HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 3 (2024)

# Genomic Potential of Bioaugmentation with Microbial $N_2O$ Reductase for Greenhouse Gas Mitigation

Seojin Jeong[1] and Jessica Cooper[#]

[1]Chattahoochee High School, USA
[#]Advisor

## ABSTRACT

Nitrous oxide ($N_2O$) is a potent greenhouse gas that significantly contributes to climate change and stratospheric ozone depletion. This study explores the genomic potential of bioaugmentation using microorganisms possessing nitrous oxide reductase ($N_2O$ reductase) to mitigate greenhouse gas emissions. We retrieved and analyzed 78 bacterial and archaeal $N_2O$ reductase protein sequences from 12 different phyla. Our phylogenetic tree and pairwise heatmap analyses identified several promising microorganisms, including previously studied strains such as *Pseudomonas stutzeri* DCP-Ps1, *Pseudomonas stutzeri* PCN-1, and *Anaeromyxobacter dehalogenans* 2CP-C, and newly identified groups such as *Euryarchaeota* and *Chloroflexota* based on high growth kinetics and efficient $N_2O$ reduction rates. Pathogenicity screening confirmed their safety for bioaugmentation applications. The study emphasizes the importance of leveraging microbial functions to combat climate change and calls for future research to explore the practical applications of bioaugmentation using these newly identified microorganisms.

## Introduction

The introduction of rapid industrialization prompted the development of many innovations and conveniences with benefits after a long period of stagnation due to the outbreak of World War. However, in recent years, mass pollution and contamination surged due to global warming, which has led to diverse detrimental effects, especially in the environment. Greenhouse gas emissions are the key cause of global warming, including carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$). These greenhouse gases can pose a serious threat to the environment as they can disrupt the balance of nature by keeping the heat, which leads to the rise of sea level, and altering biodiversity and current ecosystems.

Among these greenhouse gases, $N_2O$ is particularly detrimental due to its potent effects, being 265 times more impactful in terms of global warming potential than $CO_2$, despite comprising only 6.1% of total greenhouse gas emissions in the U.S. (US EPA, 2024). Additionally, $N_2O$ plays a critical role in depleting the stratospheric ozone layer, which protects the planet from harmful ultraviolet radiation. Recent studies underscore the urgency of addressing $N_2O$ emissions, which have surged from 270 parts per billion by volume (ppbv) in 1750 to 332 ppbv in 2019 (Pan et al., 2022). The rising levels of $N_2O$ have been linked to climatic warming and the formation of an ozone hole that impacts both oceanic and terrestrial temperatures (Pan et al., 2022).

Most of the $N_2O$ emissions originate from biological reactions (85%), including agricultural soil management (75%), manure management (4%), and wastewater treatment (6%) (US EPA, Overview of Greenhouse Gas, 2024). In the agricultural sector, a significant portion of $N_2O$ emissions comes from soil fertilizers used in farming and considerable quantities of $N_2O$ are also generated through manure management processes, where the gas forms from the nitrification and denitrification of nitrogen within the manure (IPCC, 2019). According

to a USDA report, $N_2O$ production between 1990 and 2018 increased from 95.8 to 99.4 MMT $CO_2$ eq. in grassland, from 18.7 to 26.1 MMT $CO_2$ eq. in wastewater treatment, and from 14.0 to 19.4 MMT $CO_2$ eq. in managed manure (USDA, 2020).

Figure 1 illustrates the nitrogen cycle and specifically the role of $N_2O$ reductase, highlighting the key processes of nitrogen fixation, nitrification, and denitrification. It shows the transformation of nitrogen from $N_2$ gas to ammonia $NH_3$, then to nitrate $NO_3$, and finally back to $N_2$ gas. The role of $N_2O$ reductase in this cycle is emphasized, showing its importance in the reduction of $N_2O$ to $N_2$. Unlike other greenhouse gases, $N_2O$ undergoes a process of nitrification and denitrification, which involves the oxidation of ammonia to nitrate and nitrite and the removal of nitrogen compounds. During nitrification, ammonia $NH_3$ is first oxidized to nitrite ($NO_2$) and then to nitrate ($NO_3$), a process facilitated by nitrifying bacteria. This phase of the nitrogen cycle involves the transfer of electrons, which plays a critical role in the overall nitrogen dynamics in the environment. The denitrification process is equally crucial, as it involves the reduction of nitrate back to nitrogen gas $N_2$ or nitrous oxide $N_2O$, facilitated by denitrifying bacteria. This step is essential in regulating the amount of nitrogen available in ecosystems and mitigating nitrogen pollution. The presence of $N_2O$ reductase in certain microorganisms determines their ability to reduce $N_2O$ to $N_2$, thus completing the nitrogen cycle and influencing the levels of $N_2O$ emissions in the atmosphere.

