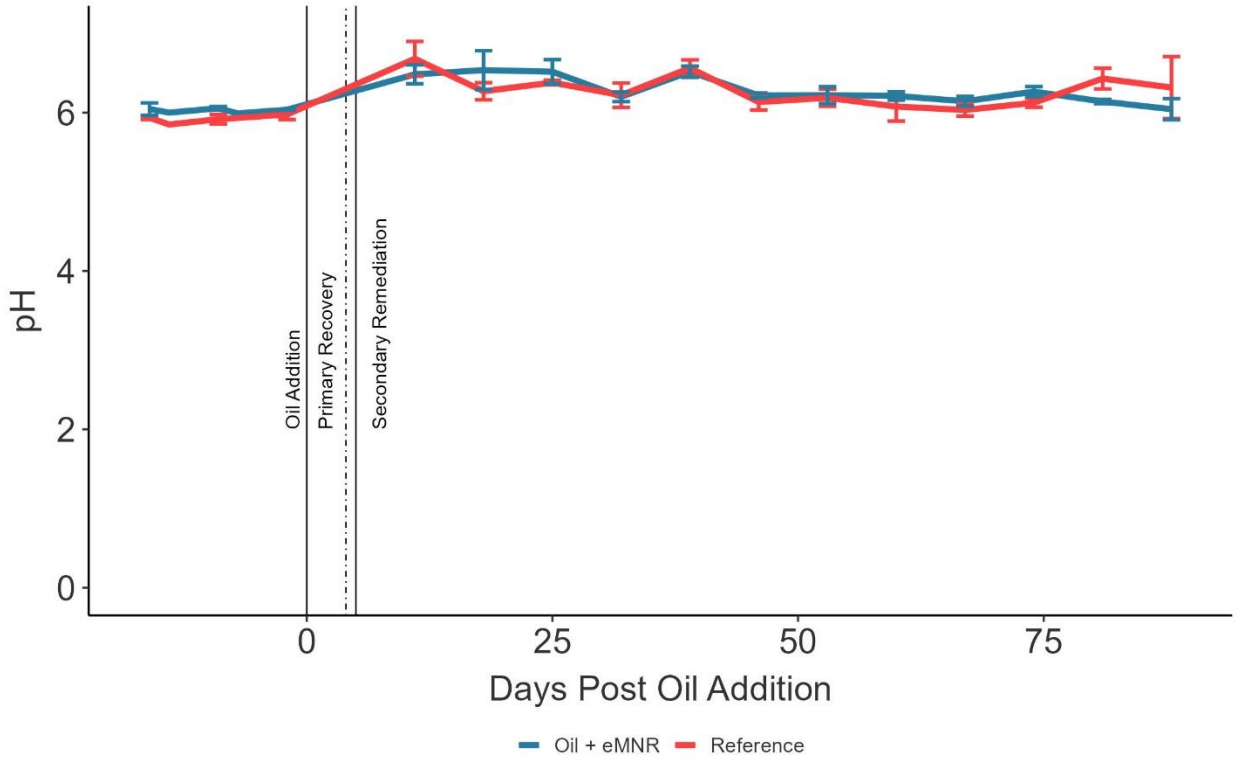
SI Figure 11: Total dissolved nitrogen concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



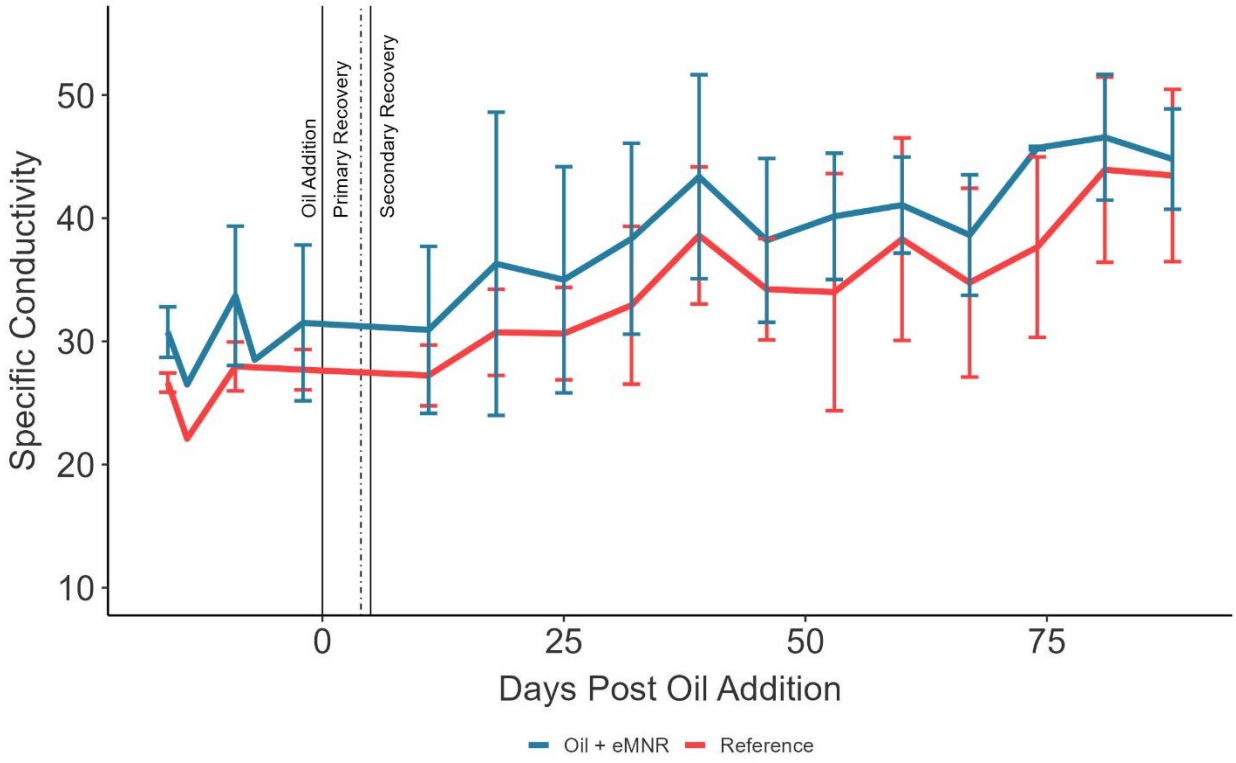
SI Figure 12: Total dissolved phosphorus concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



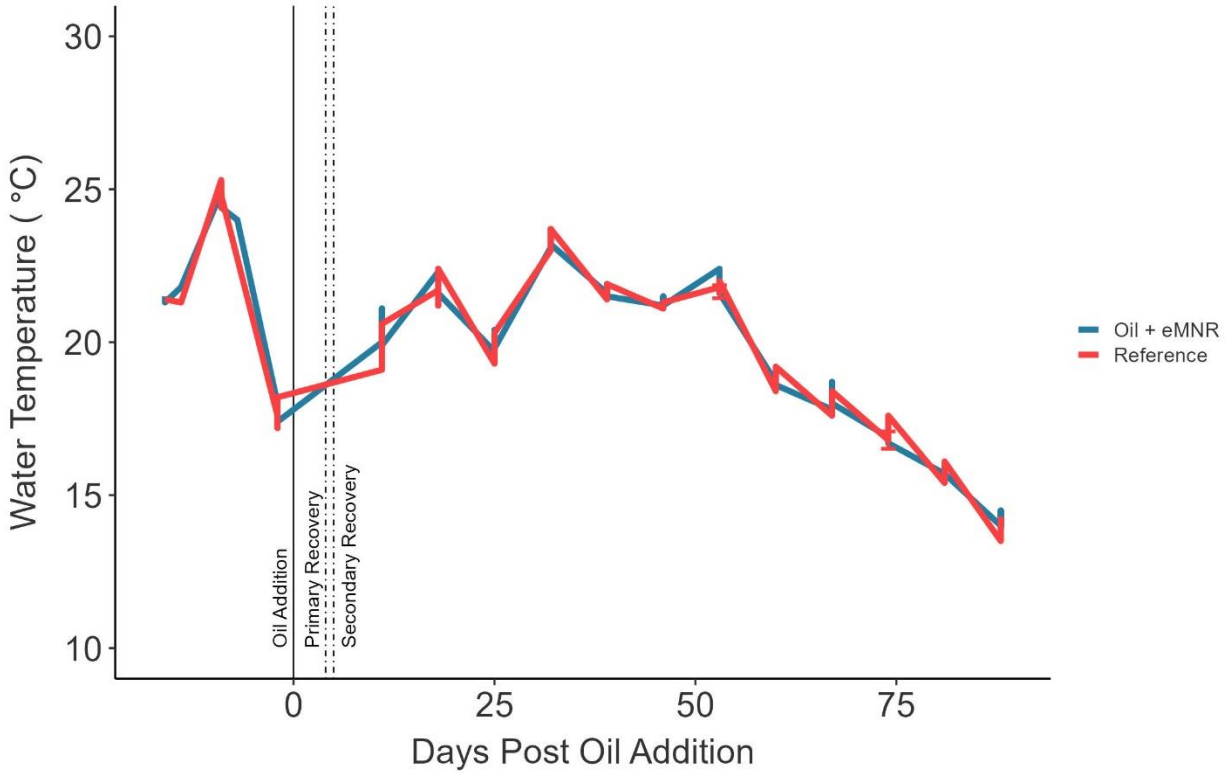
SI Figure 13: Dissolved oxygen concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



