Nitrate Effects on Dissolved Oxygen Change in *Elodea canadensis*

Research Question: To what extent does changing the sodium nitrate ($NaNO_3$) concentration in the test solution (0.00, 0.175, 0.350, 0.525, and 0.700 mM) affect the average net photosynthetic rate of freshwater *Elodea canadensis*, measured as the rate of variation in dissolved oxygen accumulation (mg L^{-1} min^{-1}).

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Content

1. Introduction

Photosynthesis is the biological process that ensures the primary productivity of plants, including submerged aquatic macrophytes, and plays a central role in the world's oxygen cycle. In freshwater ecosystems, aquatic macrophytes contribute to oxygen availability, nutrient uptake, and ecosystem stability through their presence and photosynthesis. *Elodea canadensis* is often used in laboratory experiments due to its rapid physiological response, ease of standardization, and photosynthetic activity.[4]

Nutrient availability in aquatic ecosystems is important for photosynthesis because they can influence photosynthesis. Nitrogen, a nutrient found in soil and water, is essential for the synthesis of chlorophyll, amino acids, and photosynthetic enzymes, and is required for photosynthetic capacity. In freshwater systems, nitrogen may be present naturally or through anthropogenic inputs such as sewage or agricultural runoff. Since nitrogen is an essential nutrient for plants, low nitrogen availability can limit plant performance, as shown in this study, making nitrogen availability an independent variable in studies of plant photosynthetic rates.[4][6]

Although the increase in the photosynthetic capacity of plants in aquatic ecosystems containing nitrogen is often attributed to the presence of nitrogen, the immediate responses of plants to nitrate may not always result in increased photosynthesis, but rather show an unpredictable change. At the same time, the light intensity, temperature, and the amount of inorganic carbon in the water can also affect the photosynthetic rate of plants.

To measure the change in a plant's average net oxygen accumulation rate (proxy for net photosynthesis), it is crucial to measure dissolved oxygen initially and at the end of the experiment because the average photosynthesis rate can be calculated with these numbers. In this investigation, the effects of nitrogen on the photosynthesis rate of *Elodea canadensis* was measured using sodium nitrate ($NaNO_3$) solutions with concentrations of 0.00, 0.175, 0.350, 0.525, and 0.700 mM. *Elodea canadensis* samples were placed under constant light for 15 minutes, and the change in dissolved oxygen was measured at the beginning and end. Under controlled laboratory conditions, the effects of different nitrate concentrations on the short-term changes in the photosynthesis rate of *Elodea canadensis* was investigated.

2. Background Information

Elodea canadensis, $NaNO_3$ and Photosynthesis

Aquatic plants make photosynthesis, which involves light-dependent reactions and the Calvin cycle. During light-dependent reactions, chlorophyll captures photons and mediates electron transport across the thylakoid membrane for the synthesis of ATP and NADPH. In photosystem II, water is split to produce molecular oxygen (O_2) as a byproduct. The Calvin cycle then occurs, in which ATP and NADPH are used to fix inorganic carbon into carbohydrates.

Therefore, in submerged freshwater macrophytes such as *Elodea canadensis*, oxygen production during illumination can be used as an indirect measure of photosynthetic activity.

Nitrogen availability can also affect photosynthetic capacity since nitrogen is necessary for the synthesis of chlorophyll as well as the synthesis of photosynthetic proteins and enzymes to fix carbon.

[5] In freshwater systems, nitrogen generally occurs as nitrate (NO_3^-)[6]. Nitrate is absorbed by aquatic plants and incorporated into plant amino acids and proteins.[5]However, nitrate assimilation does not occur immediately. It includes reduction steps that require energy and reducing power. Over long periods of time, increased nitrate availability can support higher chlorophyll content and enzyme abundance, increasing the potential for photosynthesis, especially when nitrogen is the main limiting nutrient.

Nevertheless, the Elodea reaction to nitrate physiologically varies greatly with time and with which variable is actually being limiting to the photosynthesis in the system. Added nitrate might not immediately be converted into increased net oxygen production in the short term due to the requirement to first reduce and be assimilated (an energy-demanding process) and because of the failure of chlorophyll/enzyme pools to increase instantly. Moreover, though the supply of nitrates may rise, the output of photosynthesis may be limited by other factors, chief among them the supply of inorganic carbon (CO_2 /HCO_3^-, in water) hence an increase in nitrate may not lead to a corresponding increase in the amount of oxygen evolved under non-carbon-normalized conditions.

It may not be possible to predict the relationship between higher nitrate levels and higher photosynthesis rate on short term scales. Photosynthesis has other limiting factors than nitrate such as inorganic carbon (CO_2/HCO_3^-) which in water is a major limiting factor unless it is standardized. In this situation changing sodium nitrate concentrations in a relatively low range (0.000–0.700 mM) may not have a beneficial effect on net oxygen accumulation if carbon fixation is limited.