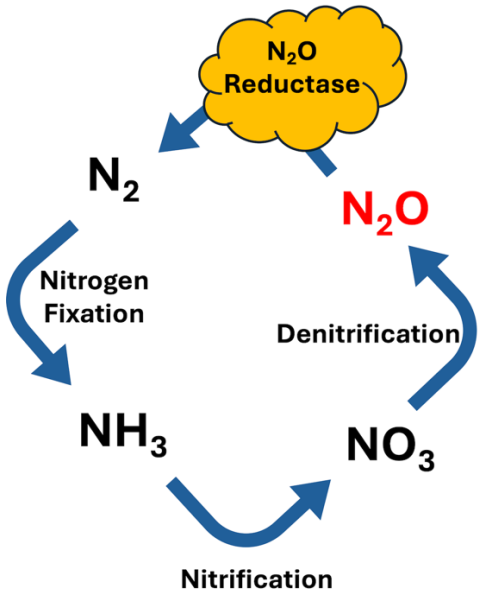


**Figure 1.** The nitrogen cycle and the role of $N_2O$ reductase

Efforts to curb $N_2O$ emissions have included the utilization of fertilizers that contain less nitrogen with split applications that increase the efficiency of plants, the use of legume crops, the prevention of waterlogging, and the implementation of nitrification inhibitors (Sudmeyer et al. 2014). In replacement to nitrogen fertilizers, genome editing in crops and the CRISPR method are utilized to increase nutrient levels and develop disease resistance in various plants without interfering with their biodiversity (Yadav et al. 2023). This method is promising to reduce greenhouse gas emissions through the use of fertilizers while maximizing the benefits of the crops.

$$N_2O + 2e^- + 2H^+ \rightarrow N_2 + H_2O$$

Mitigating $N_2O$ production can be facilitated by harnessing the $N_2O$ reductase enzyme or utilizing microorganisms containing this enzyme as biological agents for bioaugmentation. In this process, specifically, the nitrous oxide reductase (*nos*) gene, which encodes for $N_2O$ reductase in these organisms biologically transforms $N_2O$ into $N_2$. This reaction is the kinetically favorable but inert reaction, resulting in $N_2O$ accumulations in the agricultural areas. However, multiple studies have demonstrated that certain bacterial groups possess higher kinetics for converting $N_2O$ to $N_2$ (Yoon et al. 2016) and the conversion occurs more favorably under specific conditions (Robertson and Groffman. 2015). Prior screening of these microorganisms for pathogenic or other environmentally harmful traits allows their enzymes to be used as bioaugmentation agents in areas of agriculture that produce high levels of $N_2O$.

In this study, we propose a comprehensive genomic analysis of the $N_2O$ reductase (*nos*) gene across 78 archaeal and bacterial genomes in 12 different phyla that can reduce $N_2O$ emissions in agricultural sectors. The underlying hypothesis is that analyzing $N_2O$ reductases for their growth kinetics, living environments, and harmful traits in diverse microorganisms will identify more candidates of $N_2O$ reductases. This will support the feasibility of bioaugmentation in agricultural sectors to create organic fertilizers and implement $N_2O$ reductase-producing microorganisms in animals' digestive systems to minimize the greenhouse gas produced from their manure. The implications of these findings for agricultural practices are also discussed and highlighted for future applications.

## Literature Review on Microorganisms with $N_2O$ reductases

Table 1 summarizes published studies that investigate $N_2O$ reductases and the microorganisms that possess them. In this table, $N_2O$ reductases included in our phylogenetic tree and pairwise sequence analyses are marked with an asterisk (*) next to the microorganism's name.

Recently, He et al. (2024) demonstrated that *Desulfosporosinus* sp. PR can sustain bacterial $N_2O$ reduction at acidic pH, highlighting its potential for applications in acidic environments.Notably, a bioaugmentation study for $N_2O$ reduction in wastewater treatment using *Pseudomonas stutzeri* PCN-1 showed promising results, with $N_2O$ emissions reduced by an average of 36.6% in aerated zones and 41.43% in non-aerated zones (Tian et al, 2021). Hong et al. (2019) discovered that *Methylobacterium gregans* DC-1 is effective in nitrate removal without producing nitrous oxide, making it a candidate for environmentally friendly nitrogen management. The biokinetics of $N_2O$-reducing bacteria were characterized by Suenaga et al. (2018), who identified strains such as *Pseudomonas stutzeri* JCM5965, *Paracoccus denitrificans* NBRC102528, and *Azospira sp.* I13 under various oxygen levels.

The genomics and ecology of novel $N_2O$-reducing microorganisms were reviewed, noting the diversity of microbial groups involved in this process (Hallin et al., 2018). In their study, Siqueira et al. (2017) compared *Bradyrhizobium japonicum* and *Bradyrhizobium diazoefficiens*, highlighting that only the former lacks $N_2O$ reductase and the latter possesses it. Yoon et al. (2016) distinguished bacteria harboring Clade I and Clade II NosZ based on their nitrous oxide reduction kinetics, identifying *Pseudomonas stutzeri* DCP-Ps1 and *Anaeromyxobacter dehalogenans* 2CP-C respectively. Similarly, Liu et al. (2016) isolated *Marinobacter sp.* NNA5, an efficient aerobic denitrifier with zero N2O emission, underscoring its potential for mitigating nitrous oxide emissions.

A comprehensive review of nitrogen transformations, including denitrification across diverse microbial groups, was provided by Robertson and Groffman (2015). Methods to detect nitrous oxide reductase (*nosZ*) genes in soil metagenomes were developed, emphasizing their role in the nitrogen cycle (Orellana et al., 2014).

