The Impact of Freeze-Thaw Cycles on CO_2 Emissions and Subsequent Algae Growth

Adeline Niemier

Background

- **One of the biggest unknowns in climate change is the impact of thawing permafrost on the planet**
  - Permafrost, frozen soil/rock/organic matter, covers 15% of land area in Northern Hemisphere [1]
  - Permafrost contains dead plant & animal matter which is rich with carbon [2]
  - Arctic temperatures are warming 2-4 times faster than the global average, causing permafrost to thaw [3]
- **To understand how thawing of carbon-rich frozen ground impacts ecosystems & climate change, a two-phase study was conducted to investigate how the freezing and thawing of soil impacts both soil respiration and microbial activity**
  - Soil microbes eat & decompose organic matter and – during the process of soil respiration – produce CO_2 that returns to the soil and atmosphere [4]
  - As soil microbes use available carbon, they release nitrogen & phosphorous – nutrients that can drive algae growth when washed into waterways

[1] https://science.nasa.gov/kids/earth/what-is-permafrost/ ■ **[2]** Biskaborn, B. K., et al., "Permafrost is warming at a global scale," *Nature Communications*, 10(1), 264, 2019. ■ **[3]** Yonger, Sally, "NASA Helps Find Thawing Permafrost Adds to Near-Term Global Warming," *Jet Propulsion Lab*, October 2024. ■ **[4]** United States Department of Agriculture (USDA), "Soil Respiration: Soil Health – Guidelines for Educators," May 2014.

Research Questions

Phase 1: To understand if the number of freeze-thaw cycles (FTCs) impact soil respiration (CO_2 emission rates), moisture levels, or pH levels

Phase 2: To understand if the number of freeze-thaw cycles (FTCs) impact the amount or timing of algae growth

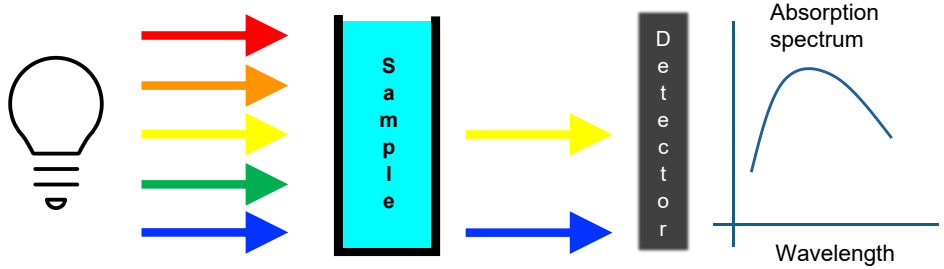
Variables

Phase 1	**Variables**
Independent	Number of times soil is frozen/thawed: 0,1,3,5,7 times
Dependent	**(1)** Amount of CO_2, **(2)** pH level, and **(3)** moisture level
Control	0 freeze condition
Phase 2	**Variables**
Independent	Number of times sample is frozen/thawed: 0,1,3,5,7 times
Dependent	Algae concentration: (spectrophotometer absorbance level, 750 nm)
Control	0 freeze condition

Materials

Phase 1: 250 mL plastic bottles ■ Soil samples from a local park ■ Vernier Go Direct CO_2 sensor ■ Vernier graphical analysis software ■ Soil pH probe ■ Freezer

Phase 2: 50 mL plastic bottles ■ Algae culture (chlorella vulgaris) ■ Algae nutrient media ■ Soil samples from a local park ■ distilled water ■ Spectrophotometer ■ coffee filters



Procedure Phase 1

- Filled 15, 250 mL bottles with 130 grams of soil and brought to room temperature
- Exposed bottles to different freeze-thaw cycles: 0,1,3,5,7 times
  - 3 bottles for each test condition

Container #s	Test Condition
1-3	Control (no freezes)
4-6	1 freeze-thaw cycle
7-9	3 freeze-thaw cycles
10-12	5 freeze-thaw cycles
13-15	7 freeze-thaw cycles

1 freeze-thaw cycle is:
- 12-hour freeze at (-14°C)
- 12-hour thaw at room temperature (18°C)

- Used sensor to measure CO_2 from each bottle over 300 second intervals
  - CO_2 readings recorded every 2 seconds
  - **Sensor records CO_2 change over time, which is used to measure soil respiration (slope of emission data)**
- Inserted soil probe to test soil pH and moisture
- Tested daily for 7 days

Procedure Phase 2

- Create supernatant (carbon, nutrients, soil microbes)
  - Mixed 1 cup of topsoil with 1 L of distilled water ■ shook 5 minutes; let settle overnight ■ decanted water 2 times with coffee filters
- Exposed supernatant to different freeze-thaw cycles:
  - 0,1,3,5,7 times
- Filled 15 50 mL test tubes with same amount of algae culture, nutrient media, & supernatant
  - 3 tubes per test condition

