Summer Project

Report

Investigating Climate Change Effects on Nitrogen Availability in High-Altitude Plant Ecosystems

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1 Introduction

During my summer internship project, I was engaged in both fieldwork and Laboratory works. The field project took place in the Kibber village of Spiti, Himachal Pradesh, located at an altitude of 4800 meters. Our primary objective in field was to establish a controlled warming experiment (Hexagonal Open Top Chambers) to simulate the effects of global warming and climate change, focusing on their influence on plant growth. Given the critical role of nitrogen as a nutrient in plant development, we collected soil samples from the experimental plots to analyze nitrogen mineralization and soil moisture content. This analysis will enable us to assess how climate change impacts nitrogen availability and soil moisture dynamics, thereby affecting plant growth. After completion of fieldwork and soil sample collection, We determined the ammonium and nitrate composition in the soil extracts. In this report, we will detail the methodologies utilized during fieldwork, the soil extraction procedures, and the laboratory protocols for estimating ammonium and nitrate levels. In addition, we will present bar graphs and conduct hypothesis tests such as ANOVA and paired T-tests to analyze the data. At the end in the Appendix session, the ammonium and nitrate concentration of each sample at T_0 and T_1 time are provided for review.

Our study aims to shed light on the potential impacts of climate change on soil nutrient dynamics and plant ecosystems in high-altitude regions.

1.1 Warming Experiment and Hexagonal Open Top Chambers (OTC)

Hexagonal Open Top Chambers (OTCs) are widely used in ecological and environmental studies to simulate and study the effects of increased temperatures on plant and soil systems. These chambers are designed with open tops to allow natural precipitation and air circulation while increasing the ambient temperature inside the chamber compared to the outside environment. In simple terms, it can be thought of as a green Housee.

The hexagonal design is optimal for maximizing the area exposed to controlled warming while minimizing edge effects. The open top allows natural weather conditions such as sunlight, rainfall, and wind to interact with the ecosystem inside the chamber. OTCs are usually constructed from transparent or semi-transparent materials, such as plastic or plexiglass or acrylic sheets, which trap heat and increase the temperature inside the chamber by a few degrees Celsius. In this project, we used OTC for warming some plots in each blocks as part of our warming experiment.

1.2 Nitrogen Cycle and Mineralization

The Nitrogen Cycle is a biogeochemical process that transforms nitrogen into various forms. This cycle moves nitrogen from the atmosphere to the soil, then into organisms, and eventually returns it to the atmosphere. The process of the Nitrogen Cycle consists of the following steps: Nitrogen fixation, nitrification, assimilation, ammonification, and denitrification. In general, the N cycle processes of fixation, mineralization and nitrification increase plant available N. Denitrification, volatilization, immobilisation, and leaching result in permanent or temporary N losses from the root zone.[1]

Nitrogen mineralization is the process by which inorganic nitrogen is obtained by decomposition of dead organisms and degradation of organic nitrogenous compounds. Mineralization is the process by which microbes decompose organic N from manure, organic matter, and crop residues into ammonium. Because it is a biological process, rates of mineralization vary with soil temperature, moisture, and the amount of oxygen in the soil (aeration). Mineralization occurs readily in warm (68-95 Â° F), well aerated, and moist soils. Manure contains N in two primary forms: ammonium and organic N. If manure is incorporated within one day, 65% of the ammonium N is retained; when incorporated after 5 days the ammonium N will have been lost through volatilization (loss of N through the conversion of ammonium to ammonia gas). Organic N in manure is not lost through volatilization, but it takes time to mineralize and become available. Immobilisation is the reverse of mineralization. All living things require N; therefore microorganisms in the soil compete with crops for N. Immobilisation refers to the process in which nitrate and ammonium are taken up by soil organisms and therefore become unavailable to crops. [2] [3]

Incorporation of materials with a high carbon to nitrogen ratio (e.g. sawdust, straw, etc.), will increase biological activity and cause a greater demand for N, and thus result in N immobilisation. For example, when the C:N ratio is less than 20:1, as is common in native soil organic matter, a net increase in available N can be expected when the microorganisms decompose that material.[4] Alternatively, when the C:N ratio is greater than 30:1, an initial temporary decrease in available N upon decomposition is likely. This is termed immobilisation, and occurs because the microbes need additional N to utilise all the carbon in the organic matter. Efficient N use also depends on a number of other factors including temperature, soil moisture, pest pressure, and soil compaction.

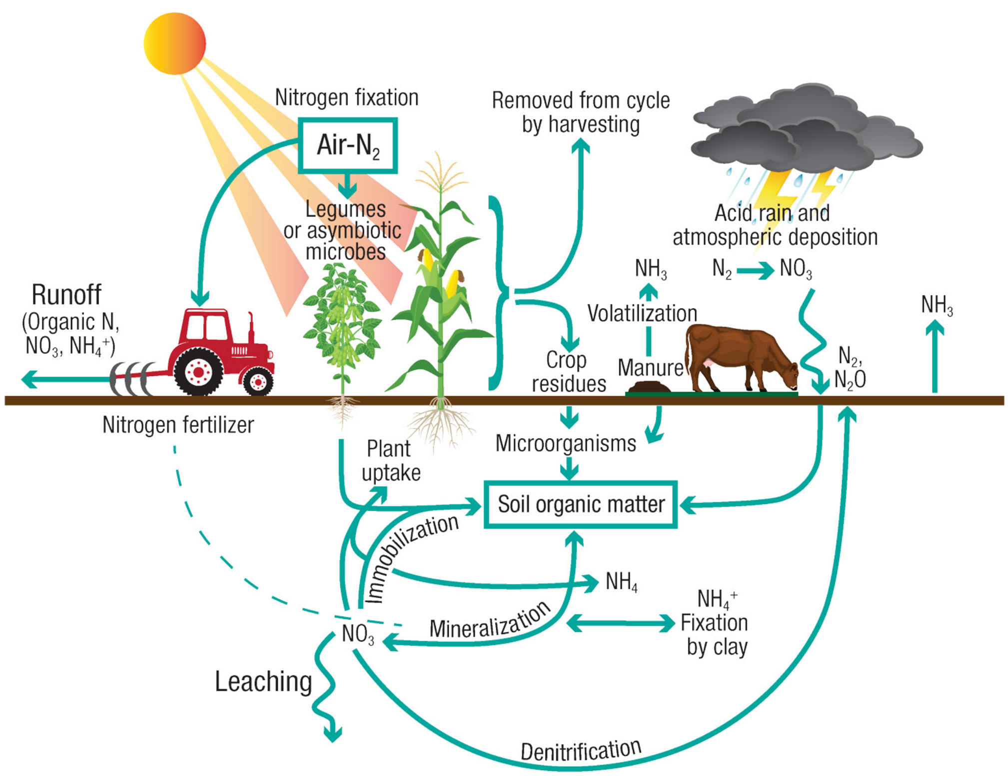

Figure 1: Illustration of Nitrogen cycle

[*Nitrogen cycle- web image link*]