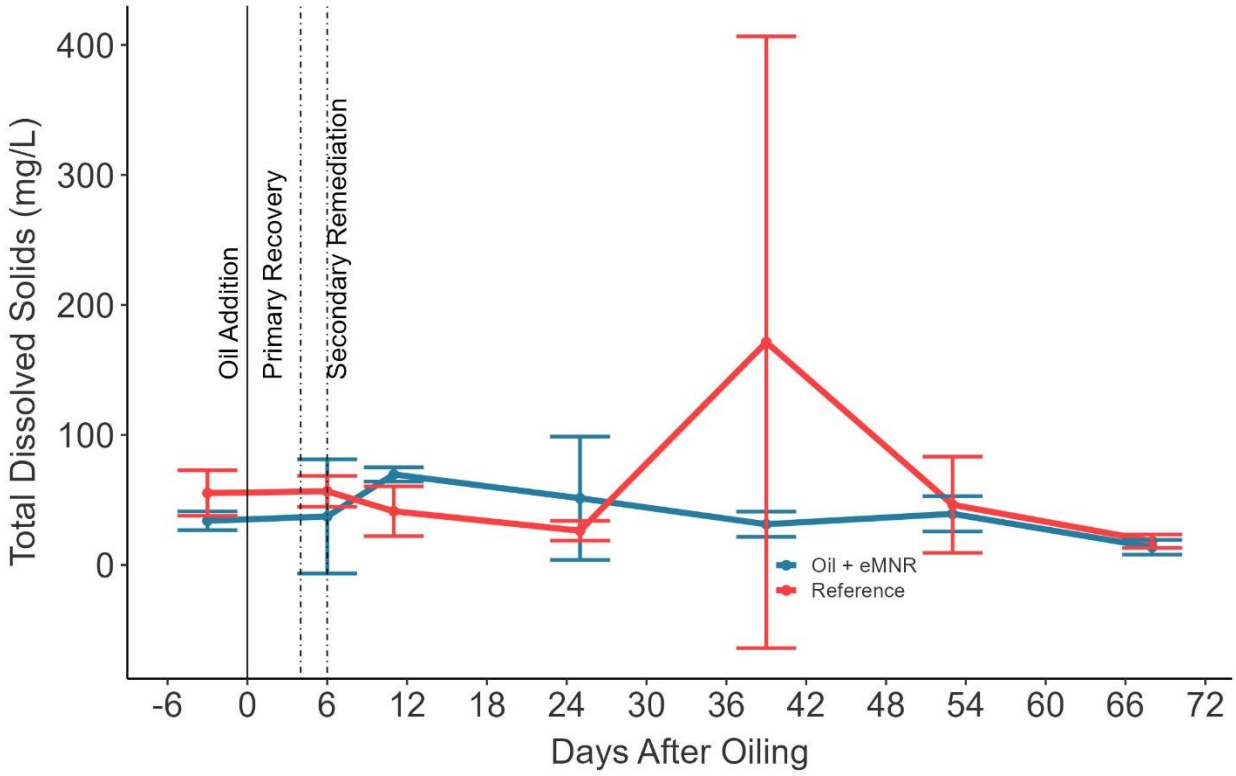
SI Figure 14: pH concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



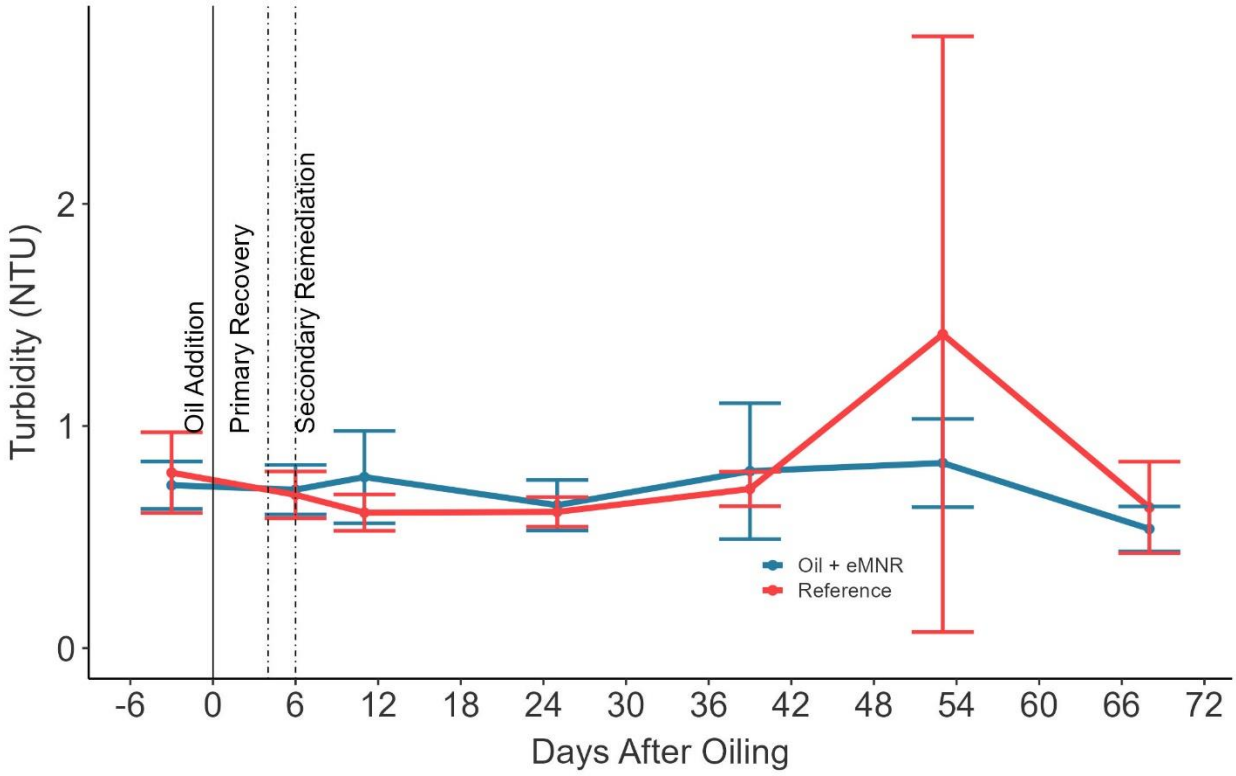
SI Figure 15: Specific conductivity concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



SI Figure 16: Water temperature concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

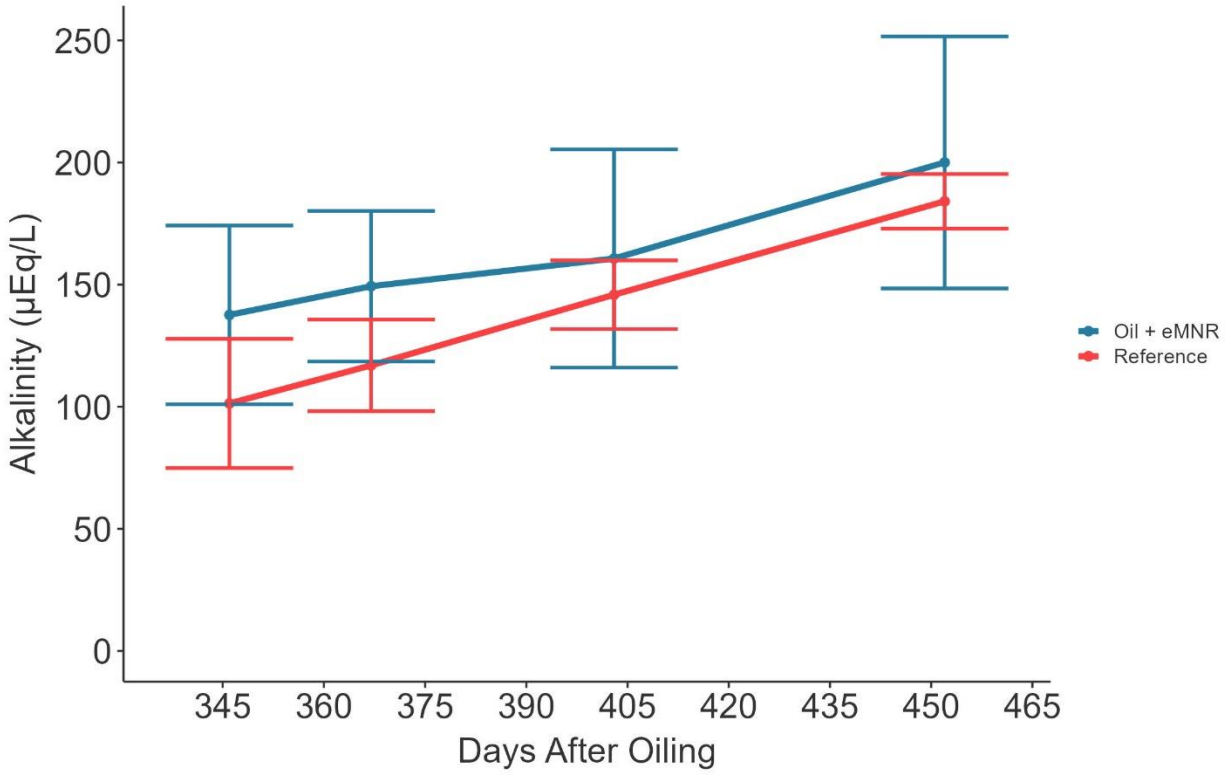


SI Figure 17: Total dissolved solids concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

