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

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Background

- Permafrost is soil/rock/organic matter that remains frozen for at least 2 consecutive years ■ some areas frozen for 1000s of years ■ 15% of land area in Northern Hemisphere [1]
- Permafrost contains dead plant & animal matter which is rich with carbon [2]
- Temperatures in the Arctic 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, it is important to study 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

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

Hypotheses

Phase 1: As number of freeze-thaw cycles (FTCs) increase:

1. More CO_2 will be released (soil respiration)
2. Moisture levels will increase
3. pH levels will decrease

Phase 2: As number of freeze-thaw cycles (FTCs) increase:

1. Algae growth (absorbance levels) will increase
2. Algae growth will be delayed

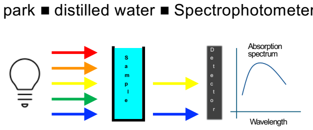
Variables

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 (removed large sticks, roots, etc.) 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

- Acquired supernatant (carbon, nutrients, soil microbes)
  - Mixed 1 cup of soil 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 cycles
- 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	Total volume
5 mL	20 mL	25 mL of supernatant that underwent {0,1,3,5,7} FTCs	50 mL

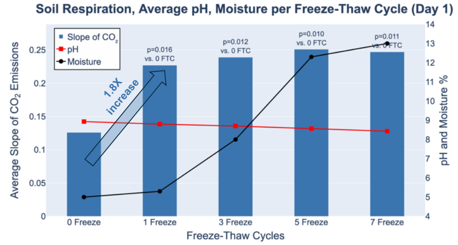
- Tested absorption at optical density of 750 nm on spectrophotometer over 18 days
  - Every 12 hours for 7 days ■ then daily for 7 days ■ then every other day for an additional 4 days

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
  - ***Increase from 0 to {1,3,5,7} FTC statistically significant** ($p < 0.05$)*
- 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 in soil is condensed leading to higher moisture levels
- When <u>soil thaws</u>:
  - Microbes consume organic material that cannot 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 the Birch Effect [5]**

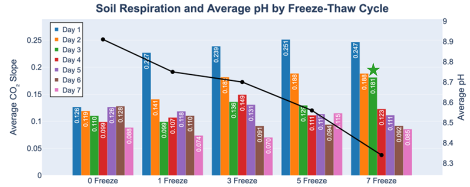
Soil Respiration, Average pH, Moisture per Freeze-Thaw Cycle (Day 1)



The 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
  - On Day 3 ★, the 7 FTC – which has the greatest amount of released nutrients as indicated by the lowest pH – has the highest respiration levels
- Over time, soil respiration declines and CO_2 levels stabilize

Soil Respiration and Average pH by Freeze-Thaw Cycle



Phase 2 Data

FTCs resulted in less and slower algae growth

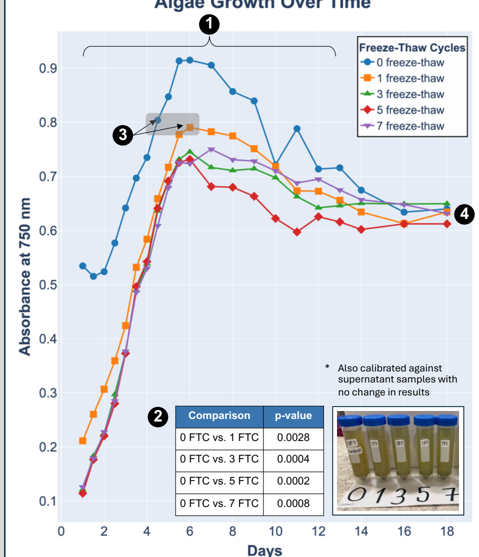
❶ Algae growth is ***highest*** in 0 FTC condition for first 12 days
- Microbes consume carbon and release nitrogen & phosphorus which promotes algae growth
- **FTCs cause microbial death, reducing microbial community size available to produce nutrients that drive algae growth**
  - May result in up to 50% reduction in microbial biomass [6]

❷ First freeze-thaw cycle has greatest impact ■ additional cycles had minimal differences
- **0 FTCs statistically significant vs. {1,3,5,7} FTCs ($p < 0.01$)**

❸ Algae growth is the ***fastest*** in 0 FTC condition for the first 12 days
- For example, the 0 FTC condition reached absorbance of ~0.8 after 4 days, while it took the 1 FTC conditions 2 additional days to reach this level of algae growth

❹ After 14 days, when all nutrients are depleted (closed-system), all samples contain similar amounts of algae

Algae Growth Over Time



❷

Comparison	p-value
0 FTC vs. 1 FTC	0.0028
0 FTC vs. 3 FTC	0.0004
0 FTC vs. 5 FTC	0.0002
0 FTC vs. 7 FTC	0.0008

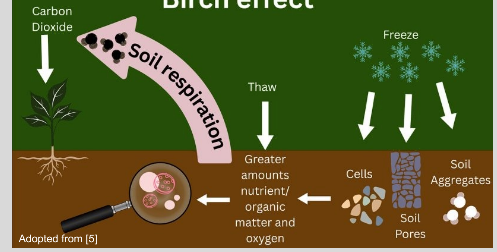
Charts created by Adeline Niemier ■ All photos taken by Adeline and Amy Niemier.

Results

Phase 1 Hypotheses	Phase 1 Results
As number of FTCs increase, more CO_2 will be released (soil respiration)	✓**Correct:** Respiration increased as the freeze-thaw cycles increased – especially on Day 1 where a large "burst" in CO_2 was shown ■ Birch Effect
As number of FTCs increase, soil moisture will increase	✓**Correct:** Moisture levels increased as the freeze-thaw cycles increased
As number of FTCs increase, pH will decrease	✓**Correct:** The 7 FTC condition had the lowest pH

Phase 2 Hypotheses	Phase 2 Results
As number of FTCs increase, algae growth (absorbance levels) will increase	**X Incorrect:** Algae growth did not increase with the number of freeze-thaw cycles ■ FTCs lead to less algae growth due to a decrease in microbe population
As number of FTCs increase, algae growth will be delayed	✓**Correct:** All samples that underwent 1 or more FTC had slower algae growth than the 0 FTC condition ■ minimal differentiation after first FTC

Birch effect



Conclusions and Implications

- **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 ■ 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
- As permafrost thaws, Arctic could change from a beneficial carbon sink to a *contributor* of global warming due to:
  1. Higher CO_2 emissions from increased soil respiration
  2. 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 hrs)

References

[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. ■ [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. ■ **[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.