2 Methodology

2.1 Spiti valley, India - Site description

The Spiti Valley, located within the Trans-Himalayan region, lies in the rain shadow of the Greater Himalayas. Covering an area of approximately 12,000 square kilometers, it encompasses the catchment area of the Spiti River. The elevation of the valley varies significantly, ranging from 3,350 meters to 6,700 meters above sea level.

Characterized by an arid climate, Spiti Valley receives an average annual precipitation of around 500 mm, predominantly as winter snowfall. During the growing season, the mean precipitation is about 200 mm. The temperature in this high-altitude region fluctuates widely, dropping to -30 Â° C in winter and rising to a maximum of 28 Â° C in summer[5].

The warming plots were set in Thinam near to Kibber village in spiti, Himachal Pradesh. The plots were at an altitude about 4800 meters. Kibber, a village nestled in Himachal Pradesh's Spiti Valley at an elevation of about 4200 meters, boasts a captivating yet harsh landscape. Dry, arid, and barren, the Kibber's terrain is dominated by rocky slopes. Summers bring a flurry of activity as locals engage in farming and construction. Barley is the main crop cultivated here. The early summer brings cold days with temperatures ranging between 5 and 10 degrees Celsius. Nights can be quite cold, dipping down to 0 to -4 degrees Celsius. However, by late summer, the weather warms up, with average temperatures reaching 20 degrees Celsius during the day and 5 degrees Celsius at night. Early summer is also the time for occasional rain and light snowfall. Afternoon winds are a common occurrence throughout the season. Despite the dry and barren landscape, Kibber surprisingly boasts a variety of short flowering plants (Caragana versicolor, Lonicera sp., Eurotia sp.). These plants come in a vibrant color and possess unique architectures, allowing them to thrive in this challenging environment. Notably, many of these plants have adapted to the rocky and sloping terrain. Ibex, blue sheep, and cattle are the main plant-eating animals (herbivores) found in Kibber. Predators (carnivores) such as snow leopards and red foxes are also present in the area. Locals traditionally graze their livestock, which includes sheep, cows, yaks, mules, horses, and donkeys. Kibber's extraordinary environment, characterized by summer sunshine, high altitude, wind, and grazing animals, has led to the evolution of fascinating adaptive measures in plant life.

2.2 Experimental design

There were 5 blocks each with 6 plots each. There were warming plots and un-manipulated plots in each blocks. Each plot was approximately 2m x 2m dimension and blocks were about 10m x 10m dimension. Inside each plot there was an area (about 1m x1m) of least interactions.