**Table 1.** Published Articles on Microorganisms Containing $N_2O$ Reductase Genes

| **Source** (Journal, Authors, Year) | **Title** | **Microorganisms that contain $N_2O$ reductase** |
|---|---|---|
| Nature Communications, He et al., 2024 | Sustained bacterial $N_2O$ reduction at acidic pH | *Desulfosporosinus* sp. PR* |
| Bioresource Technology Tian et al., 2021 | Mitigating NO and $N_2O$ emissions from a pilot-scale oxidation ditch using bioaugmentation of immobilized aerobic denitrifying bacteria | *Pseudomonas stutzeri* PCN-1 |
| Bioresource Technology Hong et al., 2019. | Efficacy of zero nitrous oxide emitting aerobic denitrifying bacterium, *Methylobacterium gregans* DC-1 in nitrate removal with strong auto-aggregation property. | *Methylobacterium gregans* DC-1 |
| Frontiers in Microbiology, Suenaga et al., 2018 | Biokinetic Characterization and Activities of $N_2O$-Reducing Bacteria in Response to Various Oxygen Levels | *Pseudomonas stutzeri* JCM5965*, *Paracoccus denitrificans** NBRC102528, *Azospira sp.* I13* |
| Trends in Microbiology, Hallin et al., 2018 | Genomics and Ecology of Novel $N_2O$-Reducing Microorganisms | Diverse microbial groups |
| Microbes and Environments, Siqueira et al., 2017 | Anaerobic Reduction of Nitrate to Nitrous Oxide Is Lower in *Bradyrhizobium japonicum* than in *Bradyrhizobium diazoefficiens* | *Bradyrhizobium japonicum* (lacks $N_2O$ reductase), *Bradyrhizobium diazoefficiens** |
| Applied and Environmental Microbiology, Yoon et al., 2016 | Nitrous Oxide Reduction Kinetics Distinguish Bacteria Harboring Clade I NosZ from Those Harboring Clade II NosZ | *Pseudomonas stutzeri* DCP-Ps1* *(Clade I), Anaeromyxobacter dehalogenans 2CP-C* (Clade II)* |
| Bioresource Technology, Liu et al., 2016 | *Marinobacter* strain NNA5, a newly isolated and highly efficient aerobic denitrifier with zero $N_2O$ emission. | *Marinobacter* sp. NNA5* |
| Soil Microbiology, Ecology, and Biochemistry, Robertson and Groffman. 2015 | Chapter 14 Nitrogen Transformations, (5) Denitrification | Diverse microbial groups |
| mBio, Orellana et al., 2014 | Detecting Nitrous Oxide Reductase (*nosZ*) Genes in Soil Metagenomes: Method Development and Implications for the Nitrogen Cycle | Diverse microbial groups |
| PNAS, Sanford et al., 2012 | Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils | *Anaeromyxobacter dehalogenans* 2CP-C* |
| Science, Galibert et al., 2001 | The composite genome of the legume symbiont *Sinorhizobium meliloti* | *Sinorhizobium meliloti* 1021* |

| | | |
|---|---|---|
| Journal of Inorganic Biochemistry, McGuirl et al., 1998 | The *nos* (nitrous oxide reductase) gene cluster from the soil bacterium *Achromobacter cycloclastes*: Cloning, sequence analysis, and expression | *Achromobacter cycloclastes* ATCC 21921=IAM 1013* |

We also reviewed expressed enzyme structures of $N_2O$ reductases from various microorganisms. Notably, *Pseudomonas nauticus* 617, *Paracoccus denitrificans*, *Achromobacter cycloclastes*, *Pseudomonas stutzeri* P1, and *Shewanella denitrificans* have been reported to harbor $N_2O$ reductases. These structures are published in the Protein Data Bank (PDB) under accession numbers 1QNI, 1FWX, 2IWF, 3SBR, and 5I5I, respectively. Figure 1 illustrates the crystal structure of $N_2O$ reductase from *Pseudomonas nauticus* 617 (Brown et al., 2000).