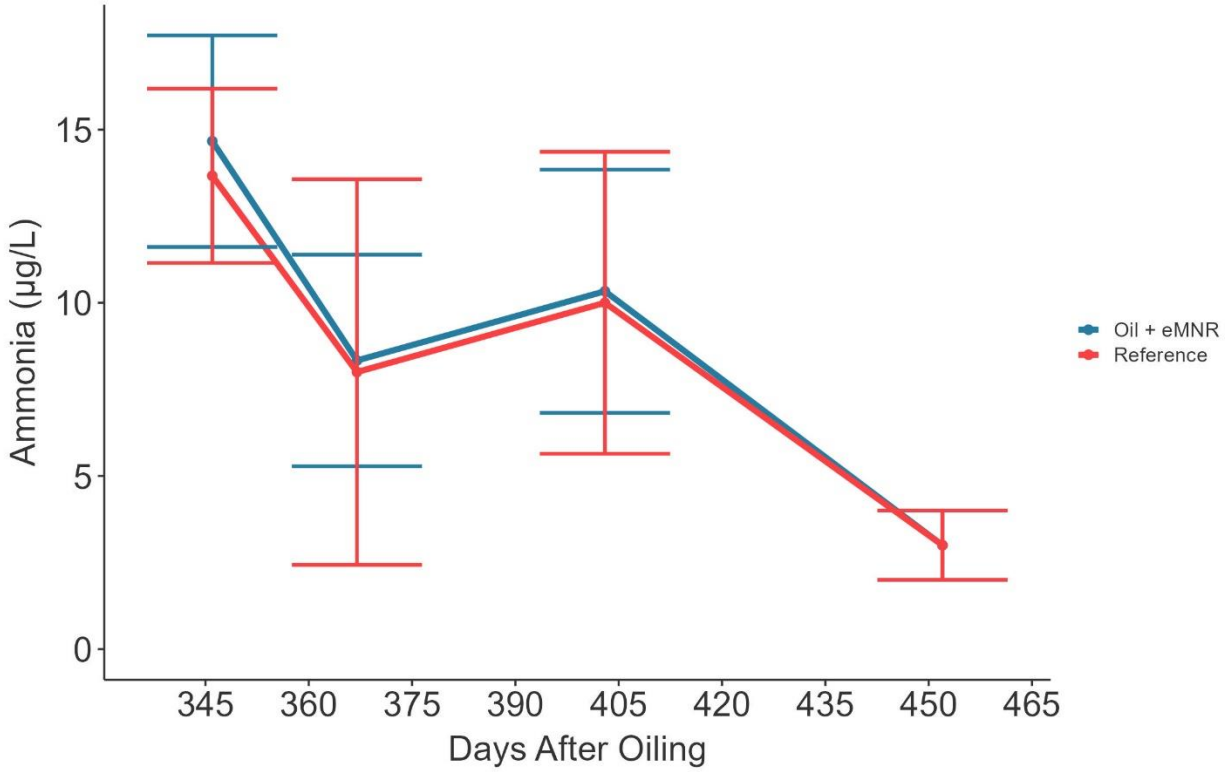


SI Figure 18: Turbidity concentration within reference and treatment enclosures during the 2021 FOReSt study. Day 0 (Oil addition date) is represented by the dashed line. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

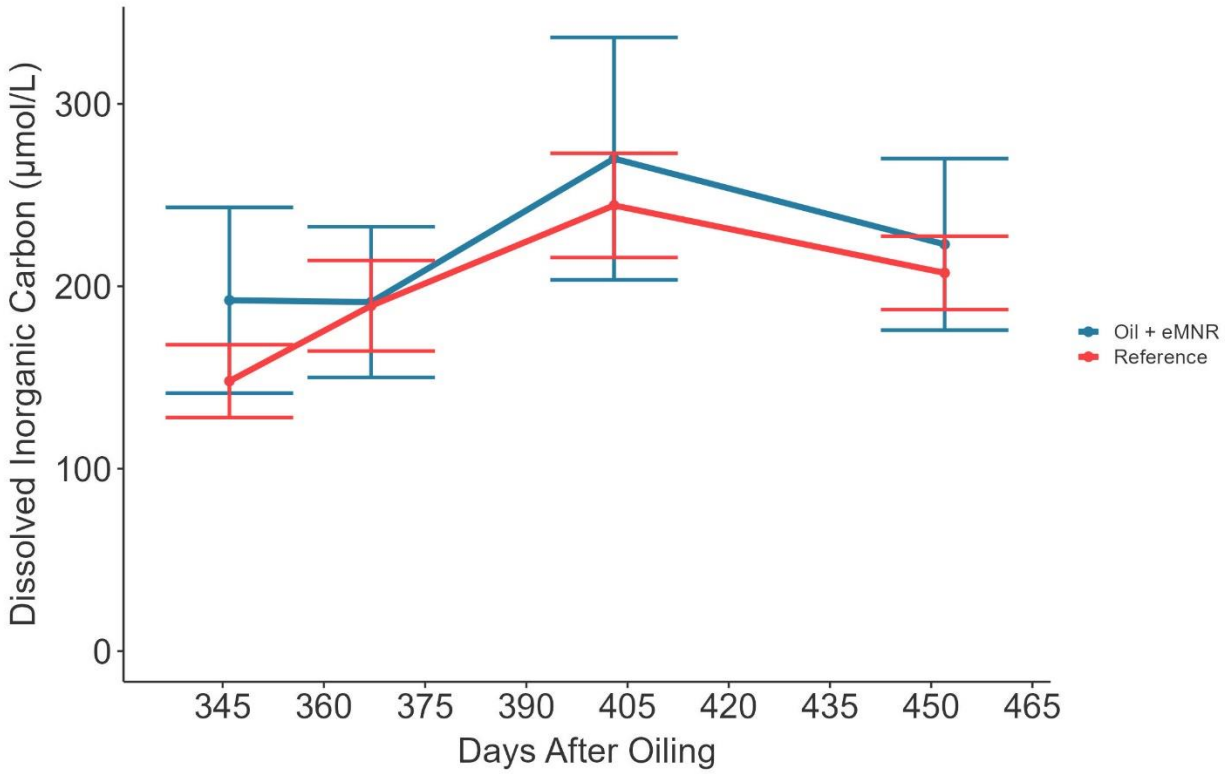
2022 Figures



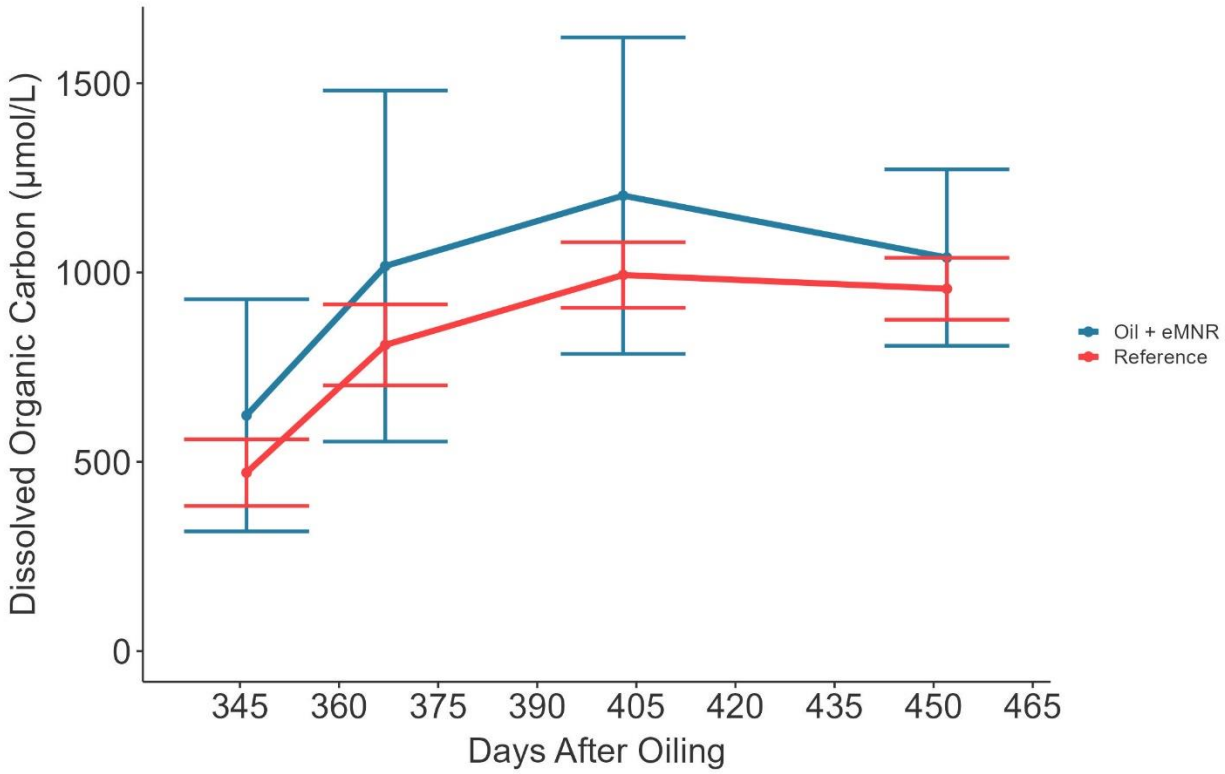
SI Figure 19: Alkalinity concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