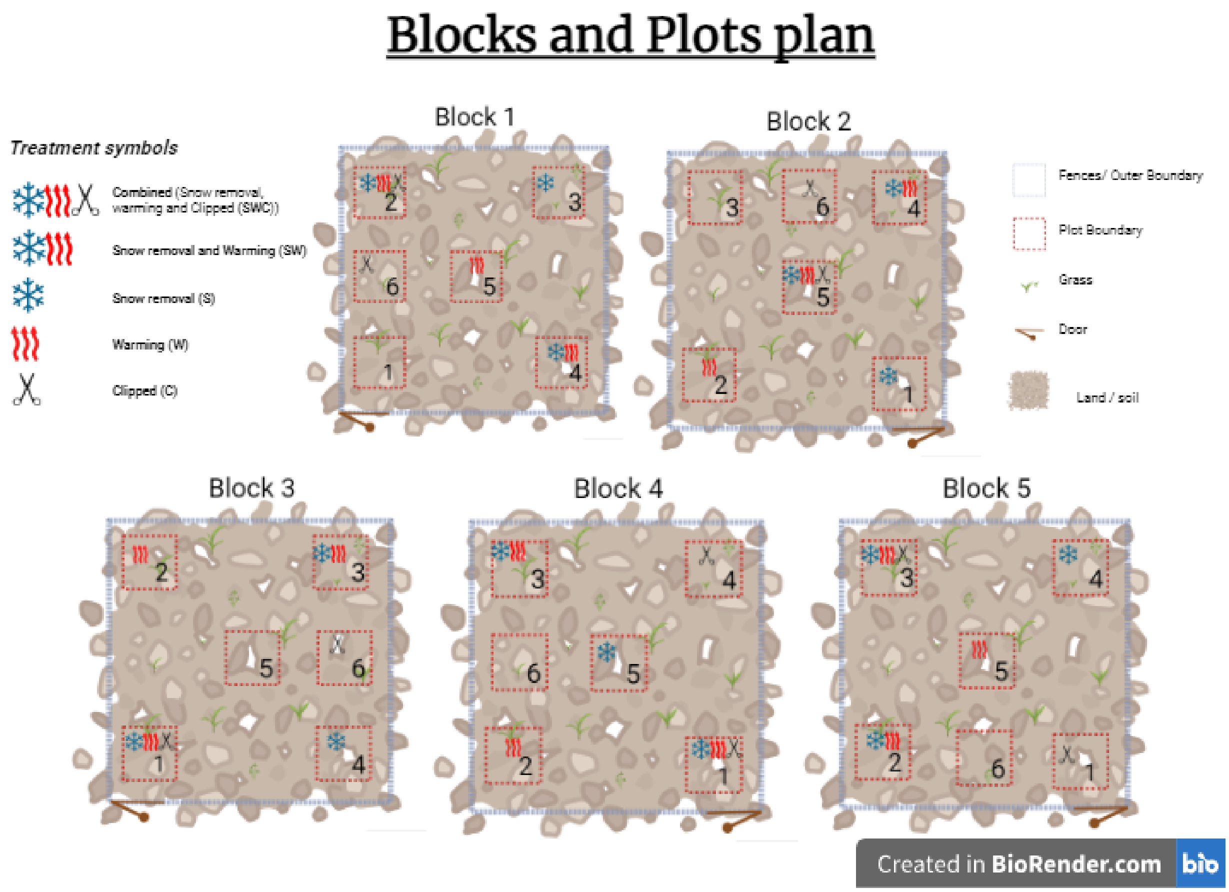

Figure 2: OTC Chamber

hexagonal open-top chambers (OTC, dimensions: 170 cm at the top, 60 cm high) with sides made of transparent acrylic sheets were built. In a previous study preceding this experiment, we found that this OTC warmed plots by almost 3 degree Celsius. Thereafter, we left one side of the hexagon open, and this reduced the warming effect to an average of 1.2 degree Celsius.

Figure 3: OTC Chamber

We deployed temperature loggers (iButton DS1921G by Maxim Integrated) taped to wooden pegs 10 to 20cm above-ground to measure air temperature difference between warmed and control plots at 30-min interval over the summer days. A single wooden stick contains two temperature loggers attached to it. The one above the ground will measure air temperature whereas the one below the ground (about 5cm deep) will measure soil temperature.

Figure 4: Temperature Loggers

3 Field, extraction and estimation protocols

3.1 Field sampling protocol

Objective

- To construct the OTC
- To collect initial or T0 soil samples and Final or T1 soil samples
- To perform $CaCl_2$ extraction

Materials Required

- Construction of OTC: Transparent glass sheets or acrylic glass sheets,Iron rods, Nuts, Bolts, Screw driver and Hammer
- For Sampling and incubation: Steel corer to collect soil, PVC pipes with Mesh covering at bottom: Length 15 cm, Paper bags, Weighing balance, Hammer, Forceps to sort out roots and stones from samples
- $CaCl_2$ extraction: Wooden funnel holders, Funnels, Whatman Filter paper, Whirl packs, Ziplock bags, Volume dispenser for dispensing 20 ml of 0.01 M $CaCl_2$ solution, Plastic bottle to carry $CaCl_2$ solution, Plastic scintillation vials

Figure 5: Labelled PVC pipes with mesh covering

Figure 6: Steel Soil Corer

Figure 7: Whirlpacks with soil

Figure 8: Funnels and Scintillation vials

Procedures

OTC construction: Acrylic sheets were cut in trapezium shapes and were mounted on Iron welded rods with the help of Nuts and bolts.

Field sampling;

- Take a soil core of 10 cm depth. Put the soil in a PVC pipe. Install the filled PVC pipe in the same place where the core was taken
- Adjacent to this core, take another soil core of 10 cm depth, collect the soil, and bring it back to the field station. This will be our T0 soil sample.
- On the same day, the collected soil samples should undergo $CaCl_2$ extraction,
  - For each soil sample, measure out 10 grams of the sieved sample (the soil sample should be devoid of large roots and stones).
  - Put this 10 grams de-stoned and de-rooted sample in a whirl-pack, and add 20 ml of 0.01 M $CaCl_2$ solution. The mixture is shaken vigorously.
  - Make blanks, Blanks are basically whirl-packs containing 20ml of 0.01 M $CaCl_2$ solution.
  - After 24 hours, each of these mixtures would be filtered through Whatman filter paper, and the filtrate should be stored in an acid-washed 20 ml plastic scintillation vial
  - For T1 soil sampling, after 15-16 days go to the field. Remove the fitted PVC pipes. Collect the soil inside. This sample will undergo $CaCl_2$ extraction the same day, using the protocol used above.

3.2 Ammonium and nitrate estimation in soil (Extraction solution 0.01 M $CaCl_2$

Objective

- To prepare various solutions for the estimation.
- To quantify Ammonium and Nitrate in soil

Materials and Equipment required

Spatula, Weighing balance, Falcon tubes, Vortex machines, Pippette and pipette tips, Tube holders, Foil, Oven, Microplates, Plate readers and spectrophotometer

3.2.1 Chemicals, reagents and reaction mixtures required for estimation

For Ammonium estimation

1. Sodium salicylate cocktail: Sodium salicylate + tri sodium citrate + sodium tartarate + sodium nitroprusside + dd water/ milliQ
2. Cyanurate: NaOH + Sodium dichloro-isocyanurate dihydrate (DCI) + dd water or MilliQ
3. Stock ammonium standard (1000mg/L): Ammonium sulphate + 0.01 M $CaCl_2$

For Nitrate and nitrite estimation

1. Sodium Hydroxide: NaOH + dd water/ MilliQ
2. $CuSO_4$ Stock: $CuSO_4$ + dd water/ MilliQ
3. $ZnSO_4$ Stock: $ZnSO_4$ + dd water/ MilliQ
4. Hydrazine Sulphate: Hydrazine sulphate + $CuSO_4$ Stock + $ZnSO_4$ Stock + dd water/MilliQ : Hydrazine reduces Nitrate to Nitrite.
5. Phosphoric acid solution: Orthophosphoric acid + Tetra sodium diphosphate decahydrate + dd water/ MilliQ
6. Color reagent: Sulphanilamide + NEDD (N-(1-Naphthyl) Ethylene Diamine Di-Hcl + Orthophosphoric acid + dd water/MilliQ : Cover the test-tube with foil (since some chemicals are light-sensitive), and put in oven at around 50oC for 15-20 minuts to dissolve the chemicals in MilliQ. This reagent reacts with nitrite to produce color.
7. Stock nitrates standard (1000mg/L): Potassium nitrate + 0.01 M $CaCl_2$
8. Stock Nitrite standard (1000mg/L): Sodium nitrite + 0.01 M $CaCl_2$

Reaction mixture for Ammonium estimation

1. 40 microlitre sample
2. 80 microlitre sodium salicylate cocktail
3. 80 microlitre cyanurate solution
4. Rest for 15 minutes
5. Absorbance at 650nm

Reaction mixture for Nitrate estimation

1. 40 microlitre sample
2. 20 microlitre Sodium hydroxide
3. 20 microlitre Hydrazine sulphate
4. 20 microlitre Phosphoric acid solution
5. 80 microlitre Color reagent
6. Immediately measure absorbance at 550nm

Chemicals and solutions required and its amount

Solution	**Components**	**Amount required**
Sodium salicylate cocktail	Sodium salicylate	3.4g
	Tri sodium citrate	2.5g
	Sodium tartrate	2.5g
	Sodium nitroprusside	0.0125g
	dd water/MilliQ	Make it up to 50ml
Cyanurate	NaOH	1.2g
	Sodium dichloro-isocyanurate dihydrate (DCI)	0.25g
	dd water or MilliQ	Make it up to 50ml
Stock ammonium standard (1000mg/L)	Ammonium sulphate	0.235g
	0.01 M CaCl2	Make it up to 50ml

Table 1: Ammonium estimation in soil (extraction solution 0.01M $CaCl_2$).