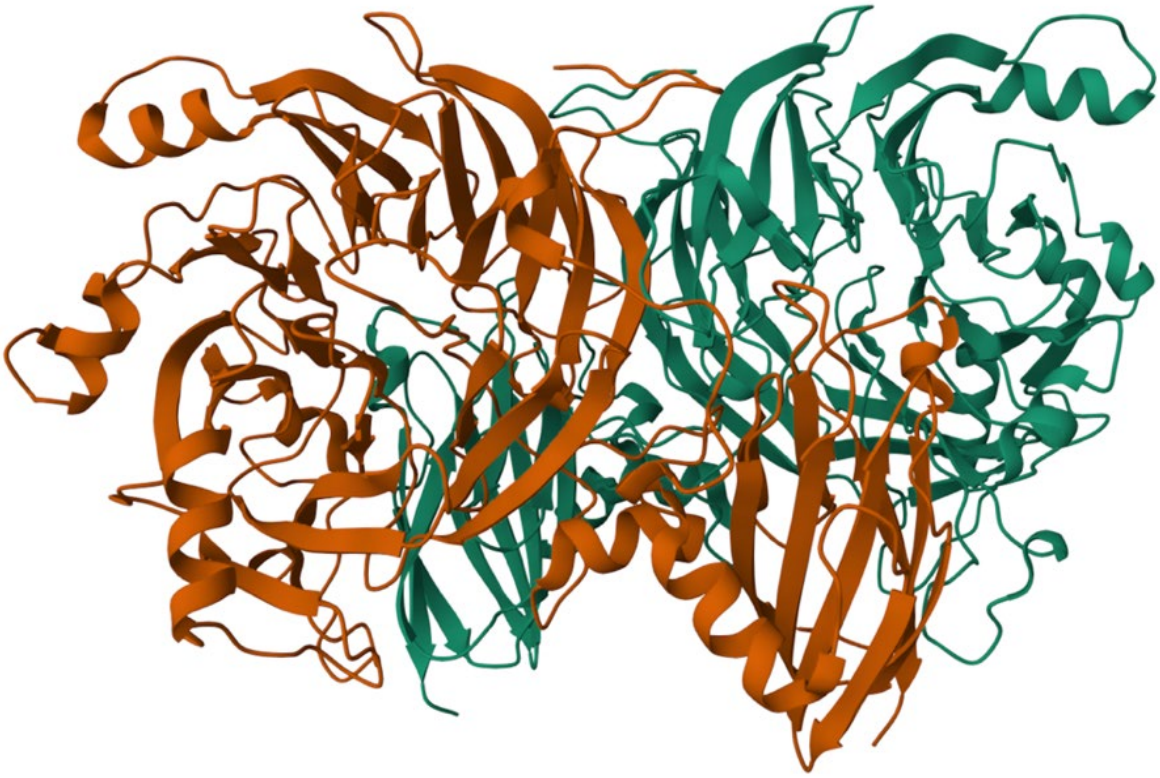

**Figure 1.** The three-dimensional structure of $N_2O$ reductase from *Pseudomonas nauticus* 617. The structure model was obtained from PDB under accession number 1QNI.

## Methods

In order to collect and analyze the $N_2O$ reductase as protein sequences, we retrieved 78 bacterial and archaeal $N_2O$ reductase protein sequences from 12 different phyla, including *Actinomyceota, Aquificota, Chloroflexota, Cyanobacteria, Deinococcota, Firmicutes, Proteobacteria, Spirochaetes, Verrucomicrobiota, Crenarchaeota, Euryarchaeota,* and *Thaumarchaeota*, from the National Center for Biotechnology Information (NCBI) GenBank by searching all bacterial and archaeal phyla.

The phylogenetic tree was constructed using MEGA (Molecular Evolutionary Genetics Analysis) to determine the evolutionary relationships among microorganisms and their direct connection to their unique ancestry. In MEGA, we analyzed and aligned the protein sequences using the ClustalW algorithm to compare their similarities and differences in the ancestry tree. The phylogenetic tree was inferred using the Neighbor-Joining method. After trimming the sequences, the gene lengths ranged approximately from 600 to 700 amino acids, and there were a total of 710 positions in the final alignment file. The tree was subsequently edited using Microsoft PowerPoint software, where the color code for phyla was created and embedded into each microorganism.

The pairwise heatmap was derived from the same aligned protein sequences used for the phylogenetic tree to compare pairwise sequence similarities among all 78 $N_2O$ reductase sequences. The pairwise heatmap analysis was performed using Python 3. Various libraries, including Biopython 1.83, NumPy, Seaborn, Matplotlib, Pandas, and SciPy, were employed to facilitate sequence manipulation, similarity calculation, and data visualization.

Figure 1 and Figure 4 were created using Microsoft PowerPoint.

## Results and Discussion

### Phylogeny of Deep-Branching $N_2O$ Reductases in Diverse Microorganisms

Figure 2 demonstrates the evolutionary relationship of 78 representative microorganisms that contain $N_2O$ reductase. This phylogenetic tree provides an overview of the diversity of bacteria and archaea with the potential to reduce $N_2O$ in the atmosphere through bioaugmentation.

We identified diverse $N_2O$ reductases across 12 different bacterial and archaeal phyla, noted on the right of the phylogenetic tree. Some microorganisms used in previous kinetics and bioaugmentation studies are dispersed throughout the tree. Notable Proteobacteria strains such as *Pseudomonas stutzeri* DCP-Ps1, *Pseudomonas stutzeri* PCN-1, *Paracoccus denitrificans*, *Bradyrhizobium diazoefficiens*, *Sinorhizobium meliloti* 1021, and *Achromobacter cycloclastes* ATCC 21921=IAM 1013 are closely related to Euryarchaeota strains such as *Natronococcus*, *Halomontanus*, *Haloplanus*, *Halopiger*, *Halovivax*, *Natronorubrum*, *Natrinema*, and *Salinirubrum*.

Among these, *Pseudomonas stutzeri* PCN-1 showed promising bioaugmentation results for $N_2O$ reduction, with an average reduction of 36.6% in aerated zones and 41.43% in non-aerated zones (Tian et al., 2021). *Pseudomonas stutzeri* DCP-Ps1 exhibited a Ks (half-saturation constant) of 35.5 ± 9.3 μM and a Vmax (maximum specific growth rate) of 4.16 ± 0.44 μmol/min/mg biomass, the highest Vmax in the absence of dissolved oxygen among tested organisms, demonstrating a high capacity for $N_2O$ reduction (Yoon et al., 2016). The similar evolutionary history between these *Proteobacteria* and *Euryarchaeota* groups possibly indicates similar functional metabolisms in their growth kinetics and bioaugmentation applications.