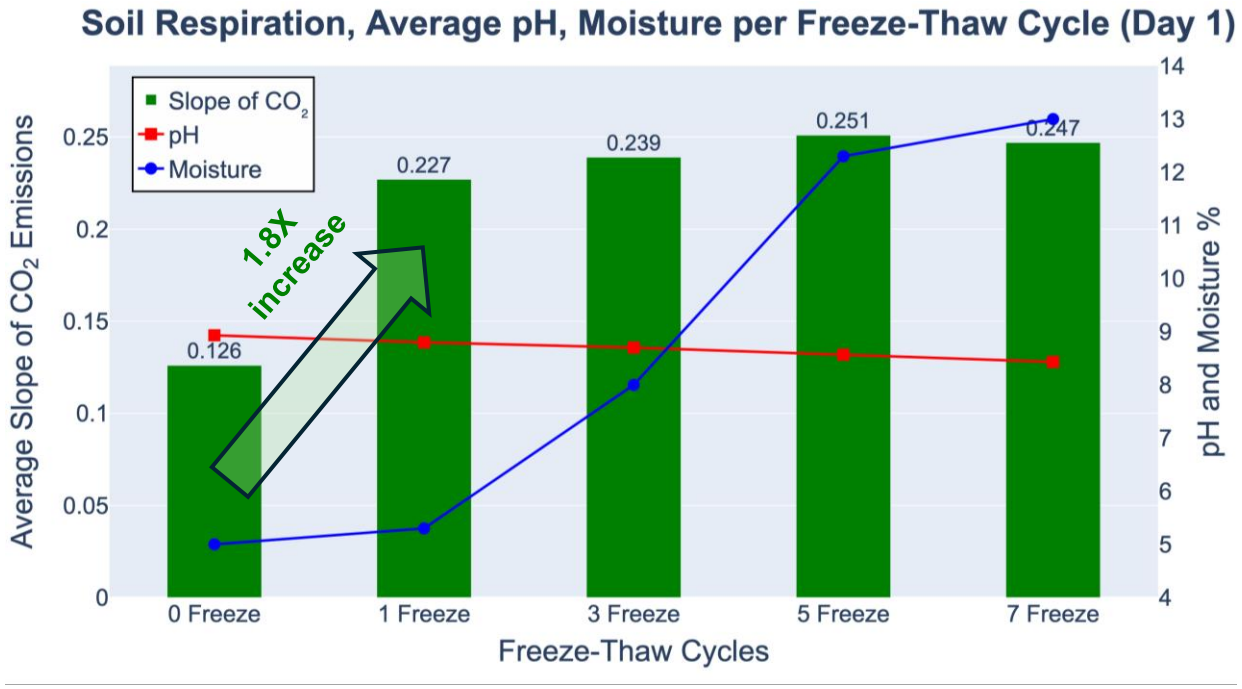
Algae Culture (mL)	Nutrient Media (mL)	Freeze/Thaw Supernatant	Distilled Water	Total volume
5 mL	20 mL	10 mL of supernatant that underwent {0,1,3,5,7} FTCs	15 mL	50 mL

- Tested absorption at optical density of 750 nm on spectrophotometer over 18 days
  - Every 12 hours for 7 days ■ then daily for 7 days ■ then every other day for an additional 4 days

Phase 1 Data

FTCs led to higher soil respiration and CO_2 emissions

- The first FTC has the greatest impact as soil respiration (slope of CO_2 emissions over time) increases dramatically from control to 1 FTC
  - ***The increase from 0 to 1 FTC is statistically significant (p=.0193)***
- Additional FTCs only slightly impact CO_2 emissions but result in lower pH & higher moisture levels
- When <u>soil freezes</u>:
  - Microbes burst (lysis) & soil particles break down, releasing carbon, nutrients, & acids – this increase in nutrients & acids lowers the pH
  - Water is soil is *condensed* leading to higher moisture levels
- When <u>soil thaws</u>:
  - Microbes eat organic material that can't decompose in frozen soil



