When you hear the term “greenhouse gas”, the first gas that comes to mind is probably carbon dioxide (CO$_2$). Society considers this heat-trapping gas as the main culprit of global warming. However, there are other greenhouse gases! While they exist at much lower levels than carbon dioxide, they are often more able to trap outgoing heat energy from the Earth. Nitrous oxide (N$_2$O) is one of these stronger greenhouse gases, and scientists are trying to figure out how to reduce its impact on the global climate.

To do so, we must first understand what produces it and how they create it. We tested different types of algae in both light and dark environments, which made us realize that only green algae make nitrous oxide from nitric oxide, and they have different ways of doing it based on the amount of light available. We also linked the nitric oxide production to fertilizers, implying that there may be a way to reduce the amount of nitrous oxide produced by algae in the future.

**Introduction**

When you hear the term “greenhouse gas”, the first gas that comes to mind is probably carbon dioxide (CO$_2$). Society considers this heat-trapping gas as the main culprit of global warming. However, there are other greenhouse gases! While they exist at much lower levels than carbon dioxide, they are often more able to trap outgoing heat energy from the Earth. Nitrous oxide (N$_2$O) is one of these stronger greenhouse gases, and scientists are trying to figure out how to reduce its impact on the global climate.

To do so, we must first understand what produces it. For some time, scientists noticed a correlation between algal blooms and nitrous oxide, but they didn’t understand how algae produced this gas. In this study, we wanted to figure out the link between algae and nitrous oxide. How do algae produce it? Does light affect this process? And is there a way that we can reduce its production?
Methods

→ **Experiment 1:**
We conducted a nitric oxide (NO) and nitrous oxide (N\textsubscript{2}O) exchange experiment by placing algae into an anaerobic environment that contained nitric oxide. Then we used a special type of mass spectrometer to measure how the amount of nitric oxide and nitrous oxide changed over time. We ran the experiment in the dark and in light to see how things changed. Then we ran the experiment again in an aerobic environment that contained nitrite (a common fertilizer) as the source of nitric oxide to better simulate what would happen naturally.

→ **Experiment 2:**
The algae species we used contained flavodiiron proteins (FLVs) and cytochrome P450 (CYP55), which are both families of enzymes. These enzymes help add electrons to nitric oxide, causing it to transform into nitrous oxide, a process known as reduction. We conducted the NO and N\textsubscript{2}O exchange experiment again, but with mutated algae species that were either missing the FLVs or CYP55. We ran the experiment in both the light and dark and compared the results to Experiment 1.

→ **Experiment 3:**
We conducted the same NO and N\textsubscript{2}O exchange experiment with nine species of algae. These species included green algae, red algae, and diatoms. Two of the nine species had both types of enzymes, two species only had the flavodiiron proteins, and five species did not have either enzymes. We ran the experiment in both the light and dark and compared the results to Experiment 1.

Results

→ **Experiment 1:**
This experiment showed that algae produced nitrous oxide in both the dark and the light. However, the rate of this production is faster in the light. Similar results occurred when we used nitrite, a common fertilizer. The algae transformed the nitrite into nitric oxide before reducing it to nitrous oxide in both the light and dark.

→ **Experiment 2:**
Algae mutants without FLVs produced about the same amount of nitrous oxide in the dark, but significantly less in the light. The algae mutants without CYP55 produced the same amount of nitrous oxide in the light, but did not produce any nitrous oxide in the dark (Figure 1).

→ **Experiment 3:**
Green algae took in nitric oxide and used it to produce nitrous oxide. The red algae and diatoms also took in nitric oxide, but they did not produce nitrous oxide from it.

![Figure 1](image_url)

The rate of nitrous oxide production in the dark and in the light for algae with both flavodiiron proteins (FLVs) and cytochrome P450 (CYP55) as well as the mutant algae species missing one of the enzyme families.

Which enzyme family is responsible for nitrous oxide production in the dark? In the light?
Discussion

Our data showed that there are two ways algae make nitrous oxide. When there was light, algae used flavodiiron proteins to reduce nitric oxide to nitrous oxide. When it was dark, cytochrome P450 produced nitrous oxide. Also, because algae produced nitrous oxide faster in light than in darkness, we know that photosynthesis aided this process.

Our results also proved that only green algae produced nitrous oxide through the reduction of nitric oxide. Since green algae have both types of enzymes, the chemical pathways we discovered are the only ones producing nitrous oxide in algae.

Conclusion

Global warming is a real concern. Increasing temperatures are already impacting both our climate and our oceans. We must lower the production of greenhouse gases, and now we know that we should also decrease the amount of fertilizer runoff into lakes and the ocean.

You can help reduce the amount of fertilizer runoff from your garden!

- Plant a garden with native plants because they need less fertilizer.
- Apply fertilizer at the right time of year and in the right amount for your plants.
- Avoid applying it right before storms so it is not carried to the closest body of water (where algae can transform it into nitrous oxide).

Check your understanding

1. How does algae both decrease and increase the amount of greenhouse gases in the atmosphere?

2. What evidence from this study supports the claim that photosynthesis is involved in the reduction of nitric oxide (NO) to nitrous oxide (N₂O)?

3. Why do only green algae produce nitrous oxide?

4. Enzymes play an important role in the production of nitrous oxide. Can you think of other processes that utilize enzymes?

5. The article suggests ways for people with gardens to reduce the impact of fertilizer. However, a significant portion of fertilizer runoff comes from agriculture. Research solutions that farms can use to reduce the input of nitrites into bodies of water. Make a list of at least three solutions, and identify which solution you think is the best choice. Explain why you selected this solution.
Glossary of Key Terms

**Aerobic environment** - an environment in which oxygen is present.

**Algal bloom** - the rapid increase in the population of algae in an aquatic ecosystem.

**Anaerobic environment** - an environment in which oxygen is absent.

**Cytochrome P450 (CYP55)** - a family of enzymes commonly found in some species of algae and in fungi.

**Diatoms** - species of algae that use silica (SiO$_2$) for their cell walls.

**Enzyme** - special types of proteins that plant and animal cells make. They help control how quickly chemical reactions happen.

**Flavodiiron proteins** - a family of enzymes commonly found in some species of algae and in bacteria. They transfer electrons to oxygen or nitric oxide to avoid the buildup of toxic compounds.

**Greenhouse gas** - a gas in the atmosphere that traps outgoing heat energy from the Earth. By preventing this energy from radiating into space, the Earth is warmed. The greenhouse gases act like a blanket. When you wrap yourself in a blanket, you warm up because it prevents your heat energy from escaping.

**Mass spectrometer** - a device used to identify the presence of chemical compounds. A mass spectrometer makes the compounds into an ion by removing electrons. Then they are moved through a magnetic field which exerts a force on them, causing them to move in a more circular path. The path they take depends on their mass. The mass spectrometer uses their path to identify what compound they are. Think about it like this: if you shoot water at a tennis ball flying by, then at a bowling ball, they’ll end up in different places because they have different masses.

**Reduction** - a chemical reaction during which electrons are added to a chemical. For example: $2\text{NO} + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O}$.

**Runoff** - liquid that drains or flows off, as rainwater flows off fields and into nearby streams and rivers.

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Acknowledgement: This article's adaptation was supported by [Gráfico Familiar Foundation].