Interestingly, *Azospira* sp. I13 in Proteobacteria is not clustered with other Proteobacteria strains but with deep-branching microbial groups such as *Chloroflexota, Actinomyceota,* and *Cyanobacteria*. Since *Azospira* sp. I13 showed the highest $N_2O$-reducing activity in a previous study (Suenaga et al., 2018), Chloroflexota, Actinomyceota, and Cyanobacteria could also be candidates for fast $N_2O$ reduction in bioaugmentation applications. Similarly, *Anaeromyxobacter dehalogenans* 2CP-C in *Proteobacteria* is grouped with *Actinomyceota* and *Chloroflexota* and closely clustered with many *Verrucomicrobia* strains. Sanford et al. (2012) reported high $N_2O$ reduction activity for *Anaeromyxobacter dehalogenans* 2CP-C, with a cell yield of about 6.4 mg dry weight per mmol $N_2O$ reduced, indicating highly efficient $N_2O$-reducing metabolism. This efficiency suggests similar $N_2O$ reductase activities in closely related microbial groups in the tree.


**Figure 2.** Phylogenetic Tree of $N_2O$ reductase genes in diverse microorganisms. The color code represents 12 different phyla and is embedded next to each microorganism to identify its phylum. Microorganisms' names in

red indicate those with $N_2O$ reductases identified in previous studies, as shown in Table 1. NCBI GenBank accession numbers are provided in parentheses.

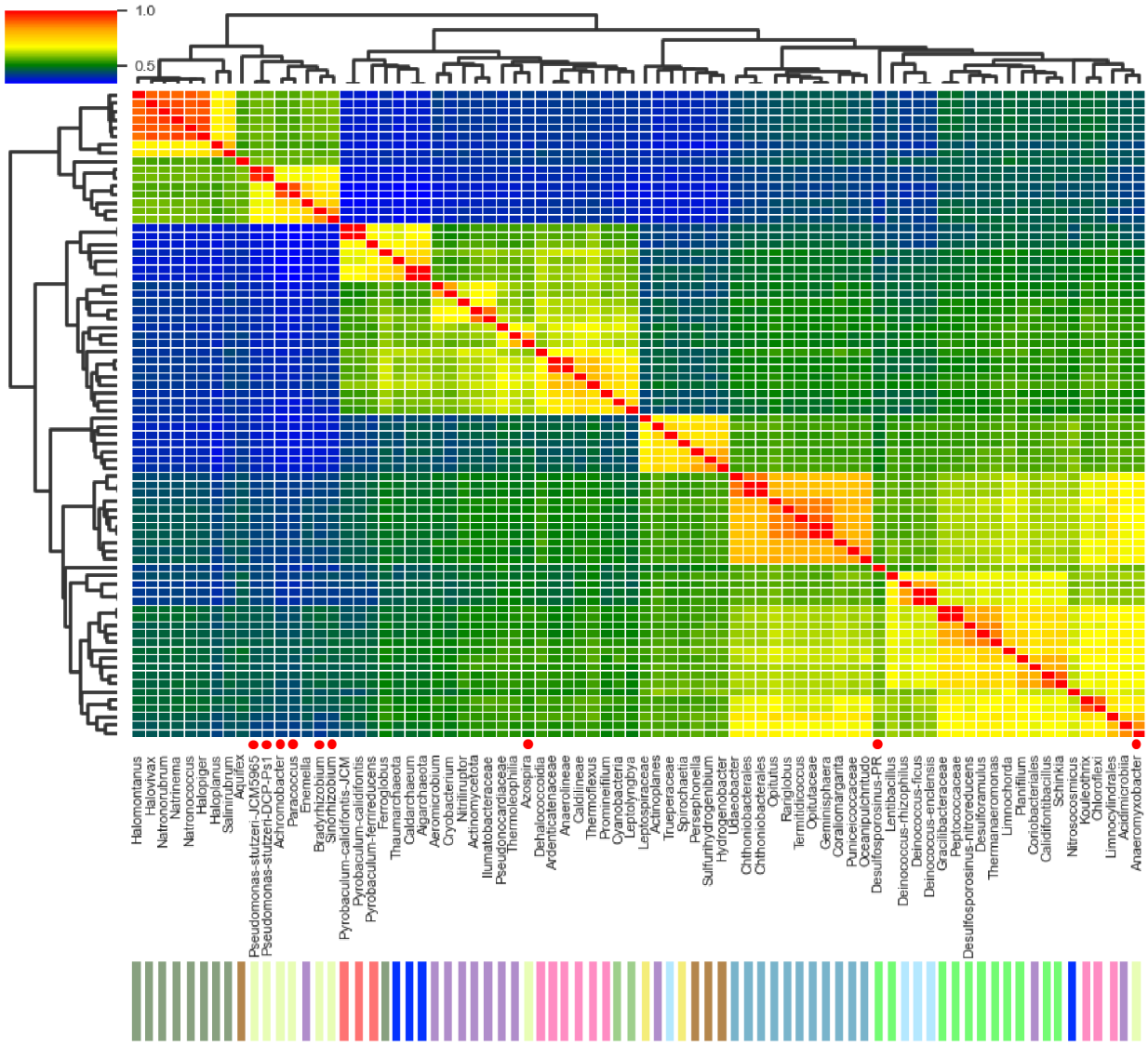


**Figure 3.** Pairwise heatmap based on the alignment file for the phylogenetic tree in Figure 2. Microorganisms' names are shown on the X-axis from left to right, and the same list on the Y-axis from top to bottom. The list on the Y-axis is omitted in the graph. Genus names are provided. If the genus is the same, species or strain names are given. Microorganisms' names marked with red dots indicate $N_2O$ reductases identified in previous studies, as shown in Table 1. Dendrograms are placed on the left and top of the pairwise heatmap to display the evolutionary history based on sequence similarity. The square boxes in the top left corner explain the color key for similarity: red indicates a value of 1 (identical), and blue indicates a value of 0 (distinct).