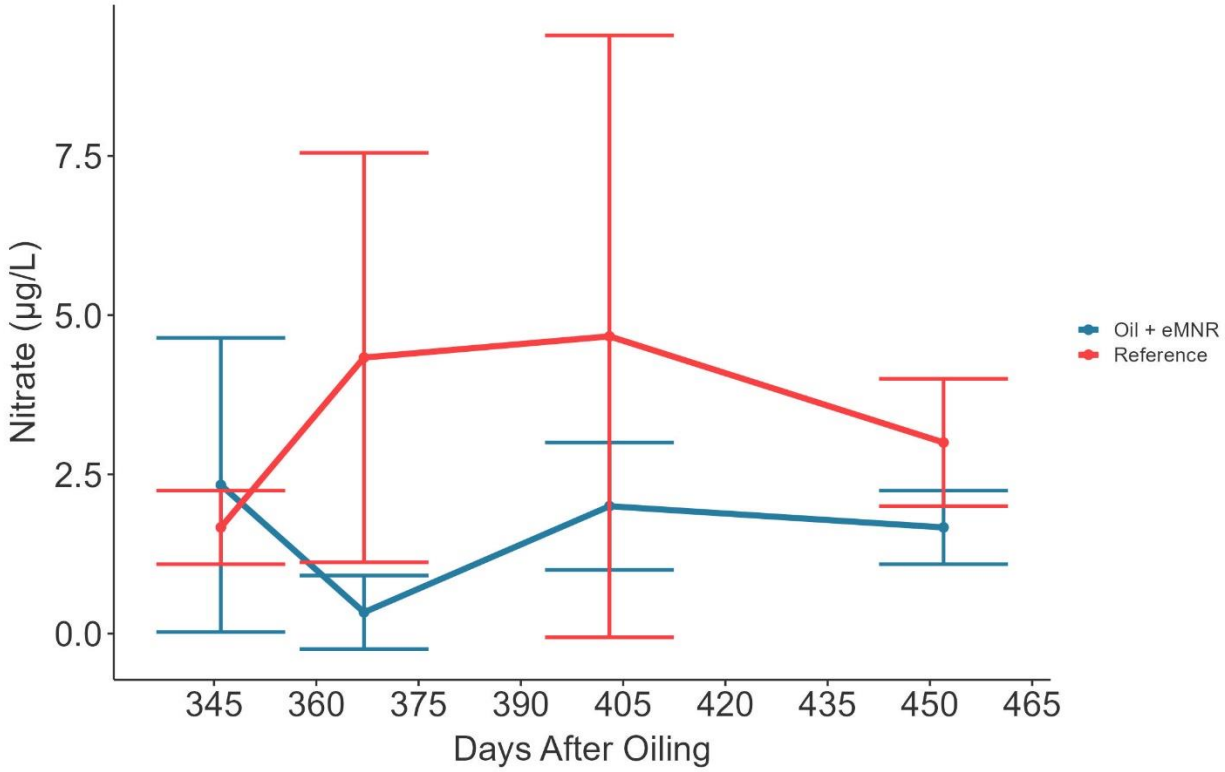
SI Figure 20: Ammonia concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



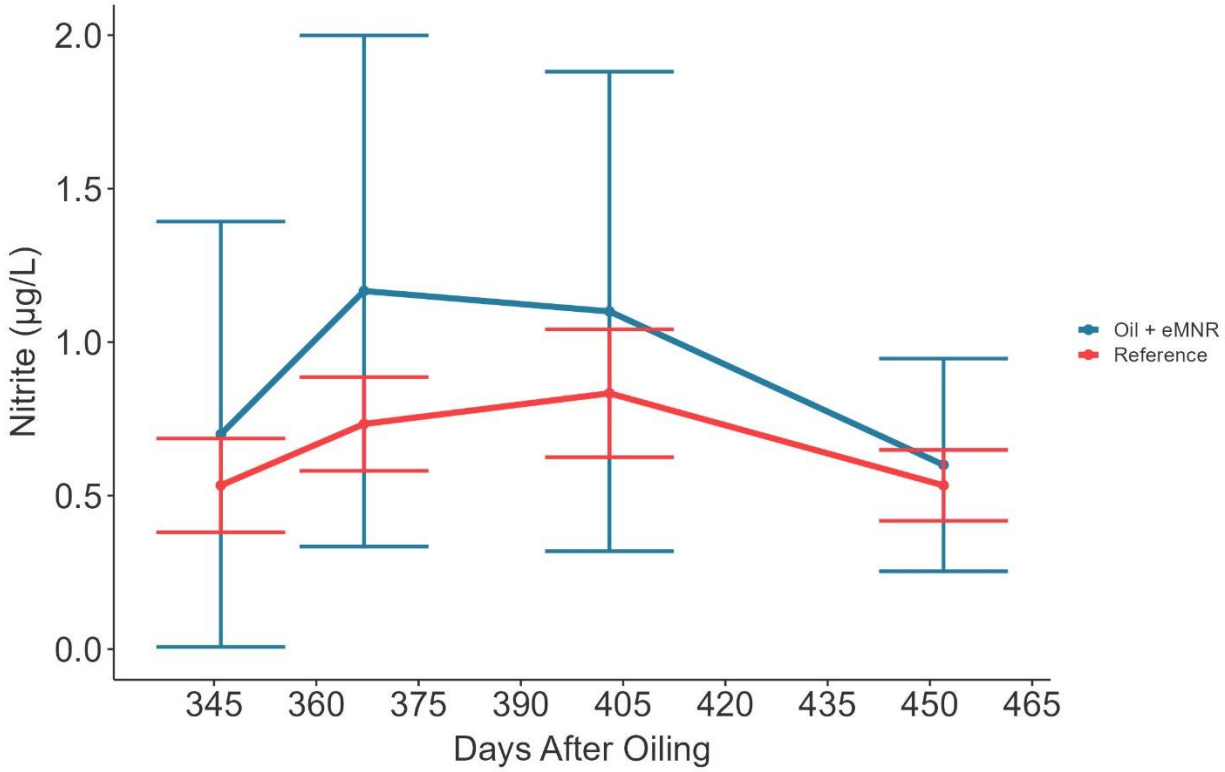
SI Figure 21: Dissolved Inorganic Carbon (DIC) concentration within reference and treatment enclosures during 2022 the FOReSt study Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