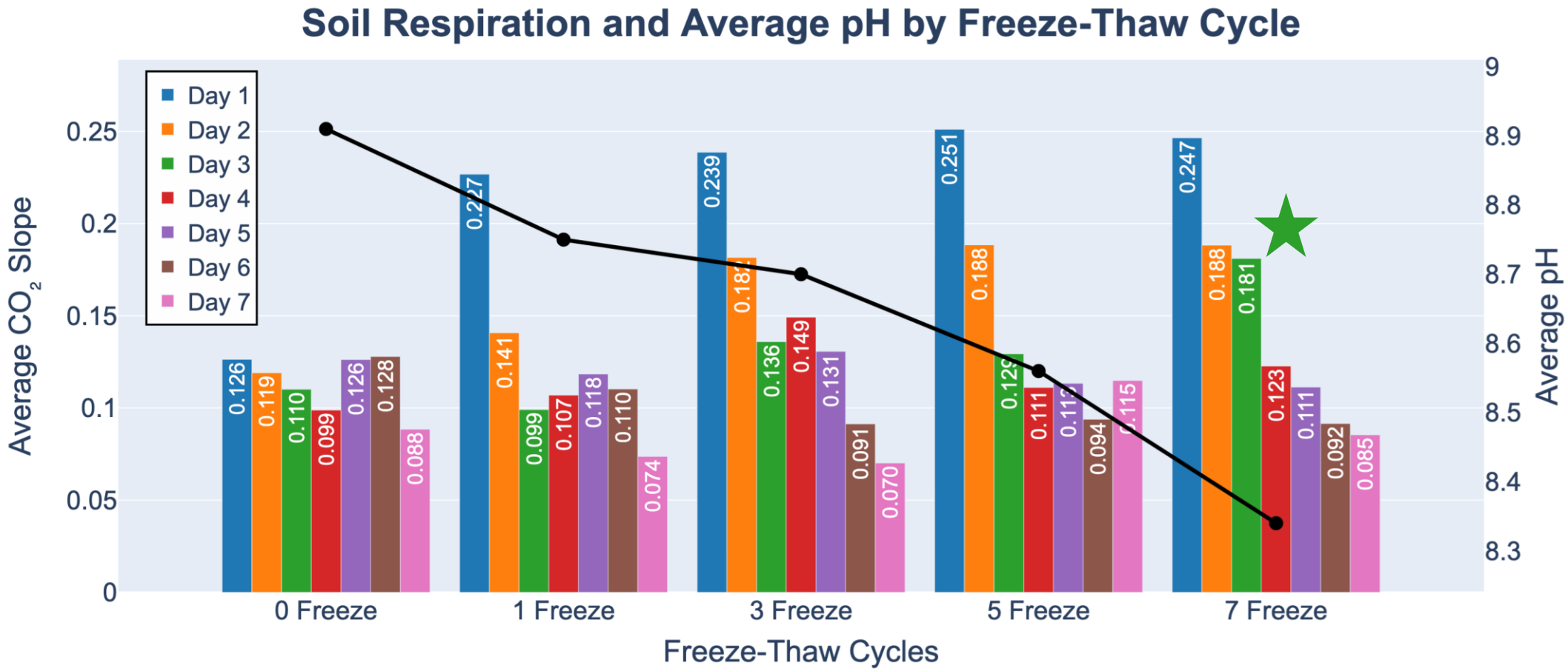
These conditions lead to a rapid increase of microbial activity which results in a burst of soil respiration and CO_2 emissions ■ Often referred to as Birch Effect [5].

[5] Fraser, F.C., et al., "On the Origin of Carbon Dioxide Released from Rewetted Soils," *Soil Biology and Biochemistry, p. 1-5,* Volume 101, October 2016.

Phase 1 Data

Number of FTCs impacts amounts of nutrients available & duration of "respiration burst"

