IRON'S IMPACT ON NITROGEN CYCLING IN AN OPEN WATER WETLAND TO PROMOTE SUSTAINABLE WATER TREATMENT

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Introduction and Objectives

Constructed wetlands offer a sustainable complement to traditional engineered water treatment, which is expensive monetarily and energetically. Open water wetlands have been demonstrated to be particularly effective at removing nitrate and other bioavailable nitrogen from wastewater-impaired river water to mitigate eutrophication and economic burdens on municipal water treatment[1]. To design the wetland to most efficiently denitrify – reduce nitrate to dinitrogen gas – while limiting the emission of greenhouse gases such as carbon dioxide and nitrous oxide, research has been done on the microorganisms in the sedimentary biomat of the wetland. Beneath the biomat's photosynthetic layer, microorganisms can reduce nitrate using various electron donors such as ferrous iron, sulfide, and methane. In the absence of oxygen, bacteria traditionally perform denitrification using an organic carbon source (heterotrophy), producing carbon dioxide and nitrous oxide. However, some alternate bacteria can use inorganic carbon such as carbon dioxide as their carbon source (autotrophy). Autotrophy acts as a carbon dioxide sink, which reduces greenhouse gas emissions. In the absence of autotrophy, heterotrophy takes organic carbon to carbon dioxide. Quantifying autotrophic activity can help us mechanistically understand what controls greenhouse gas emissions to potentially modify designs to favor autotrophy (or apply the system to alternate waters such as mining).

We hypothesized that heterotrophic denitrification would be the dominant denitrification pathway. Secondarily, low iron concentrations would experience autotrophic denitrification while high iron concentrations would cause a shift towards dissimilatory nitrate reduction to ammonium (DNRA). While denitrification removes bioavailable nitrogen in the system, the DNRA process retains it in the form of ammonium. Ammonium retention likely supports anammox, a separate pathway that consumes ammonium and produces dinitrogen gas, contributing to the removal of bioavailable nitrogen. Since the role of iron in the partitioning of these processes was previously unknown, this investigation evaluated the effect of different concentrations of ferrous iron on the metabolic processes within the biomat system.

Methods

The experiment was conducted in microcosms with sample biomat from a shallow open water engineered wetland in Orange County, CA that selects for a benthic microbial community by preventing plant growth[2]. Ongoing research has shown that organisms that can use iron as an electron donor in denitrification are potentially active in the biomat (in prep), and this research queried iron's potential to serve as a prominent electron donor for the organisms' use. Foiled 240mL glass serum vials in triplicate contained the anaerobic microcosms (He flushed and overpressurized to ~6psig) and were placed on a shaker table at 200rpm and 21°C. After 15 days of conditioning, the system was spiked with concentrations of ferrous iron ranging from 0, 5, 100 to 1000 μM Fe2+, nitrate at a concentration of 2mM, and acetate at a concentration of 1mM. Aqueous sampling conducted twice a week monitored concentrations of species of interest using standard colorimetric methods[3], the Ferrozine reaction[4], ion chromatography, and a Shimadzu TOC-L analyzer. Species included nitrate, nitrite, ammonium, sulfide, ferrous iron, ferric iron, total organic and inorganic carbon, and acetate. Additional sampling once a week included gas, mineral and microbial sampling. Gas analysis will be conducted on a GC-ECD to quantify nitrous oxide and methane production; mineral sampling tested for ferric oxides; microbial sampling used PCR techniques to identify the presence of functional genes for denitrification, DNRA, and anammox to link the chemistry with the microbiology.

Results and Discussion

The concentration of nitrate decreased after the nitrate and acetate spike at 15 days. Possible electron donors analyzed were ferrous iron and total organic carbon (TOC) (Figure 1). The loss in nitrate corresponds with a loss in TOC in all conditions and a loss in ferrous iron in the high iron condition, indicating that both organic carbon and ferrous iron may have served as electron donors (Figure 1). The nitrate and TOC trend also indicates heterotrophic denitrification.