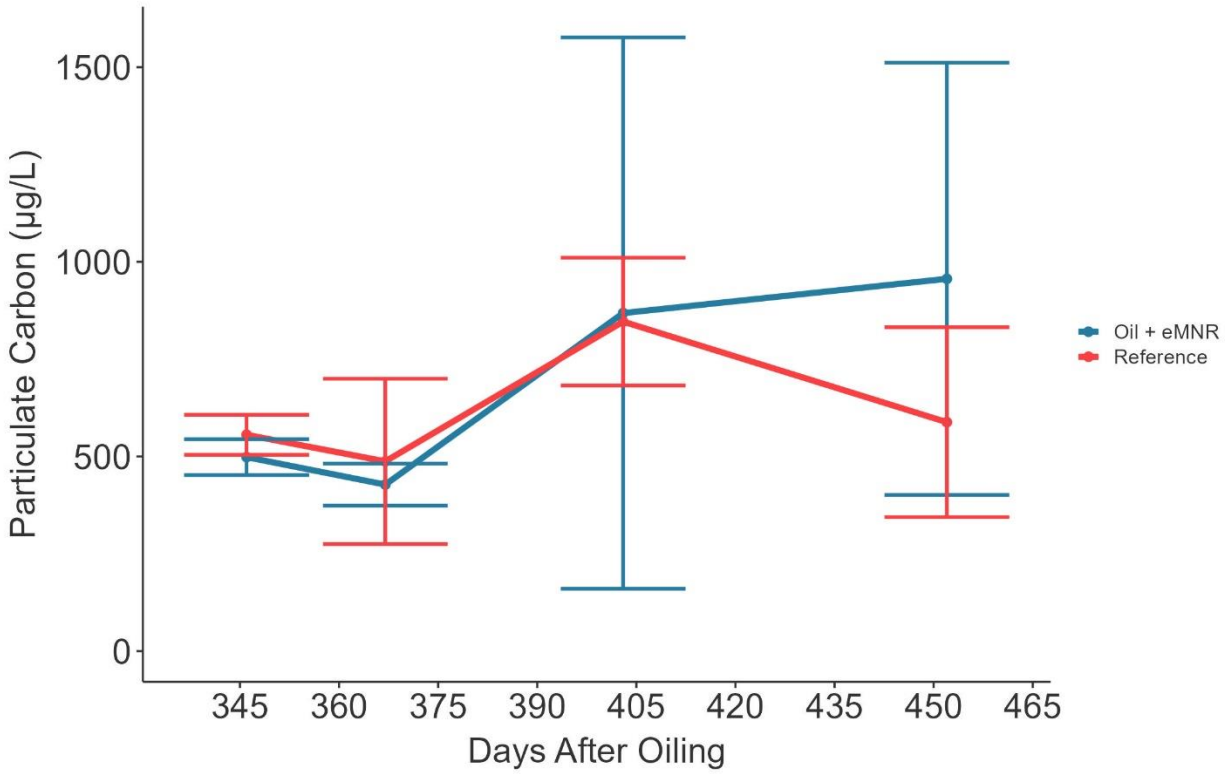
SI Figure 22: Dissolved Organic Carbon (DOC) concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



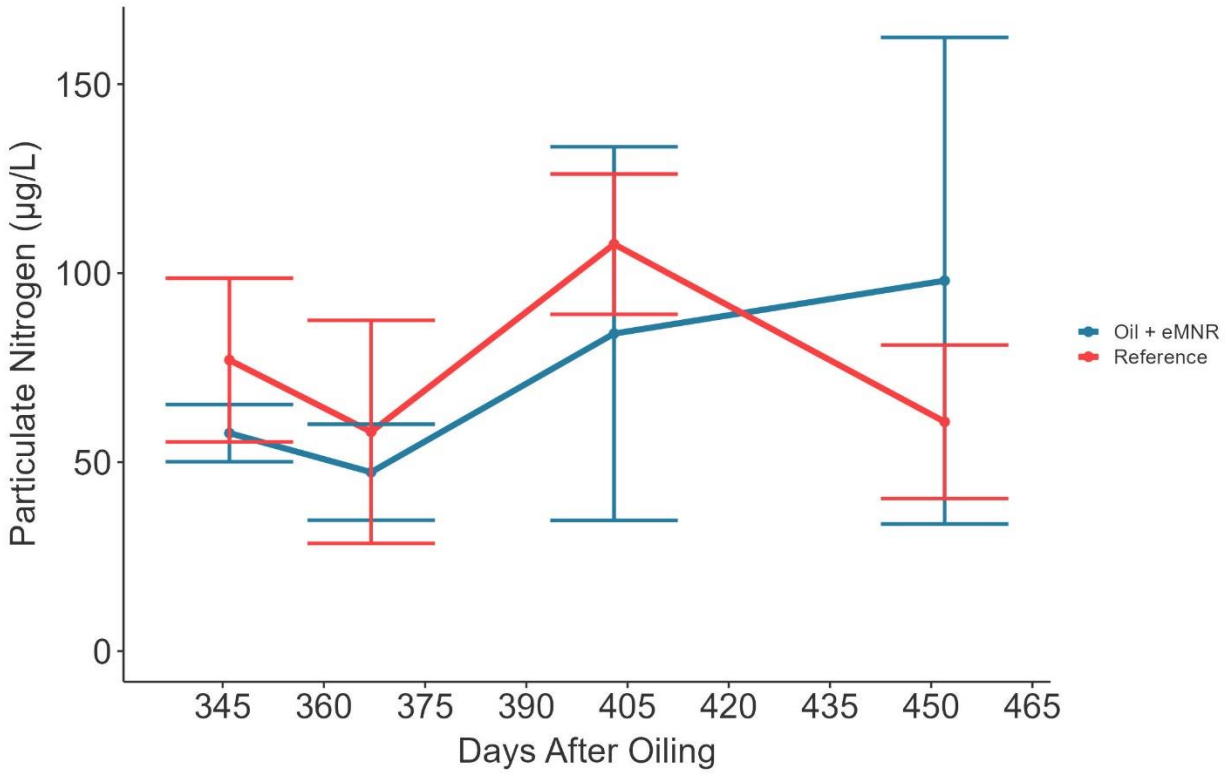
SI Figure 23: Nitrate concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



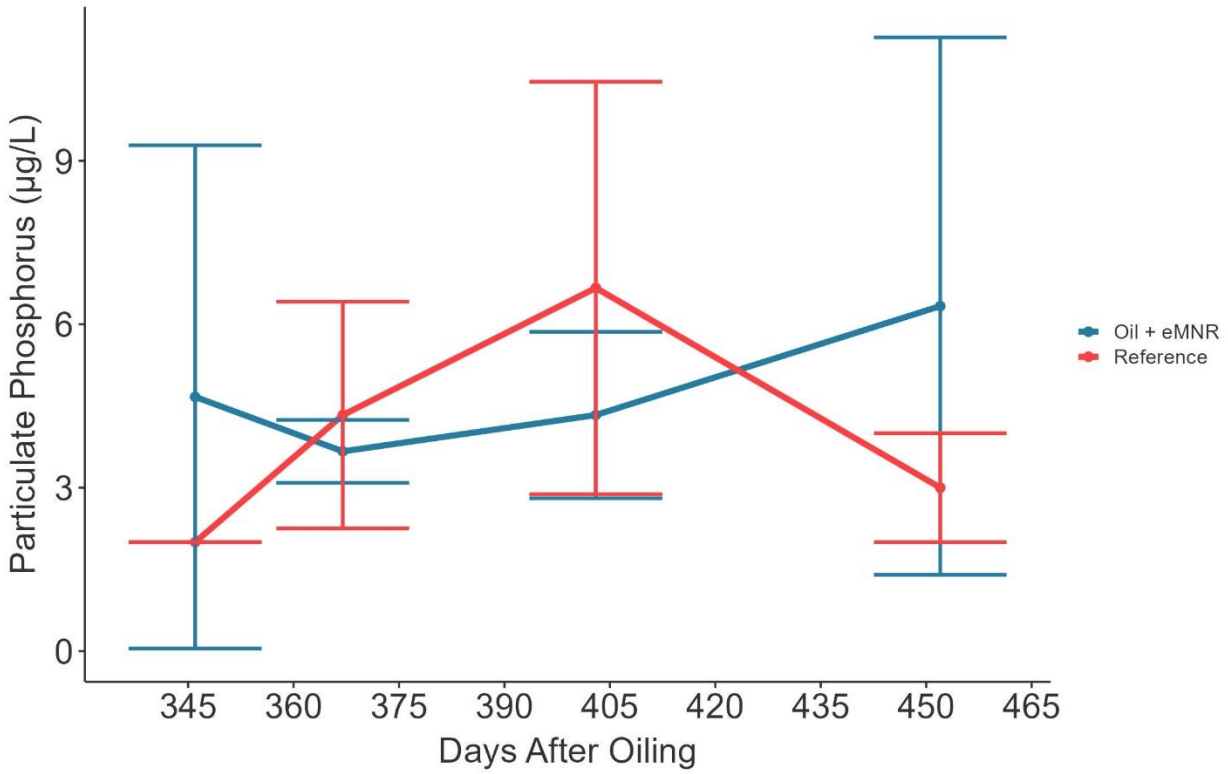
SI Figure 24: Nitrite concentration within reference and treatment enclosures during the 2022 FOReSt study Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post oil addition.