Solution	**Components**	**Amount required**
Sodium Hydroxide	NaOH	2g
	dd water/MilliQ	Make it up to 50ml
$CuSO_4$ stock	$CuSO_4$	0.05g
	dd water/MilliQ	Make it up to 50ml
$ZnSO_4$ stock	$ZnSO_4$	0.5g
	dd water/MilliQ	Make it up to 50ml
Hydrazine Sulphate	Hydrazine sulphate	3g
	$CuSO_4$ stock	0.7ml or 700μl
	$ZnSO_4$ stock	2ml
	dd water (or MilliQ)	Make it up to 50ml
Phosphoric acid solution	Ortho-phosphoric acid	0.15ml or 150μl
	Tetra-sodium-diphosphate decahydrate	0.2g
	dd water/MilliQ	Make it up to 50ml
Color Reagent	Sulphanilamide	0.5g
	NEDD (N-(1-Naphthyl) Ethylene Diamine Di-Hcl	0.025g
	Ortho-phosphoric acid	25ml
	dd water/MilliQ	Make it up to 50ml
Stock Nitrates standard (1000mg/L)	Potassium nitrate	0.3609g
	0.01 MM $CaCl_2$	Make it up to 50ml
Stock nitrite standard (1000mg/L)	Sodium nitrite	0.2465g
	0.01M $CaCl_2$	Make it up to 50ml

Table 2: Nitrate and nitrite estimation in soil (extraction solution 0.01M $CaCl_2$)

3.2.2 Ammonium and nitrate standards

Ammonium standards curve: 8, 6, 4, 2, 1, 0 mg/L

Standard Concentration	Volume (mL)	stock volume required (uL)	Volume of 0.01 M $CaCl_2$
8 mg/L	10	80	9.92
6mg/L	10	60	9.94
4 mg/L	10	40	9.96
2 mg/L	10	20	9.98
1 mg/L	10	10	9.99
0 mg/L (Blank)	10	0	10

Table 3: Ammonium Standards

Nitrate standard curve: 3, 2.5, 2, 1.5, 1, 0.5, 0 mg/L

Standard Concentration	Volume (mL)	stock volume required (uL)	Volume of 0.01 M $CaCl_2$
3 mg/L	10	30	9.97
2.5mg/L	10	25	9.975
2 mg/L	10	20	9.98
1.5 mg/L	10	15	9.985
1 mg/L	10	10	9.99
0.5 mg/L	10	5	9.995
0 mg/L (Blank)	10	0	10

Table 4: Nitrate Standards

4 Visualization

Following the protocol and using the multimodal plate reader, the ammonium and nitrate concentrations for each plot at T0 and T1 time are tabulated and stored as a data sheet. Further analyses are performed on the collected data. Mainly bar graphs are plotted to understand the mineralization rate among different time intervals for ammonium, nitrate, and total conditions.

