Goldschmidt 2023 Abstract
https://doi.org/10.7185/gold2023.15890

# Nitrate-dependent anaerobic methane oxidation (N-DAMO) as a bioremediation strategy for waters affected by agricultural runoff

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Agricultural drainage ditches are subjected to high anthropogenic nitrogen input leading to eutrophication and greenhouse gas emissions. Nitrate-dependent anaerobic methane oxidation (N-DAMO) has been proposed as a promising remediation strategy to decrease methane ($CH_4$) emissions and nitrate ($NO_3^-$) concentration simultaneously. Until now, however, there was no study assessing the efficiency of the N-DAMO process. Therefore, we aimed to evaluate the potential of N-DAMO to remove excess $NO_3^-$ and decrease $CH_4$ release from agricultural drainage ditches common in the Dutch landscape. Microcosm experiments were conducted using sediment and surface water collected from three different sites: a sandy-clay ditch (SCD), a freshwater-fed peatland ditch (FPD), and a brackish peatland ditch (BPD). The microcosms were inoculated with an N-DAMO enrichment culture dominated by *Candidatus* Methanoperedens and *Candidatus* Methylomirabilis and supplemented with $^{13}CH_4$ and $^{15}NO_3^-$. The concentration of $CH_4$, formation of $^{13}CO_2$, and evolution of N species were followed over the incubation period. Additionally, archaeal and bacterial community composition was analyzed halfway through the experiment when the $NO_3^-$ reduction was the most prominent. The results showed that a significant decrease in $CH_4$ and $NO_3^-$ concentration was only observed in the BPD sediment. In freshwater sediments (FPD and SCD) the effect of N-DAMO inoculation on $CH_4$ and $NO_3^-$ removal was negligible, likely because N-DAMO microorganisms were outcompeted by heterotrophic denitrifiers consuming $NO_3^-$ much faster. Overall, our results suggest that bioaugmentation with N-DAMO might be a potential strategy for decreasing $NO_3^-$ concentrations and $CH_4$ emission in brackish ecosystems with increasing agricultural activities where the native microbial community is incapable of efficient denitrification.