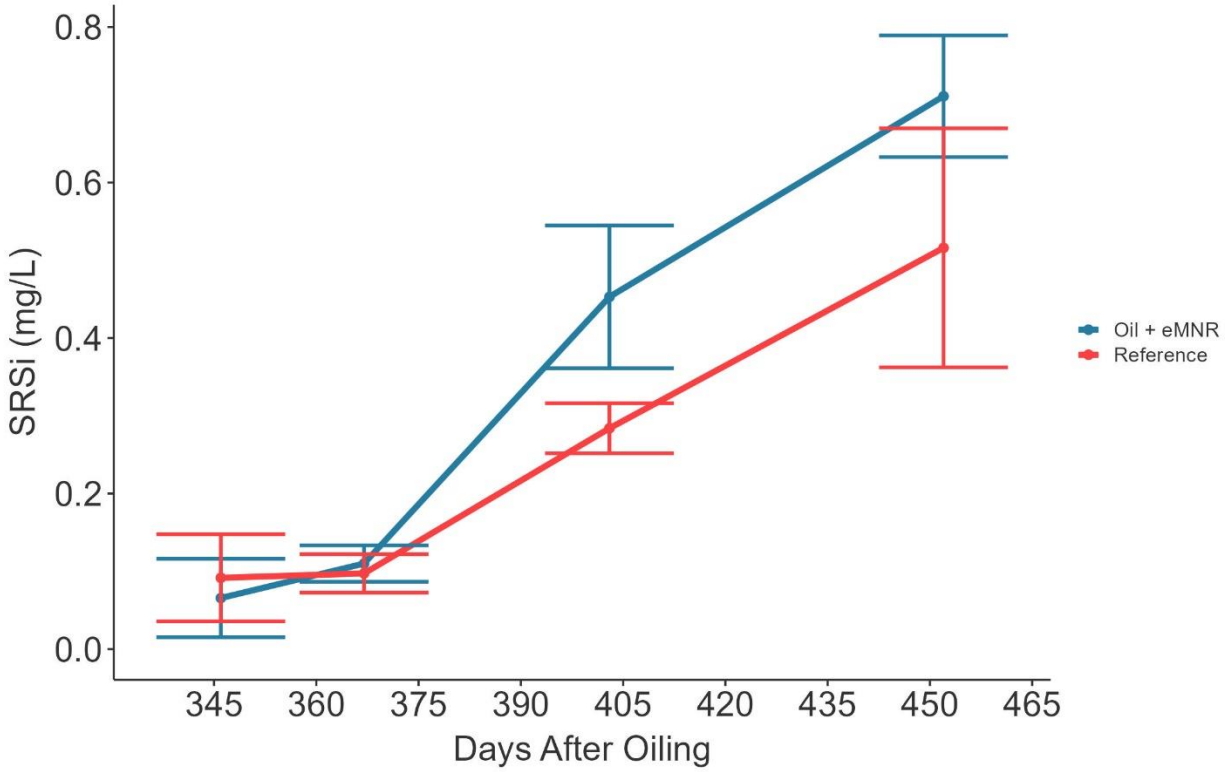
SI Figure 25: Particulate carbon (PC) concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



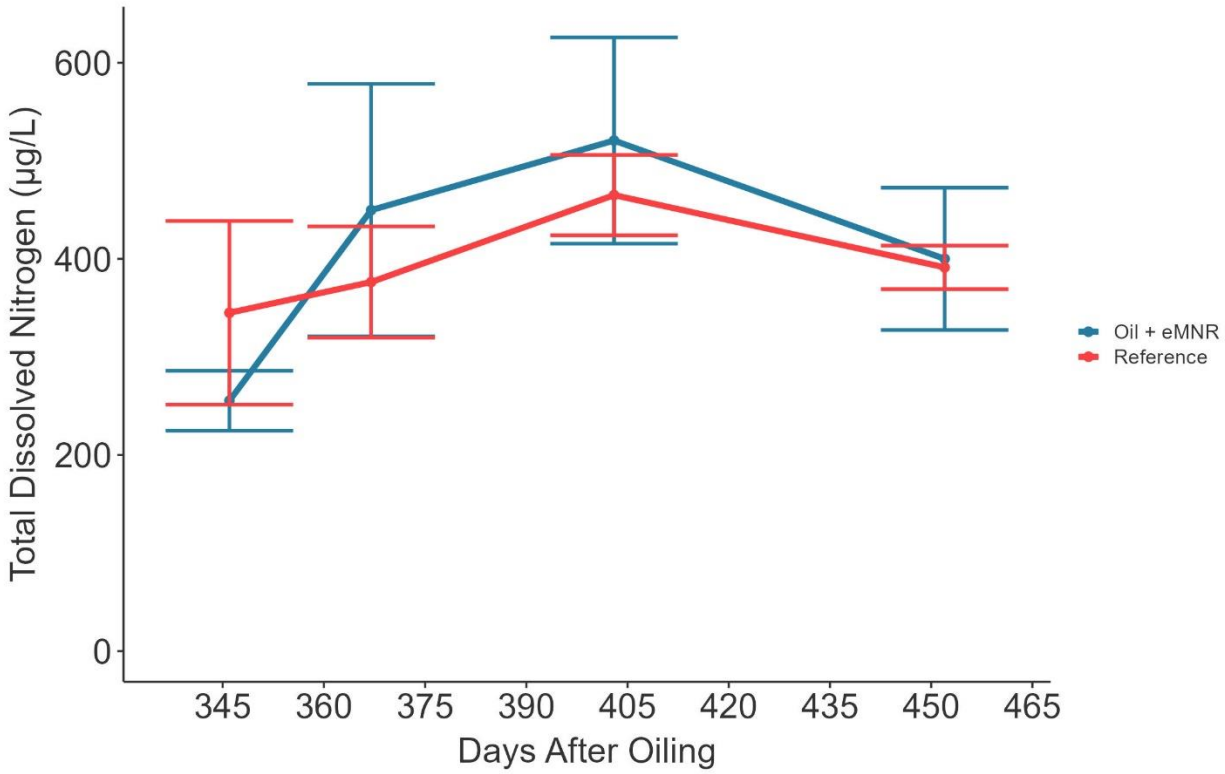
SI Figure 26: Particulate nitrogen (PN) concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



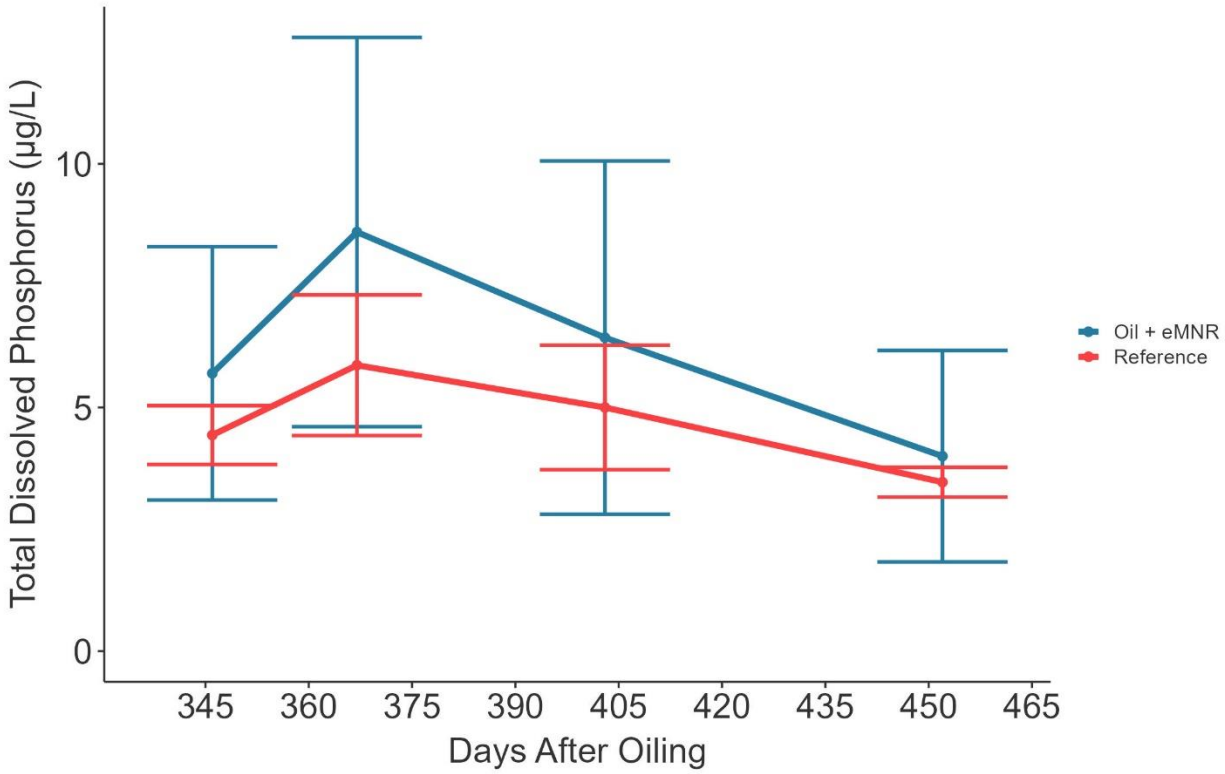
SI Figure 27: Particulate phosphorus (PP) concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