4.1 To and T1 Ammonium Concentration and its mineralization Rate: Bar graph

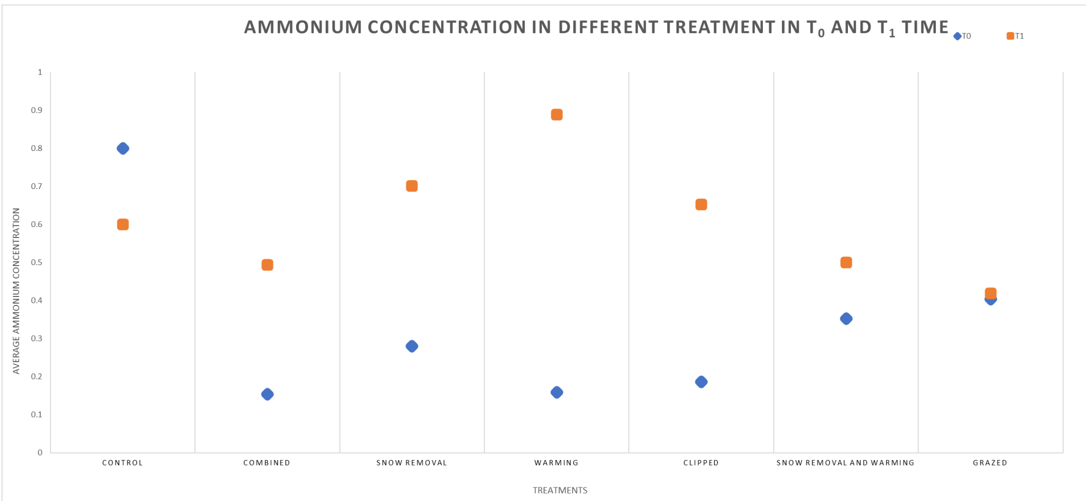

Figure 9: T0 and T1 Ammonium concentration

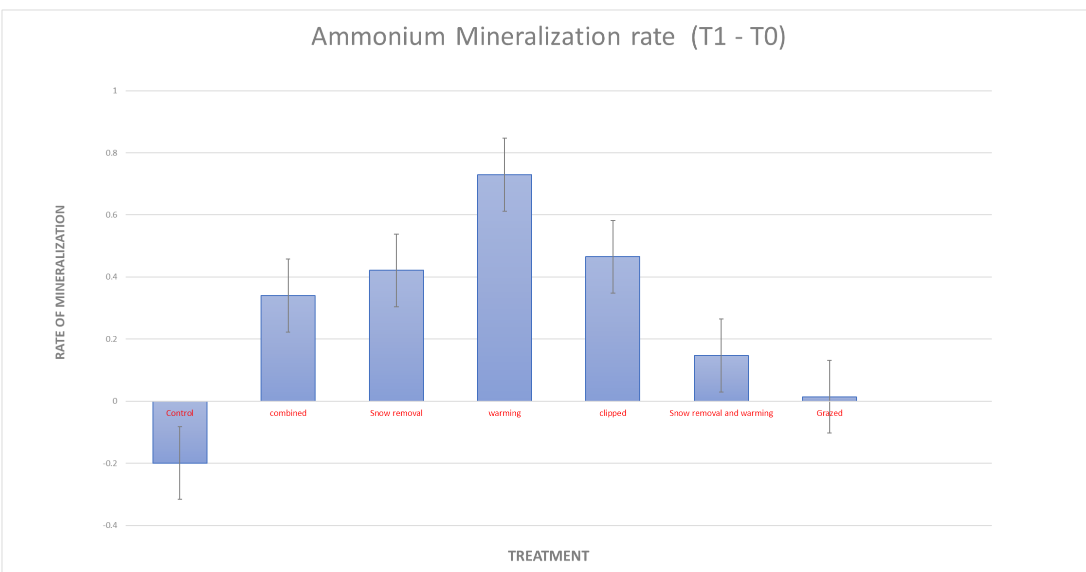

Figure 10: Ammonium: Mineralization Rate

Interpretation;

- From the concentration graph, the concentration of ammonium in T1 is always greater than T0 value in all treatment except Control treatment.
- Warming treatment shows a greater Mineralization rate compared to other treatment.
- Combined, Snow removal and clipped almost shows similar rate.
- Clipped and grazed shows a different rate, even though their treatment is mostly same on outside.
- Combined (Snow removal + warming + clipping): Positive rate (0.340) shows increased mineralization, likely due to combined effects of treatments enhancing microbial activity.
- Warming: Highest increase (0.729), indicating that warming significantly boosts ammonium mineralization, possibly by accelerating microbial processes.
- Snow Removal and Warming: Lower increase (0.147) compared to individual treatments, indicating potential interaction effects that might reduce overall mineralization.

4.2 To and T1 Nitrate Concentration and its mineralization Rate: Bar graph

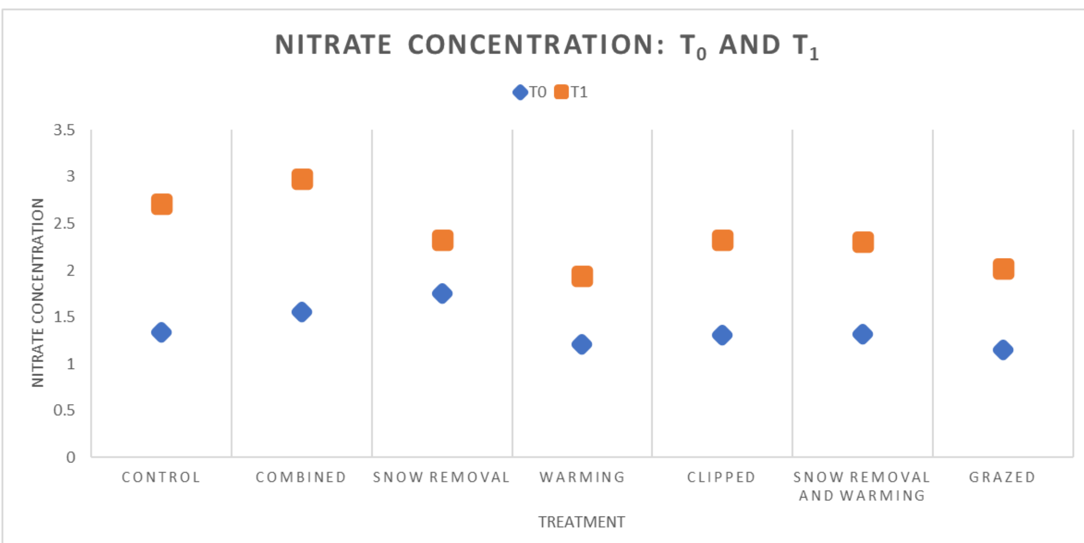

Figure 11: T0 and T1 Nitrate concentration

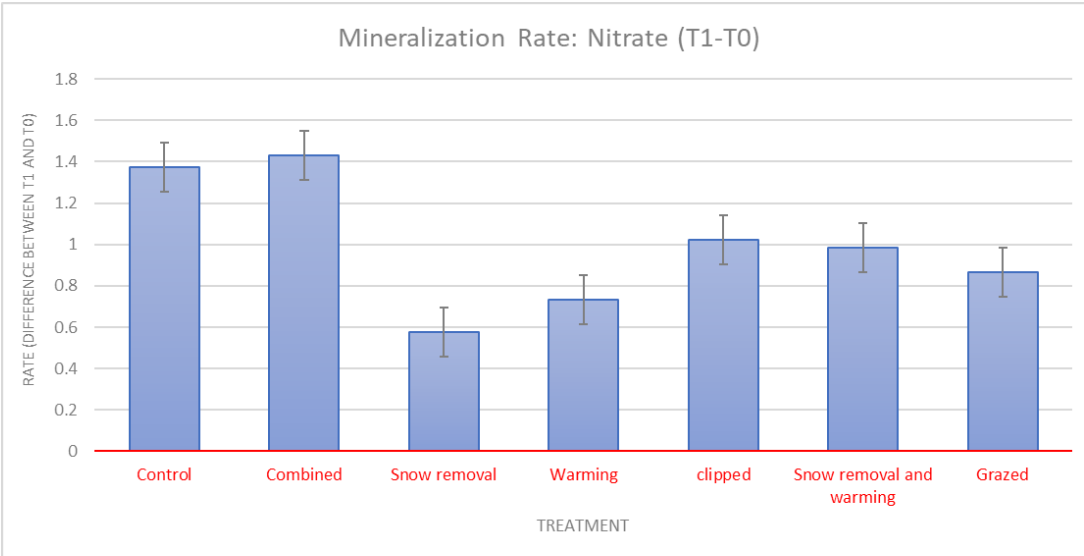

Figure 12: Nitrate: Mineralization Rate

Interpretation;

- The concentration of nitrate in T1 is always greater than T0 value in all treatments.
- Control: High rate (1.373), indicating substantial nitrate mineralization under natural conditions.
- Combined: Slightly higher rate (1.430) than control, suggesting combined treatments marginally enhance nitrate mineralization
- Snow Removal: Lower rate (0.575), indicating snow removal alone reduces nitrate mineralization.
- Mineralization rate in control treatment and Combined treatment are almost equal but combined shows a slightly more rate.
- Clipped and grazed treatment shows around equal mineralization rate.
- Rate in combined, warming and snow removal varies a lot, suggesting some correlation between them
- Warming: Moderate rate (0.733), suggesting warming promotes nitrate mineralization but not as strongly as ammonium.
- Snow Removal and Warming: Moderate rate (0.985), suggesting combined effects are less than individual treatments.