As Figure 3 illustrates, we analyzed the pairwise sequence similarities of $N_2O$ reductases to find similarities and distinctions between two $N_2O$ reductases. The heatmap delineated the relationship of $N_2O$ reductases' protein sequence similarities between 78 microorganisms. In addition to the phylogenetic tree that indicated the connection in their $N_2O$ reductases' evolutionary relationships, we looked for their pairwise similarities or dissimilarities. This was similar to the relationship between *Proteobacteria* such as *Pseudomonas stutzeri*

DCP-Ps1 and *Pseudomonas stutzeri* PCN-1, and *Euryarchaeota* such as *Haloplanus* and *Natronococcus*. As shown on the top left, the two groups, *Proteobacteria* and *Euryarchaeota*, shared relatively high similarity of approximately 60 to 70%. Similar to *Pseudomonas stutzeri* DCP-Ps1 and *Pseudomonas stutzeri* PCN-1 that were used for bioaugmentation and microbial kinetics studies, which indicated promising growth rates and bioaugmentation efficiency, those *Euryarchaeota* strains could be a platform for bioaugmentation based on $N_2O$ reductase protein sequence similarities. Another notable microorganism was *Azospira* in the *Proteobacteria* phylum, which was identified adjacent to non-*Proteobacteria* such as *Actinomycetota* and *Chloroflexota* in the phylogenetic tree. This microorganism also showed high sequence similarities with them, represented in green, yellow, and orange colors, indicating approximately 40 to 70% similarity. Moreover, *Anaeromyxobacter dehalogenans* 2CP-C indicated significantly high $N_2O$ reductase sequence similarity with two other microorganisms, *Acidimicrobiia* and *Limnocylindrales*, showing yellow to red, approximately 70 to 80% of protein sequence similarity.

## Bioaugmentation with $N_2O$ Reductase-Harboring Microorganisms in Agricultural Sectors

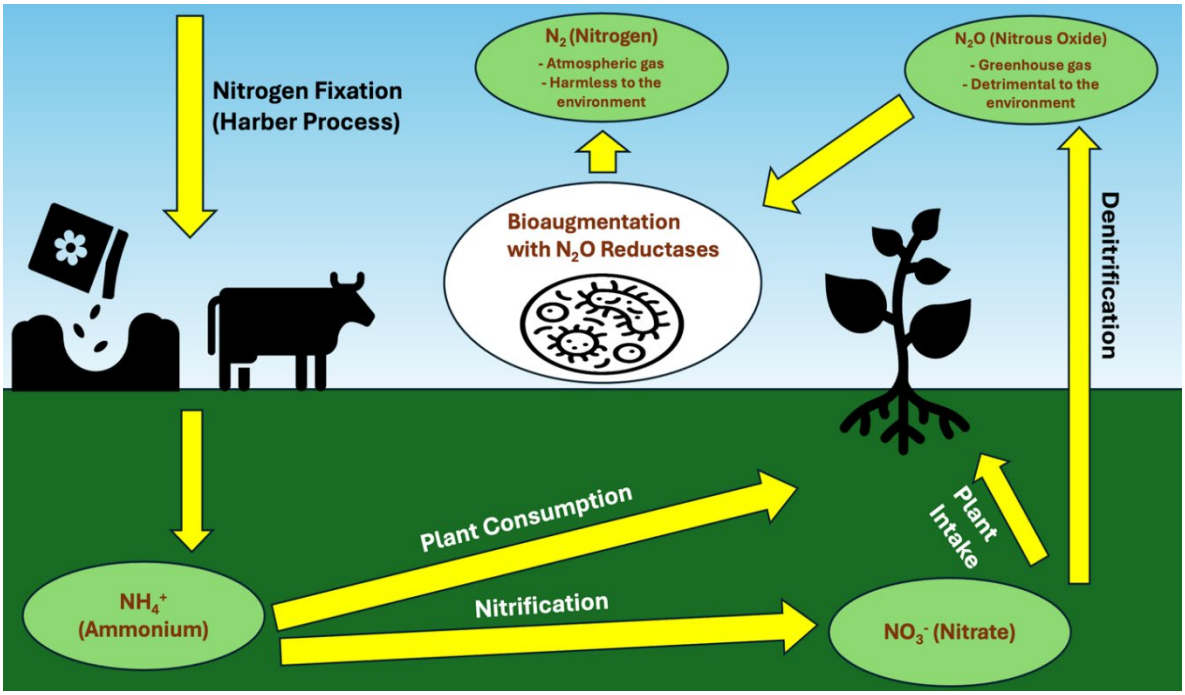


**Figure 4.** Conceptual design of bioaugmentation using microorganisms with $N_2O$ reductase in agricultural sectors

The conceptual diagram in Figure 4 illustrates how bioaugmentation can be employed in agricultural sectors. As reviewed in the nitrogen cycle, the ultimate product is the harmless, atmospheric gas $N_2$. Fixed nitrogen from the Haber process undergoes nitrification and denitrification processes, as well as plant consumption. $N_2O$ is produced during the denitrification process and converted to $N_2$ by $N_2O$ reductases in diverse microorganisms. As the diagram shows, denitrification is essential for the removal of $N_2O$ and the reduction of greenhouse gas emissions in agricultural fields.