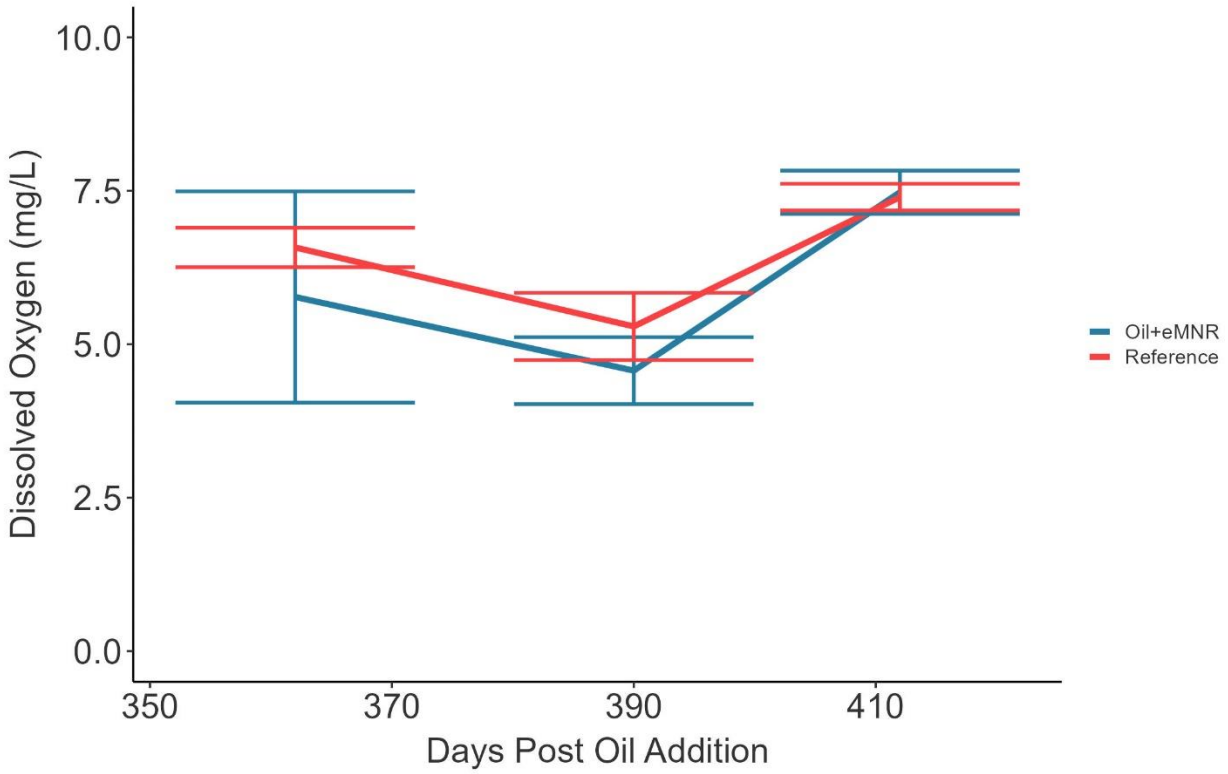
SI Figure 28: SRSi concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



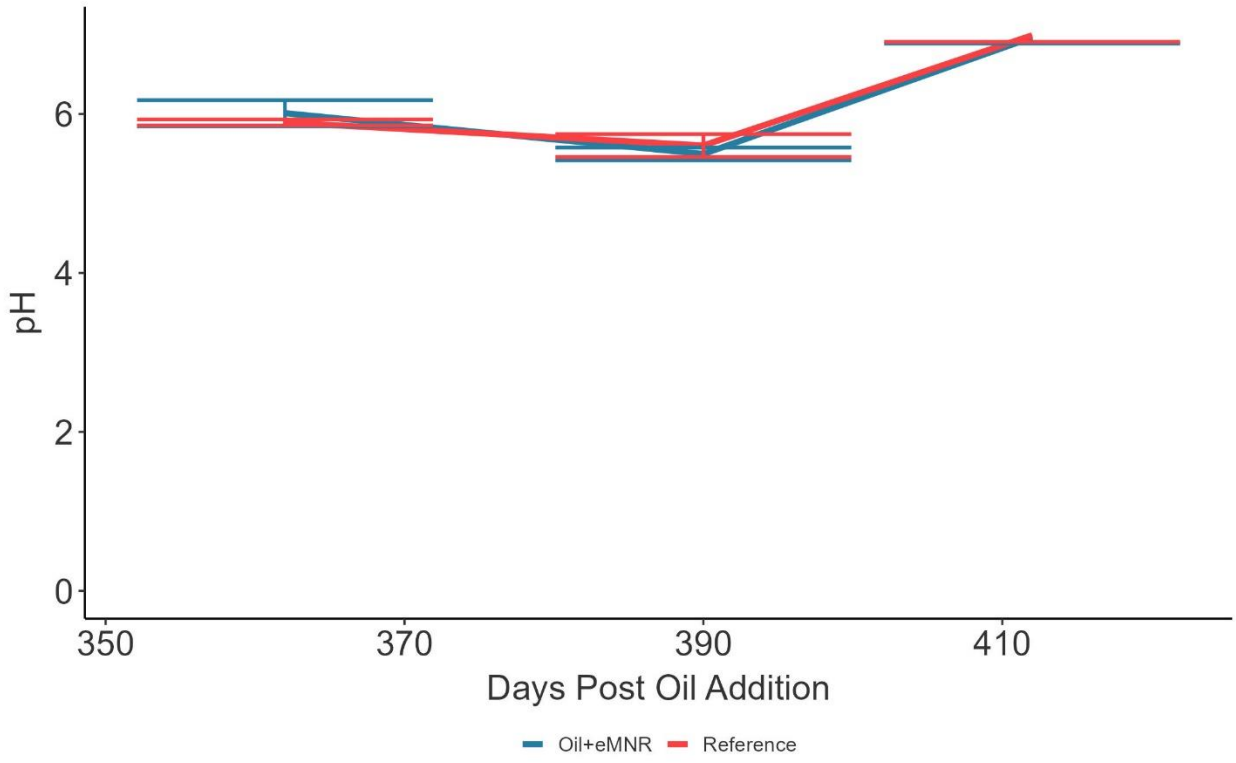
SI Figure 29: Total dissolved nitrogen concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



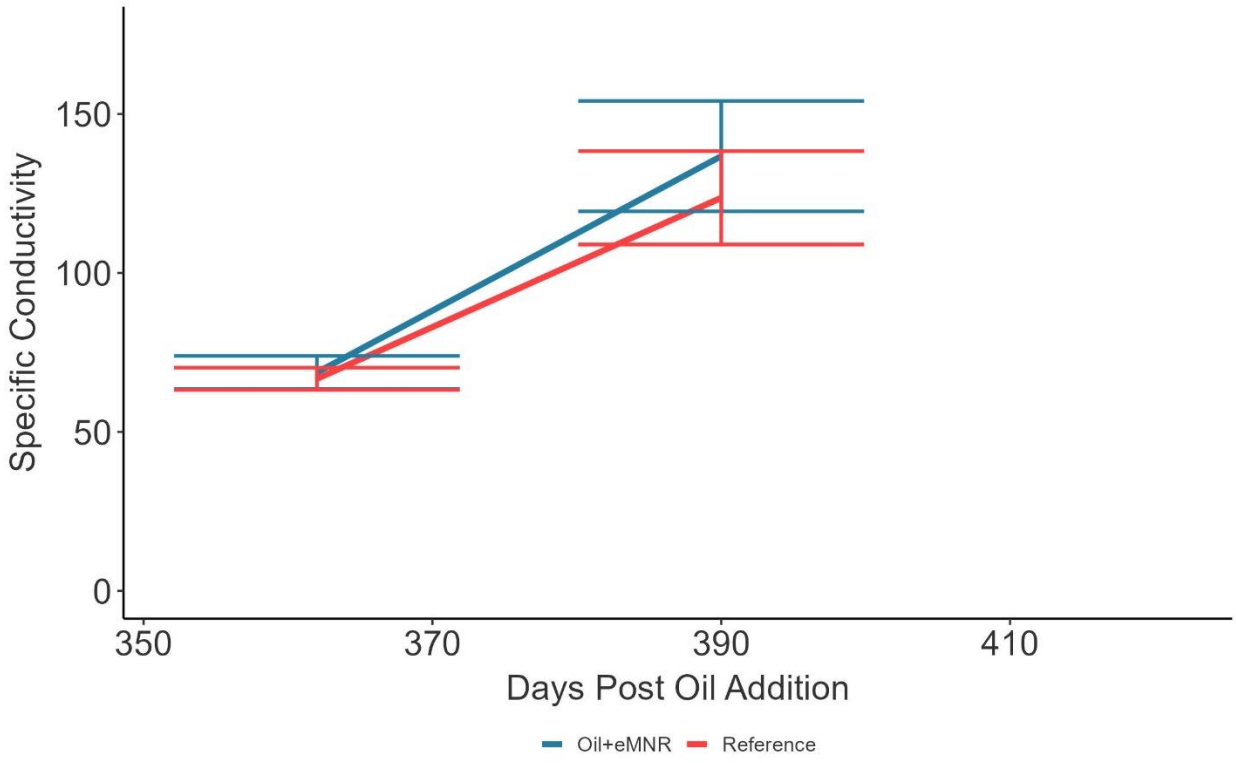
SI Figure 30: Total dissolved phosphorus concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