4.3 Total (Ammonium+Nitrate) mineralization Rate: Bar graph

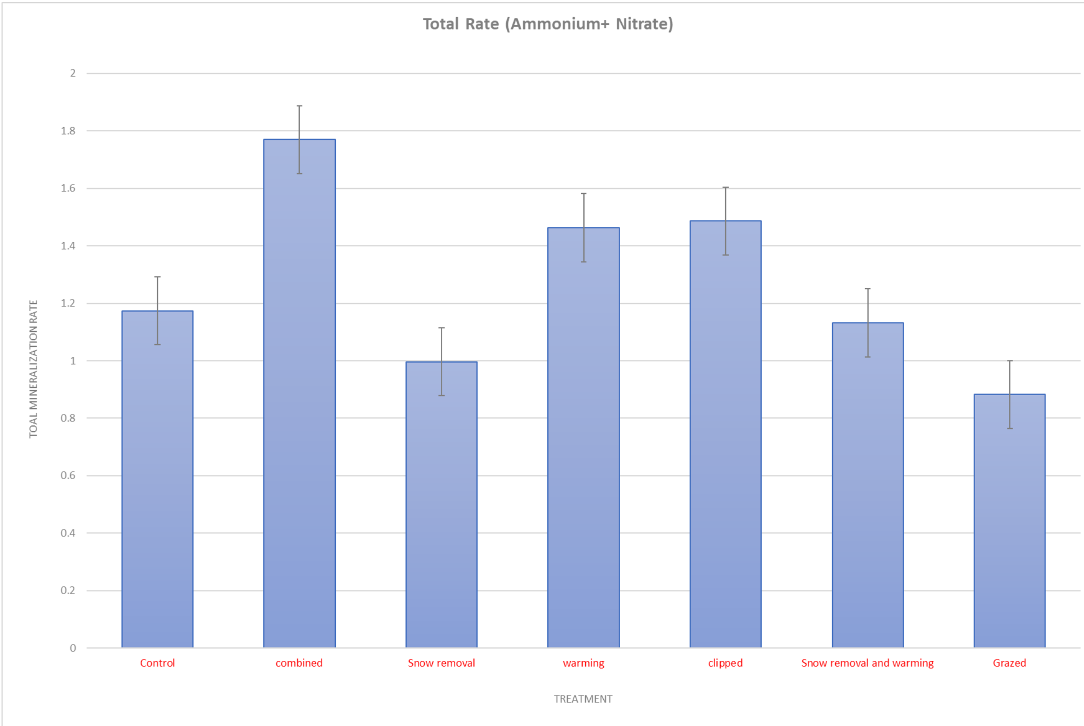

Figure 13: Total Mineralization rate

Interpretations;

- Among all treatments, combined treatment shows a greater mineralization rate compared to others, suggesting the role of the combined effect of snow removal, warming, and clipping.
- Warming treatment also shows a higher minerlization rate compared to the reference plot.
- Control: Baseline rate (1.173), representing natural mineralization.
- Combined: Highest rate (1.770), indicating combined treatments significantly enhance overall mineralization.
- Snow Removal: Moderate rate (0.996), suggesting snow removal alone has a moderate effect.
- Warming: High rate (1.463), indicating warming strongly promotes total mineralization.
- Clipped: High rate (1.486), suggesting clipping significantly enhances total mineralization.
- Snow Removal and Warming: Moderate rate (1.132), indicating combined effects are less than individual treatments.
- Grazed: Lower rate (0.882), suggesting grazing has a limited effect on total mineralization.

5 Anova Test and Paired T Test

To test whether there are significant differences between treatments in ammonium, nitrate and total mineralization rates, I performed a one-way analysis of variance (ANOVA) and a paired T test. The normality of the data was checked by visualizing a Q-Q plot (Quantile- Quantile plot). In the Q-Q plot, the data points were falling along the reference line, suggesting normality.

5.1 ANOVA: Ammonium Mineralization Rates

	Sum of squares	degree of freedom	Mean square	F	p-Value	Significant
Between	3.143000	6.0	0.524	1.491	0.21735	False
Within	9.834000	28.0	0.351			False
Total	12.976936	34.0				False

Table 5: ANOVA: Ammonium

5.2 ANOVA: Nitrate Mineralization Rates

	Sum of squares	degree of freedom	Mean square	F	p-Value	Significant
Between	2.961000	6.0	0.494	0.671	0.6735	False
Within	20.584000	28.0	0.735			False
Total	12.976936	34.0				False

Table 6: ANOVA : Nitrate

5.3 ANOVA: Total Mineralization Rates

	Sum of squares	degree of freedom	Mean square	F	p-Value	Significant
Between	2.318000	6.0	0.533	0.933	0.487	False
Within	16.603000	28.0	0.593			False
Total	19.920492	34.0				False

Table 7: ANOVA: Total

Inferences from ANOVA
The results from the ANOVA tests indicate that there are no statistically significant differences among the group means for ammonium, nitrate, and total nitrogen levels. This suggests that, when considering all treatments together, the variations in nitrogen mineralization rates are not large enough to be considered statistically significant.
The lack of significant differences among treatments in the ANOVA tests suggests that the overall variability

in nitrogen mineralization rates within each treatment group is not substantial enough to distinguish one treatment from another at a statistically significant level.
High variability within treatment groups might be a reason for it, small sample sizes, or the treatments having similar effects on nitrogen mineralization.