Bioaugmentation for $N_2O$ reduction has been proposed primarily for wastewater treatment plants. However, our research introduces a novel approach to bioaugmentation in agricultural fields by using (i) organic

fertilizer or (ii) synthetic food for animals' digestive systems, both amended with $N_2O$-reducing microorganisms. Given that denitrification occurs in anaerobic or low-oxygen conditions, bioaugmentation with *Pseudomonas stutzeri* PCN-1 and *Pseudomonas stutzeri* DCP-Ps1 as an organic fertilizer and as a synthetic food amendment for ruminants could be a promising strategy. Both strains have demonstrated successful bioaugmentation and growth kinetics for $N_2O$ reduction in low-oxygen conditions (Tian et al., 2021; Yoon et al., 2016). This is pertinent because both soil environments and the digestive systems of ruminants are typically considered oxygen-limited or anaerobic.

As identified in our phylogenetic tree and pairwise heatmap analyses, bioaugmentation could be applied with similarly performing microbial groups, such as *Euryarchaeota* close to *Pseudomonas*. Among the *Euryarchaeota*, several strains like *Halomontanus*, *Haloplanus*, *Halopiger*, and *Halovivax* have been studied as halophiles, which can be cultivated in hypersaline conditions (Mitra et al., 2021). Certain soil environments are considered moderately saline with higher conductivity (Corwin, 2005). Despite the slower growth kinetics of *Euryarchaeota*, they offer the advantage of being cultivable without sterilization in hypersaline conditions (Mitra et al., 2021), potentially reducing the cost of producing these $N_2O$ reductase-producing microorganisms.

### Environmental and Human Health Implications of Bioaugmentation

As the final step, we analyzed the potential pathogenicity of microorganisms and their implications for human health as identifying bacteria containing the *nos* gene is crucial, but investigating their pathogenicity through protein sequencing is equally vital. In most cases, microorganisms mitigate $N_2O$ without being pathogenic, such as *Pseudomonas stutzeri*, known for its safety to human health and its isolation in non-pathogenic contexts. Consistently, in our study, no pathogenicity was identified in previously studied microorganisms such as *Pseudomonas stutzeri* DCP-Ps1, *Pseudomonas stutzeri* PCN-1, *Paracoccus denitrificans*, *Bradyrhizobium diazoefficiens*, *Sinorhizobium meliloti* 1021, *Achromobacter cycloclastes* ATCC 21921 = IAM 1013, *Azospira* sp. I13, and *Anaeromyxobacter dehalogenans* 2CP-C. Similarly, closely related microorganisms and promising strains, including *Euryarchaeota*, *Chloroflexota*, *Actinomyceota*, *Cyanobacteria*, *Firmicutes*, and *Verrucomicrobia*, did not exhibit pathogenic traits.

However, the phylum *Firmicutes* contains several pathogens in the *Bacillus* genus, such as *Bacillus cereus* and *Bacillus subtilis*, which warrants caution when considering *Firmicutes* for bioaugmentation applications. Therefore, pre-cultivation of $N_2O$ reductase-producing microorganisms should include thorough genomic and physiological screenings against pathogenicity. If feasible, enzyme purification processes can be considered to mitigate potential pathogenic risks and align with economic platform configurations.

## Conclusion

Our research has successfully identified several microbial groups with significant potential for bioaugmentation to mitigate greenhouse gas emissions through $N_2O$ reductase activity. Using phylogenetic tree and pairwise heatmap analyses, we pinpointed both previously studied microorganisms, such as *Pseudomonas stutzeri*, and newly identified microbial groups, including *Euryarchaeota* and *Chloroflexota*. These microorganisms exhibited high growth kinetics and efficient $N_2O$ reduction rates, highlighting their suitability for bioaugmentation applications in various environmental contexts. Future research should focus on the practical implementation of bioaugmentation using these microorganisms, particularly in agricultural sectors and animal digestive systems, ensuring their safety and economic feasibility.

## Acknowledgments

I would like to thank my advisor, Mrs. Jessica Cooper.

## References

Brown, K., Tegoni, M., Prudêncio, M., Pereira, A. S., Besson, S., Moura, J. J., Moura, I., & Cambillau, C. (2000). A novel type of catalytic copper cluster in nitrous oxide reductase. *Nature Structural Biology, 7*(3), 191-195. https://doi.org/10.1038/73288

Corwin, D. L., & Lesch, S. M. (2005). Apparent soil electrical conductivity measurements in agriculture. *Computers and Electronics in Agriculture, 46*(1-3), 11-43. https://doi.org/10.1016/j.compag.2004.10.005

Galibert, F., Finan, T. M., Long, S. R., Puhler, A., et al. (2001). The composite genome of the legume symbiont Sinorhizobium meliloti. *Science, 293*, 668-672.