Distinctions among conditions occurred predominantly between the control and high iron

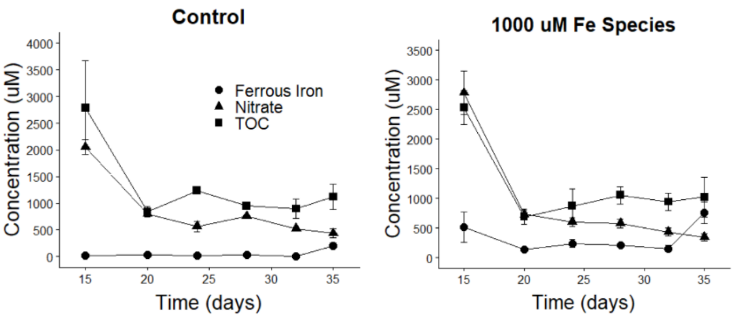

Fig. 1: Concentrations of nitrate, ferrous iron and organic carbon for the (left) control condition and (right) 1000 µM condition.

condition. Ammonium production showed no difference among the conditions (Figure 2A). A lack of differential ammonium production in the high iron concentrations serves to refute the hypothesis that high iron conditions favored DNRA.

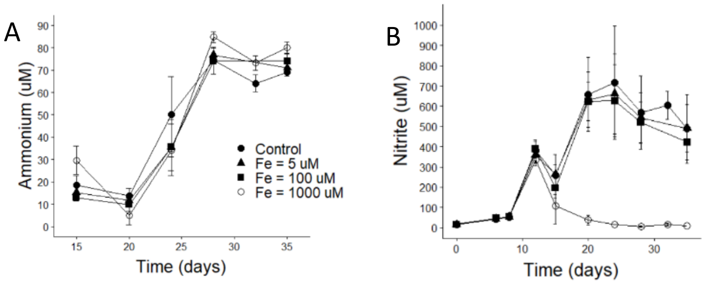

Fig. 2: Concentrations across conditions of A) Ammonium B) Nitrite.

The most compelling distinction between the low and high iron conditions after iron was added at 15 days occurred for nitrite (Figure 2B). The decrease in nitrite corresponded with a visible iron oxidation in the high condition (Figure 3).

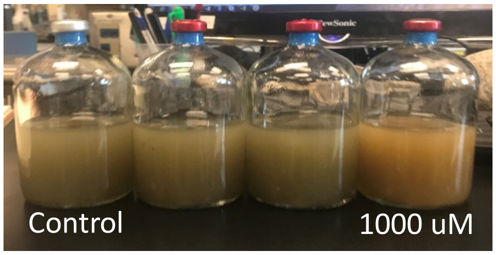

Fig. 3: Unfoiled microcosms at the final time point. The red hue in the high iron condition indicates iron oxidation.

Conclusions and Larger Implications

The nitrate and organic carbon data serve to support the hypothesis that heterotrophic denitrification dominated. With the data gathered, there was no strong evidence for or against autotrophic denitrification. The ammonium results serve to disprove the hypothesis that high iron concentrations would cause a shift towards DNRA.

The reduction in nitrite coupled with iron oxidation indicates that a process such as abiotic iron oxidation with nitrite reduction[5] may occur above a threshold iron concentration. Nitrite was the key species affected by the addition of high amounts of iron.

Continuing research from this experiment will analyze gathered gas samples, acetate data and microbial data. Given the conclusion of heterotrophic denitrification, gas samples should contain carbon dioxide and nitrous oxide. Acetate data could further support the heterotrophic denitrification conclusion with stoichiometric ratios. In addition, DNA sequencing of microbial samples will analyze for community shifts. If the nitrite reduction is indeed abiotic, there should be no significant community shift observed. Future research to expand upon this investigation could explore the abiotic component of denitrification by heat-exposing a sample before spiking with iron and nitrate, killing microbes.

The most relevant benefactor of this research would be iron-impaired mining water industries. The highest iron condition in this experiment is relevant to concentrations seen in mining water. This research showed that iron-impaired water can still be effectively treated for nitrate removal and especially nitrite removal. Results from this investigation provide insight into nitrogen reduction processes of the Prado open water wetland in the presence of iron.

Sources

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