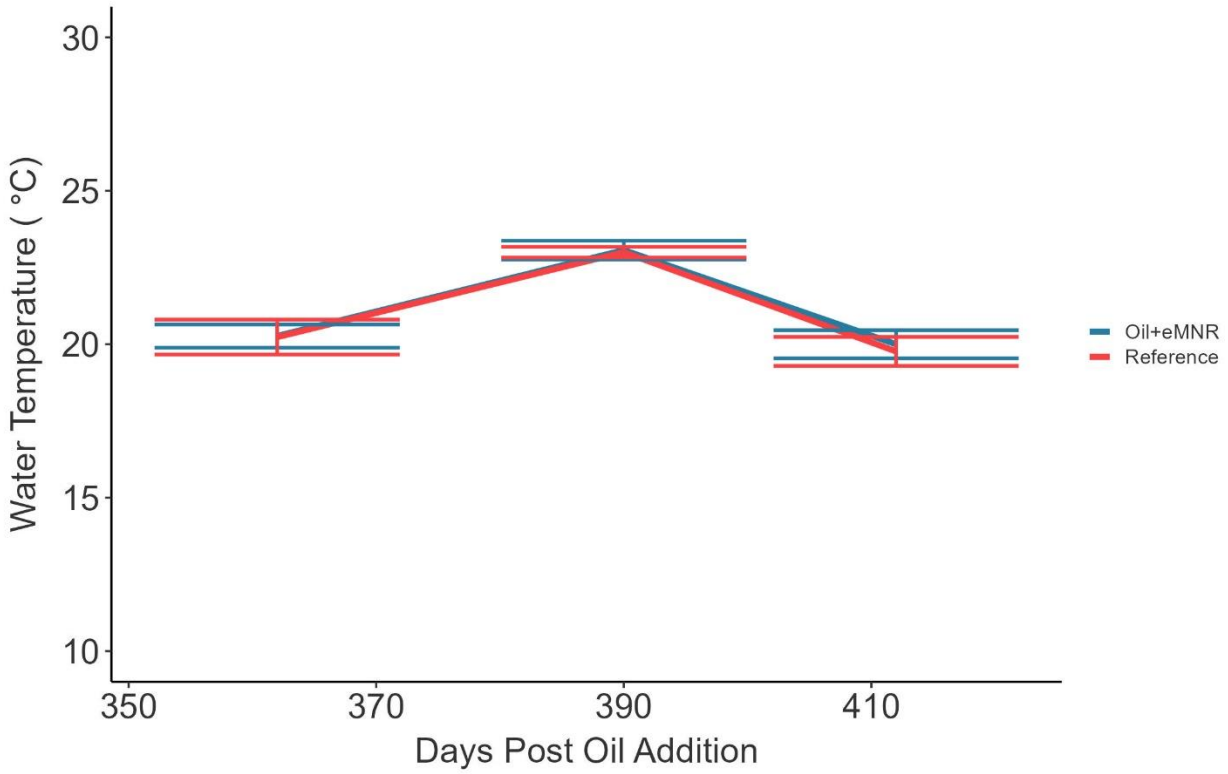
SI Figure 31: Dissolved oxygen concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



SI Figure 32: pH concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



SI Figure 33: Specific conductivity concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.



SI Figure 34: Water temperature concentration within reference and treatment enclosures during the 2022 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

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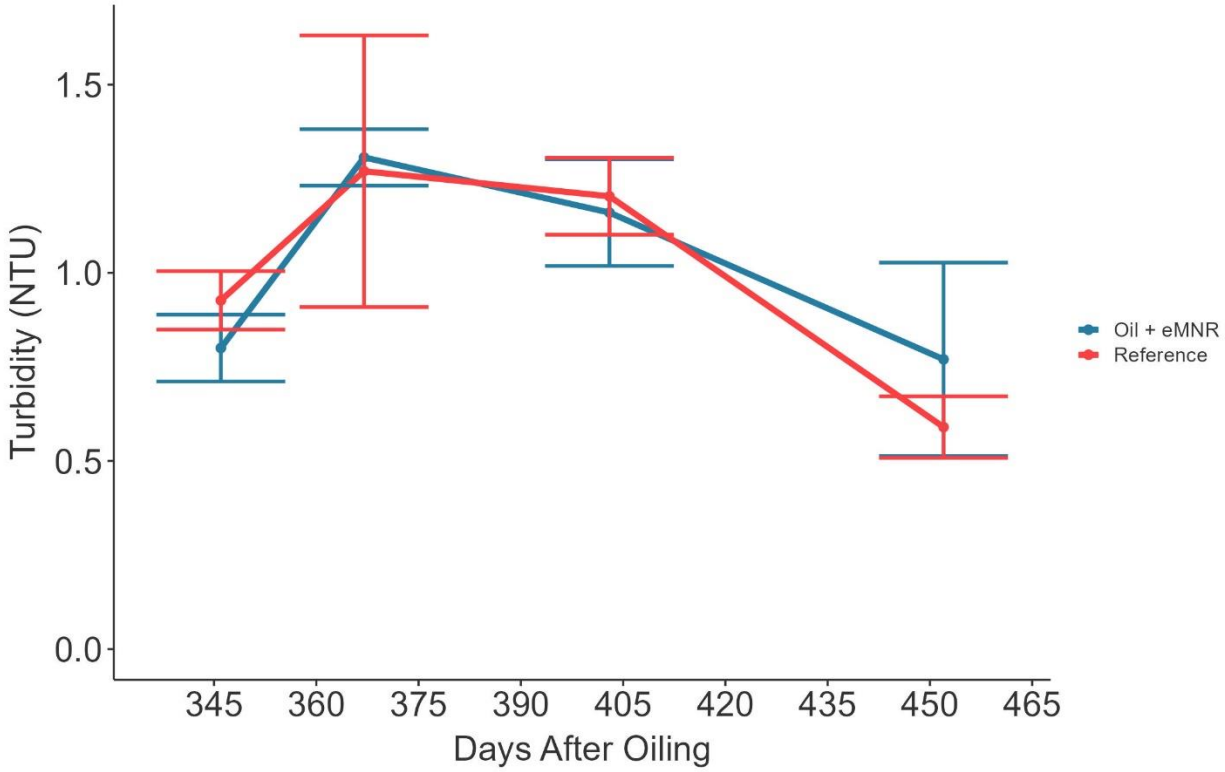

SI Figure 35: Turbidity concentration within reference and treatment enclosures during the 2021 FOReSt study. Each point is the mean (n=3), and error bars represent the standard deviation. There was no statistical difference between treated and reference enclosures pre-or post-oil addition.

	biofilm chlla conc in strip ug cm 2			sqr afdm		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	36.88	-101.17 – 174.94	0.618	11.38	5.55 – 17.21	**<0.001**
treatment [Oiled]	-17.40	-289.99 – 255.18	0.875	-5.90	-17.42 – 5.62	0.251
fac study day [13]	205.45	71.73 – 339.16	**0.006**	3.11	-2.39 – 8.60	0.282
fac study day [27]	326.88	193.17 – 460.60	**<0.001**	6.92	1.43 – 12.41	**0.022**
fac study day [41]	239.34	105.62 – 373.05	**0.002**	9.47	3.98 – 14.96	**0.003**
fac study day [55]	319.85	186.13 – 453.56	**<0.001**	11.18	5.63 – 16.73	**0.001**
fac study day [69]	286.85	153.14 – 420.57	**<0.001**	16.88	11.39 – 22.37	**<0.001**
fac study day [83]	418.45	284.74 – 552.17	**<0.001**	20.03	14.53 – 25.52	**<0.001**
treatment [Oiled] × fac study day [13]	196.21	7.11 – 385.31	0.055	5.77	-2.00 – 13.53	0.161
treatment [Oiled] × fac study day [27]	116.82	-72.28 – 305.92	0.241	6.24	-1.52 – 14.01	0.131
treatment [Oiled] × fac study day [41]	105.31	-83.79 – 294.41	0.289	7.47	-0.29 – 15.24	0.073
treatment [Oiled] × fac study day [55]	173.67	-15.43 – 362.77	0.087	9.20	1.41 – 16.98	**0.031**
treatment [Oiled] × fac study day [69]	154.84	-34.27 – 343.94	0.124	3.48	-4.28 – 11.25	0.391
treatment [Oiled] × fac study day [83]	17.41	-171.70 – 206.51	0.859	1.42	-6.35 – 9.18	0.726
N	7 study_day 6 enclosure			7 study_day 6 enclosure		
Observations	126			126		

	biofilm chlla conc in strip ug cm 2			sqr afdm		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	314.18	108.31 – 520.05	**0.006**	10.80	4.66 – 16.94	**0.002**
treatment [Oiled]	-27.99	-426.56 – 370.59	0.863	2.92	-8.97 – 14.80	0.556
fac study day [391]	198.58	-32.05 – 429.21	0.098	8.78	2.13 – 15.43	**0.021**
fac study day [410]	-3.97	-234.61 – 226.66	0.971	16.25	9.60 – 22.90	**0.001**
treatment [Oiled] × fac study day [391]	263.04	-63.12 – 589.21	0.118	-2.05	-11.45 – 7.36	0.649
treatment [Oiled] × fac study day [410]	328.82	2.66 – 654.98	0.060	0.03	-9.37 – 9.43	0.995
N	3 $_{\text{study\_day}}$ 6 $_{\text{enclosure}}$			3 $_{\text{study\_day}}$ 6 $_{\text{enclosure}}$		
Observations	54			54		

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