Hallin, S., Philippot, L., Sanford, R. A., & Jones, C. M. (2018). Genomics and Ecology of Novel N2O-Reducing Microorganisms. *Trends in Microbiology, 26*(1), 43-55. https://doi.org/10.1016/j.tim.2017.07.003

He, G., Chen, G., Xie, Y., Swift, C. M., Ramirez, D., Cha, G., Konstantinidis, K. T., Radosevich, M., & Löffler, F. E. (2024). Sustained bacterial N2O reduction at acidic pH. *Nature Communications, 15*(4092). https://doi.org/10.1038/s41467-024-48236-x

Hong, P., Shu, Y., Wu, X., Wang, C., Tian, C., Wu, H., Donde, O. O., & Xiao, B. (2019). Efficacy of zero nitrous oxide emitting aerobic denitrifying bacterium, Methylobacterium gregans DC-1 in nitrate removal with strong auto-aggregation property. *Bioresource Technology, 293*, 122083. https://doi.org/10.1016/j.biortech.2019.122083

IPCC. (2019). Emissions from Livestock and Manure Management. In *2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Volume 4: Agriculture, Forestry and Other Land Use*. https://www.ipcc-nggip.iges.or.jp/public/2019rf/pdf/4_Volume4/19R_V4_Ch10_Livestock.pdf

Liu, Y., Ai, G. M., Miao, L. L., & Liu, Z. P. (2016). Marinobacter strain NNA5, a newly isolated and highly efficient aerobic denitrifier with zero N2O emission. *Bioresource Technology, 206*, 9-15. https://doi.org/10.1016/j.biortech.2016.01.066

McGuirl, M. A., Nelson, L. K., Bollinger, J. A., Chan, Y. K., & Dooley, D. M. (1998). The nos (nitrous oxide reductase) gene cluster from the soil bacterium Achromobacter cycloclastes: Cloning, sequence analysis, and expression. *Journal of Inorganic Biochemistry, 70*(2), 155-169. https://doi.org/10.1016/S0162-0134(98)10001-6

Mitra, R., Xu, T., Xiang, H., & Han, J. (2020). Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. *Microbial Cell Factories, 19*(86). https://doi.org/10.1186/s12934-020-01342-z

Orellana, L. H., Rodriguez-R, L. M., Higgins, S., Chee-Sanford, J. C., Sanford, R. A., & Konstantinidis, K. T. (2014). Detecting nitrous oxide reductase (nosZ) genes in soil metagenomes: method development and implications for the nitrogen cycle. mBio, 5(5), e01193-14. https://journals.asm.org/doi/pdf/10.1128/mbio.01193-14

Pan, S. Y., He, K. H., Lin, K. T., Fan, C., & Chang, C. T. (2022). Addressing nitrogenous gases from croplands toward low-emission agriculture. *npj Climate and Atmospheric Science, 5*(43). https://doi.org/10.1038/s41612-022-00265-3

Robertson, G. P., & Groffman, P. M. (2015). Nitrogen Transformations. In *Soil Microbiology, Ecology, and Biochemistry* (pp. 421-443). Elsevier Inc. http://dx.doi.org/10.1016/B978-0-12-415955-6.00014-1

Siqueira, A. F., Minamisawa, K., & Sánchez, C. (2017). Anaerobic Reduction of Nitrate to Nitrous Oxide Is Lower in Bradyrhizobium japonicum than in Bradyrhizobium diazoefficiens. *Microbes and Environments, 32*(4), 398-401. https://doi.org/10.1264/jsme2.ME17081

Sudmeyer, R., Parker, J., Nath, T., & Ghose, A. (2014). Carbon farming in relation to Western Australian agriculture, *Bulletin 4856*, Department of Agriculture and Food, Western Australia. https://www.agric.wa.gov.au/sites/gateway/files/Carbon%20farming%20in%20relation%20to%20Western%20Australian%20agriculture%20-%20Bulletin%204856%20%28PDF%201.4MB%29.pdf

Suenaga, T., Riya, S., Hosomi, M., & Terada, A. (2018). Biokinetic Characterization and Activities of N2O-Reducing Bacteria in Response to Various Oxygen Levels. *Frontiers in Microbiology, 9*, 697. https://doi.org/10.3389/fmicb.2018.00697

Tian, T., Li, Q., Zhuang, Z., Zhuang, G., & Sun, H. (2021). Bioaugmentation enhanced the resistance to nitrite and reduced N2O generation via nitrite reduction. *Bioresource Technology, 337*, 125364. https://www.sciencedirect.com/science/article/abs/pii/S0960852421010452#:~:text=Bioaugmentation%20enhanced%20the%20resistance%20to,O%20generation%20via%20nitrite%20reduction

United States Department of Agriculture (USDA). (2020). U.S. Agriculture and Forestry Greenhouse Gas Inventory: 1990-2018. *Technical Bulletin No. 1957.* https://www.usda.gov/sites/default/files/documents/USDA-GHG-Inventory-1990-2018.pdf

United States Environmental Protection Agency. (2024). Overview of Greenhouse Gas. Accessed 04 May 2024. https://www.epa.gov/ghgemissions/overview-greenhouse-gases#:~:text=Total%20U.S.%20Emissions%20in%202022,of%20these%20greenhouse%20gas%20emissions.

Yadav, R. K., Tripathi, M. K., Tiwari, S., Tripathi, N., Asati, R., Chauhan, S., Tiwari, P. N., & Payasi, D. K. (2023). Genome Editing and Improvement of Abiotic Stress Tolerance in Crop Plants. *Life, 13*, 1456. https://doi.org/10.3390/life13071456

Yoon, S., Nissen, S., Park, D., Sanford, R. A., & Löffler, F. E. (2016). Nitrous oxide reduction kinetics distinguish bacteria harboring clade I NosZ from those harboring clade II NosZ. *Applied and Environmental Microbiology, 82*(13), 3793-3800. https://doi.org/10.1128/AEM.00409-16