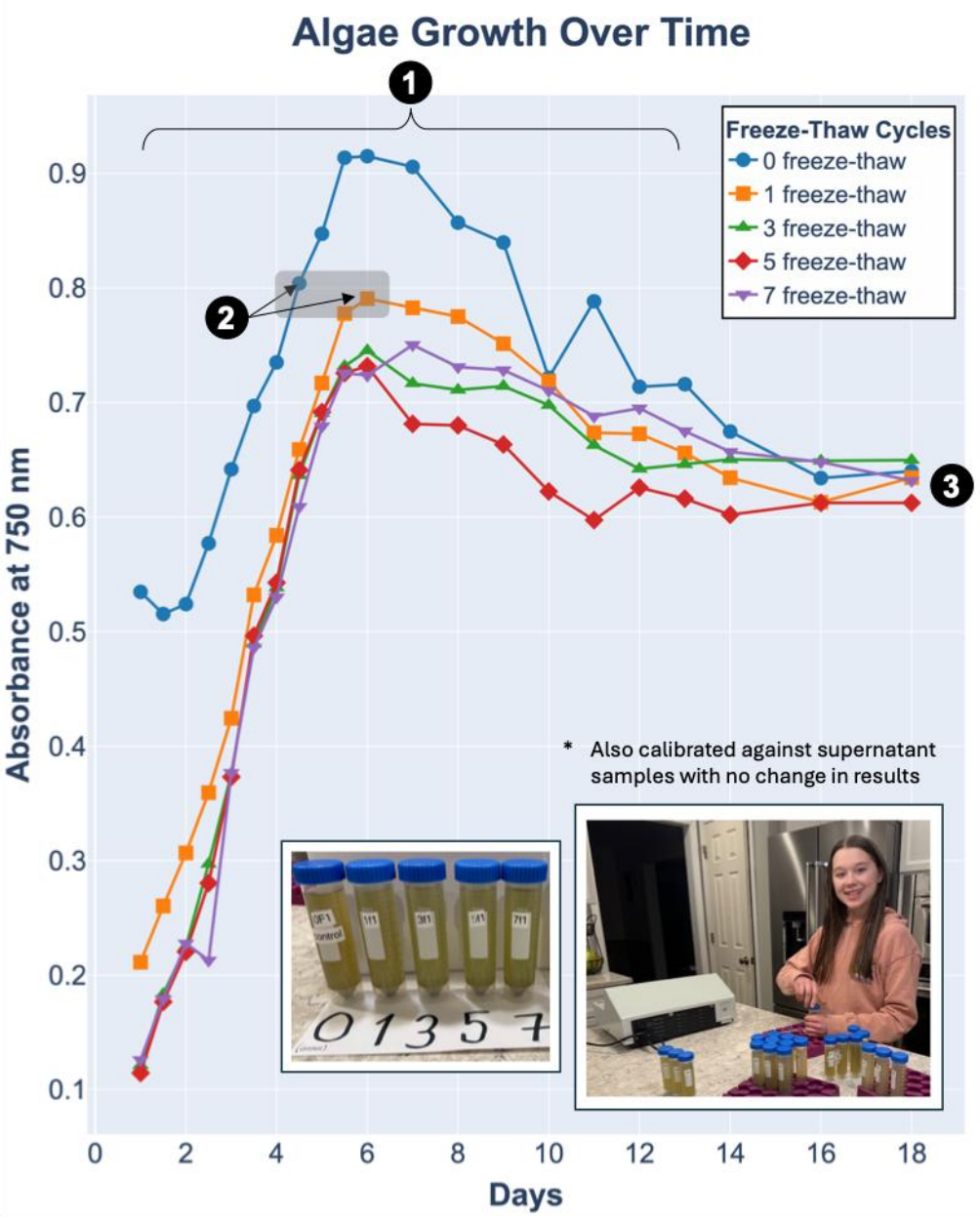
- As number of FTCs increase, respiration remains elevated for a longer period of time
  - The 7 FTC condition – which has the greatest amount of released nutrients (as indicated by lowest pH) – has the highest respiration levels on Day 3 ★
- Over time, soil respiration declines and CO_2 levels stabilize



Phase 2 Data

FTCs resulted in less, slower algae growth

1. Algae growth is ***highest*** in 0 FTC condition for first 300 hours
   - Soil microbes consume carbon and respire nitrogen & phosphorus which promote algae growth
   - **FTCs cause microbial death, reducing the size of microbial community available to produce nutrients that drive algae growth**
     - May result in up to 50% reduction in microbial biomass [6]
   - Similar to effect of FTCs on soil respiration, the first freeze-thaw cycle had the greatest impact and additional cycles had minimal differences
2. Algae growth is ***fastest*** in 0 FTC condition for first 300 hours
   - For example, the 0 FTC condition reached absorbance of ~0.8 after 108 hours, while it took the 1+ FTC conditions an additional 36 hours to reach this level of algae growth
3. After 14 days, when all nutrients are depleted, all samples contain similar amounts of algae



[6] Skogland, T., Lomeland, S., and Goksøyr, J., "Respiratory burst after freezing and thawing of soil: Experiments with soil bacteria," *Soil Biology & Biochemistry*, p. 851-856, 20(6), 1988.

Conclusions

- **CO_2 emissions increased after FTCs due to greater nutrients & increased moisture**
  - Microbes lyse and soil aggregates break apart releasing nutrients for remaining microbes to consume which, along with higher moisture levels, increases soil respiration & CO_2 emissions
  - First FTC has greatest impact n additional FTCs have less of an effect
- **Freeze-thaw cycles did not increase algae growth**
  - FTCs lead to microbial death, reducing size of microbial biomass available to release nutrients that drive algae growth
- FTCs result in less & slower algae growth

Implications

- As permafrost thaws, Arctic could change from a beneficial carbon sink to a *contributor* of global warming due to:
  - Higher CO_2 emissions from increased soil respiration
  - Slower and less CO_2 absorption due to photosynthesis impact of delayed and reduced algae growth

Future Work

- Investigate different methods of creating supernatant to determine what best replicates real-world nutrient runoff
  - Freeze soil before adding to water
  - Use carbon-rich peat moss instead of soil
- Test impact of different freeze-thaw lengths (12 vs. 24 hours)