6 Paired T test

Two set of paired t tests are perfromed,
1) Paired t-test against a baseline zero :This test determines whether the mean difference between paired observations (e.g., before and after treatment) is significantly different from zero. It is used to assess if there is a significant effect or change due to the treatment.
2) Paired t-test against a control group: It compares the difference between two related groups (treatments and control). It is performed to evaluate whether the treatment group differs significantly from the control group, which can be crucial for understanding the relative effect of the treatment.

6.1 Against a baseline of zero

Paired t-tests to compare the means of ammonium, nitrate, and total nitrogen levels for different treatments against a baseline of zero.

Treatment	Ammonium t statistic	Ammonium p-value	Nitrate t statistic	Nitrate p-value	Total Nitrogen t statistic	Total Nitrogen p -value
Control	-0.373121	0.72799	2.076182	0.106482	3.129067	0.035217
Combined	5.225337	0.006404	3.8607	0.018136	5.2222	0.006418
Snow removal	1.890728	0.13164	1.185539	0.30142	1.903924	0.129651
Warming	3.710622	0.020643	3.112998	0.035768	9.76221	0.000617
Clipping	5.683244	0.004732	5.054427	0.007209	7.070627	0.002111
Snow removal and warming	0.603727	0.57859	3.550265	0.023793	3.157696	0.03426
Grazed	-0.342335	0.74933	3.907458	0.017431	2.439764	0.071227

Figure 14: Paired T test- baseline zero

6.1.1 Inferences

Ammonium Mineralization

- Significant Increases: Combined, Warming, Clipping
- No Significant Changes: Control, Snow Removal, Snow Removal and Warming, Grazed
- Combined, Warming, and Clipping treatments significantly increase ammonium concentrations, indicating these treatments enhance ammonium mineralization

Nitrate Mineralization

- Significant Increases: Combined, Warming, Clipping, Snow Removal and Warming, Grazed
- No Significant Changes: Control, Snow Removal
- Combined, Warming, Clipping, Snow Removal and Warming, and Grazed treatments significantly increase nitrate concentrations, indicating these treatments enhance nitrate mineralization

Total Nitrogen Mineralization

- Significant Increases: Combined, Warming, Clipping, Snow Removal and Warming
- No Significant Changes: Control, Snow Removal, Grazed
- Combined, Warming, Clipping, and Snow Removal and Warming treatments significantly increase total nitrogen concentrations, indicating these treatments enhance overall nitrogen mineralization.

6.2 Against Control

Treatment	Ammonium t-statistic	Ammonium p-value	Nitrate t-statistic	Nitrate p-value	Total Nitrogen t-statistic	Total Nitrogen p-value
Combined	0.994729	0.37617	0.081269	0.939132	1.303776	0.262281
Snow removal	1.46264	0.21739	-0.932245	0.403984	-0.250748	0.814362
Warming	1.995732	0.116684	-1.155822	0.31209	0.629802	0.563008
Clipping	1.173146	0.305828	-0.592482	0.585398	0.631385	0.562071
Snow removal and warming	0.572284	0.597758	-0.635473	0.559657	-0.066366	0.950271
Grazed	0.207129	0.846026	-0.996262	0.375509	-0.605175	0.577718

Figure 15: Paired T test- control

6.2.1 Inferences

The above given table shows the t-statistics and p-values for paired t-tests comparing each treatment to the control for ammonium, nitrate, and total nitrogen
None of the treatments show a statistically significant difference from the control for ammonium, nitrate, or total nitrogen, as all p-values are above the threshold of 0.05. This indicates that the treatments do not significantly alter nitrogen levels compared to the control under the conditions studied (treatments).

7 Result

The results from the bar graphs shows variation in the mineralization rates while comparing different treatments. Among them, Combined treatments (snow removal + warming + clipping) show the highest total mineralization rate, indicating some synergistic effects (these treatments work together in a way that enhances nitrogen mineralization more than any single treatment alone). Warming and clipping treatments generally improve both ammonium and nitrate mineralization rates. Snow removal alone has a moderate effect, while grazing shows minimal impact on ammonium but a noticeable effect on nitrate mineralization. Warming treatment in Ammonium mineralization rate shows significantly higher mineralization rate compared to other treatments. This shows the effect of warming in nitrogen mineralization.
The result of ANOVA showed that there are no statistically significant difference among the group means for ammonium, nitrate and total mineralization levels. Results from paired t-test against control also aligns with the ANOVA result. Results from Paired t-tests against a base line zero suggested that each treatment results in a significant change from the initial state (zero difference).

8 Summary and Discussion

This study analyzed the mineralization rate across different treatment. Key findings include:

1. Combined treatments (snow removal + warming + clipping) show the highest total mineralization rate, indicating synergistic effects.

2. Warming treatment significantly improves the mineralization rate of ammonium and nitrate.

3. Mineralization Rate in combined, warming and snow removal varies a lot, suggesting some correlation between them

4. The results from the ANOVA tests indicate that there are no statistically significant differences among the group means for ammonium, nitrate, and total nitrogen mineralization rate levels.

5. Paired t tests against a baseline zero showed a significant increases in ammonium, nitrate, and total nitrogen in Warming, Combined and clipped treatments consistently, highlighting their strong influence on nitrogen cycling

6. Paired t-test against control showed that None of the treatments show a statistically significant difference from the control for ammonium, nitrate, or total nitrogen, as all p-values are above the typical threshold of 0.05.

Discussion;
Our findings highlight the importance of understanding the role of warming treatment in the mineralization rate. From the graph, we found that the combined treatment of snow removal, warming, and clipping showed the highest total mineralization rate. In my opinion this synergistic effect might be due to several reasons, like;

1. Snow removal can expose the soil to more sunlight and reduce insulation, leading to higher soil temperatures. This can stimulate microbial activity and decomposition processes, increasing nitrogen mineralization.
2. Warming directly increases soil temperature, which can accelerate microbial metabolism and decomposition of organic matter
3. Clipping plants can increase the amount of organic matter (like plant residues) added to the soil.

When these warming, snow removal and clipping treatments are combined, their individual effect can interact and amplify mineralization rates. Enhanced microbial activity, increased organic matter, and improved soil conditions can increase the mineralization rate.

