

# Can Molecular Biological Tools Characterize Methane and Nitrous Oxide Flux in Engineered Environmental Systems?

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**Background/Objectives.** Engineered environmental systems such as in situ groundwater remediation, landfills and wastewater treatment are designed to benefit the environment. Nevertheless, these systems contribute to climate change by producing potent greenhouse gases, including methane, which is 25 times more potent than carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O), which is over 300 times more potent than CO<sub>2</sub>.

Methane is produced by anaerobic microorganisms called methanogens and is a major driver of global warming. Landfills are estimated to emit over 17% of anthropogenic methane emissions. Other environmental systems with the potential to emit methane, include in situ bioremediation and wastewater treatment operations. Denitrification is a beneficial microbial process where nitrate is converted to inert nitrogen gas. Unfortunately, in wastewater treatment systems, and in groundwater, partial denitrification can lead to the production of N<sub>2</sub>O. The U.S. EPA has estimated wastewater treatment alone produces as much as 3% of total N<sub>2</sub>O emissions and is the sixth largest N<sub>2</sub>O contributor overall. Wastewater treatment represents a significant and ongoing source of greenhouse gas emissions that could make achieving “net zero” even more challenging if not addressed. Optimization of environmental systems to reduce their greenhouse gas production, or to encourage removal of these compounds, such as encouraging the growth of methane eating microbes (methanotrophs), has the potential to improve the sustainability of these systems.

**Approach/Activities.** Application of molecular biological tools (MBTs) in environmental scenarios is becoming routine and includes targeted quantitative PCR for denitrification pathway genes, next generation sequencing (NGS) profiles that can highlight key microbial groups. We have reviewed MBTs data sets from a variety of environmental systems including bioremediation, landfill and wastewater treatment sites and were able to detect and identify and quantify key microbial groups including methanogens, methanotrophs and denitrifiers, and functional genes, such as the nitrous oxide reductase gene (*nosZ*) that converts N<sub>2</sub>O to harmless nitrogen.

**Results/Lessons Learned.** Methanogen abundance varies tremendously in response to geochemical conditions, and interestingly, aerobic methanotrophs are routinely co-detected with anaerobic methanogens, representing a potential mitigating factor for methane. Denitrification genes, and associated microbial taxa, are detected in varying proportions that represent the potential for both complete and incomplete denitrification. This presentation will provide an overview of findings related to these fundamental microbial groups and identify knowledge gaps to improve how MBTs can be used to better understand and optimize engineered environmental systems to reduce their global warming impacts.