In the case of warming treatments, the increase in temperature can enhance the microbial activity. This can create optimal conditions for microbial activity by maintaining higher soil temperatures and reducing moisture content. This can lead to a more active microbial community that decomposes organic matter more efficiently.

In the Ammonium mineralization rate bar graph, Warming treatment shows the highest rate. It may suggest a higher level of microbial activity. This level of microbial activity might be due to the initial rise in temperature level. The initial increase in microbial activity may be temporary, as extended exposure to higher temperatures could lead to a decline in microbial activity. Consequently, we can anticipate a further reduction in the mineralization rate due to this decline in microbial activity with prolonged exposure. This trend highlights the impact of climate change on mineralization rates and, subsequently, on plant growth.

Both the results from ANOVA and paired t-test against control aligns in similar way and indicates that the treatments do not significantly alter nitrogen levels compared to the control under the conditions studied. This might be due to low sampling size, time period for incubation and due to initial sampling error.

Questions and doubts;

Upon examining the total mineralization rate bar graph, one can observe a significant variation between the clipped and grazed mineralization rates. Ideally, both treatments should function similarly, with clipping done by machines and grazing by herbivores. The disparity in these values is concerning. In my opinion, it could be attributed to the disposal of organic matter in the clipped plots.

Future works;

This study only examines mineralization over a 15-day period, covering just the initial phase of the warming treatment. To draw definitive conclusions about the effect of warming on nitrogen mineralization, further studies should be conducted after one month, and then two to three months and so on.

9 Acknowledgements

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10 Appendix

Ammonium and nitrate concentration value for T0 and T1 time period are gathered in a table below fro reference. These concentration are calculated from the straight line equation of ammonium standards and nitrate standards. Their absorbance value were collected using multi mode plate reader.
(In the below given Table, the labels stands for; U:un manipulated or control plots SWC: Combined (Snow removal+warming+ clipped) treatment S:Snow removal treatment SW: Snow removal and warming treatment W:Warming treatment C: Clipped treatment G: Grazed treatment)
B1P1 stands for Block1 plot 1 sample similarly B1P2,...B5P6. B1G stands for Block 1 grazed similarly others. These name are just for identification purpose

10.1 Ammonium estimation data: Ammonium concentration values in mg/L

Samples	T_0 Concentration	T_1 Concentration	Label
B1P1	2.721417069	0.447535771	U
B1P2	0.093800322	0.463434022	SWC
B1P3	0.394524196	0.179252782	S
B1P4	0.346618357	0.648648649	SW
B1P5	0.072463768	0.342209857	W
B1P6	0.082125604	0.669713831	C
B1G	0.320450886	0.35254372	G
B2P1	0.092190016	1.016693164	S
B2P2	0.073671498	0.598171701	W
B2P3	0.40821256	1.035771065	U
B2P4	0.125201288	0.302066773	SW
B2P5	0.106280193	0.53934815	SWC
B2P6	0.387278583	0.771065183	C
B2G	1.189613527	0.426470588	G
B3P1	0.054750403	0.271462639	SWC
B3P2	0.076489533	1.272444833	W
B3P3	0.168679549	0.839030207	SW
B3P4	0.16626409	0.337042925	S
B3P5	0.329307568	0.89266804	U
B3P6	0.07158615	0.520667727	C
B3G	0.134863124	0.734499205	G
B4P1	0.20531401	0.718203498	SWC
B4P2	0.132850242	0.583863275	W
B4P3	1.062399356	0.290143084	SW
B4P4	0.048309179	0.736893893	C
B4P5	0.25684328	0.555246423	S
B4P6	0.280192337	0.453497615	U
B4G	0.32568348	0.15892512	G
B5P1	0.345008002	0.561208267	C
B5P2	0.059178744	0.418918919	SW
B5P3	0.30958132	0.4769475	SWC
B5P4	0.489130	1.4157392	S
B5P5	0.436795	1.64069	W
B5P6	0.257246377	0.170508744	U
B5G	0.04750		G
BL1	0.231078905	0.114864865	
BL2	0.157407	0.132352941	

Table 9: Sample Ammonium Concentration Data

10.2 Nitrate estimation data: Nitrate concentration values in mg/L

Samples	T_0 Concentration	T_1 Concentration	Label
B1P1*	1.042216359	4.5847	U
B1P2	1.19659921	2.375576037	SWC
B1P3	1.998900616	2.544009217	S
B1P4	0.775065963	2.647695853	SW
B1P5	0.663808267	2.133645031	W
B1P6	1.111257696	2.736865399	C
B1G	1.138742304	2.735483711	G
B2P1	2.336631486	1.351843318	S
B2P2	1.416226913	2.260599078	W
B2P3*	1.953190202	3.7447	U
B2P4	1.5323219	1.901382448	SW
B2P5	1.713720317	2.812902326	SWC
B2P6	1.438434477	1.9640553	C
B2G	0.704485488	1.201150274	G
B3P1	0.760773967	1.590092166	SWC
B3P2	1.430299033	2.117281016	W
B3P3	1.590149516	2.712211982	SW
B3P4	1.313984169	1.499769585	S
B3P5	1.189753738	0.69142244	U
B3P6	1.076956904	2.098156682	C
B3G	1.723175022	2.199078341	G
B4P1	1.299032542	2.450691244	SWC
B4P2	1.110115831	1.784331797	W
B4P3	1.264511873	2.411059862	SW
B4P4	1.528144239	2.179262673	C
B4P5	1.437994723	2.717751521	S
B4P6	1.035400176	1.84308755	U
B4G	1.326956904	1.929493088	G
B5P1	1.364335972	2.64516129	C
B5P2	1.4382146	1.851619203	SW
B5P3*	2.7794635	5.6691	SWC
B5P4*	1.651934916	3.5027	S
B5P5	1.471326297	1.408961875	W
B5P6	1.476912929	2.702995392	U
B5G	0.848504837		G
BL1	0.076737027	0.13640553	
BL2	0.066622691	0.088018433	

Table 10: Nitrate Concentration Data of Samples at T_0 and T_1 Time Points

[Here "*" indicates that those samples are further (50:50)diluted and recalculated due to the greater absorbance value in the first round (when absorbance value is greater than the absorbance of the greatest standard concentration, then linearity breaks down)]

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