Another major aspect of this experiment is the interpretation of dissolved oxygen data. Changes in dissolved oxygen represent the system's behavior and the balance between the oxygen produced through photosynthesis and the oxygen consumed by plant tissues and microorganisms through respiration. In other words, the change in dissolved oxygen in the water over a specific time interval provides the average net oxygen accumulation rate, rather than directly giving the photosynthesis rate. This difference allows us to analyze trends related to sodium nitrate concentration in the water by considering metabolic demand and respiration in addition to photosynthetic capacity, thus enabling us to more accurately interpret the effects of concentration changes.

3. Hypothesis

H_0: Increasing nitrate levels does not increase rate of photosynthesis.

H_1: Increasing nitrate levels does increase rate of photosynthesis.

4.Variables

4.1 Independent Variable: $NaNO_3$ concentration 0.00, 0.175, 0.350, 0.525, 0.700 mM in the test solution. This low concentration range was selected to represent environmentally realistic nitrate enrichment levels while avoiding extreme stress or toxicity that could confound photosynthetic responses. Each set of concentration was produced by mixing 70 mL of $NaNO_3$ and 130 mL water and the 70 mL of $NaNO_3$ mixed in had concentrations of 0.0, 0.5, 1.0, 1.5, and 2.0 mM so the final concentration became 35 percent of the original concentrations.

4.2 Dependent Variable: Average net oxygen accumulation (mg L^{-1} min^{-1}) over a 15-minute illumination period.

4.3 Controlled Variables

Controlled Variable	How it was controlled	Effect if not controlled
Light Intensity	Lamps used for each set of beakers were kept at a fixed distance of 20 cm.	Light could have limited or benefited light dependent reactions like photosynthesis which would alter oxygen accumulation in the water.
Photoperiod	Illumination time was fixed at 15 minutes for each trial and by using a timer time was measured.	Different exposure times to light could affect photosynthesis rates of *Elodea canadensis* resulting in distort calculated rates.
Temperature	Temperature was kept at 22°C which was the room temperature and it was measured by a thermometer.	Enzyme activity and membrane processes which are temperature dependent would be affected which would affect dissolved oxygen in the water.
Total Solution Volume	70 mL $NaNO_3$ and 130 mL water was put into every solution and they were measured with graduated cylinders.	Different volume would affect oxygen capacity therefore different mg L^{-1} readings would occur.
Plant Size	Elodea canadensis were standardised at 12 cm length which was measured by a ruler.	Biomass/leaf area changes photosynthetic and respiratory demand, shifting net dissolved oxygen.
Probe Position	Probe was kept at the same place at each trial.	Different spacing could have biased readings.
Measurement Timing and Stabilisation	Dissolved oxygen in the water was measured before the *Elodea canadensis* was put in the solution and after 15 minutes of constant illumination.	Unstable readings would produce systematic errors.
Inorganic Carbon Availability	Same base water was used and no CO_2/HCO_3^- was added.	CO_2/HCO_3^- could have become a limiting factor so the dissolved oxygen rates would be different than normal.

5. Methodology

5.1 Materials

-36 *Elodea canadensis* fragments 12 cm.

-$NaNO_3$ stock solutions: 0.0, 0.5, 1.0, 1.5, 2.0 mM each 70 mL

-Measuring Cylinder 200 mL

-Beakers 250 mL

-Dissolved Oxygen Probe With Data Logger

-A Lamp

-Thermometer

-Timer

-Distilled Water for Rinsing

-Notebook and Pencil

5.2 Procedure

5.2.1 Preparation of Solution

1.Test solutions of 200 mL volume were prepared by mixing 70 mL of appropriate $NaNO_3$ and 130 mL water and they were checked to be 22 °C.

2.The solution was gently swirled for 5 seconds just before each reading to reduce local oxygen gradients.

5.2.2 Preparation of Experimental Setup

1.For all trials lamps were fixed at 20 cm away from each beaker. The beakers were arranged as if forming a crescent.
2. 36 pieces of 12 cm Elodea canadensis were cut and made into similar sized pieces, then they were all placed in separate beakers.

5.2.3 Taking Measurement

1.With the oxygen probe, 1 of each set of solutions was measured, and the initial dissolved oxygen amount was noted.

2.The plants were illuminated for 15 minutes.

3.After 15 minutes the solutions were swirled again for 5 seconds, and then dissolved oxygen after 15 minutes was recorded after stabilization.

4.The same procedure was done for 6 of each 5 sets of trials and the probe was distilled between every measurement.

5.3 Ethic and Environmental

In the experiment, the main thing was plants so there weren't any ethical overrules. After the experiment, the used plants and liquids were disposed of according to the laboratory rules.

6. Data

6.1 Raw Data

The following table will show the initial dissolved oxygen concentrations and the dissolved oxygen concentrations for each trial of each $NaNO_3$ concentration. (Table continues on the next page)

Sets of Trials	$NaNO_3$ (mM)	Trial	DO_0 (mg L^{-1})	DO_{15} (mg L^{-1})
1	0.00	1	8.44	9.39
	0.00	2	8.44	9.40
	0.00	3	8.44	9.34
	0.00	4	8.44	9.03
	0.00	5	8.44	9.10
	0.00	6	8.44	8.56
2	0.175	1	8.55	9.63
	0.175	2	8.55	9.13
	0.175	3	8.55	9.10
	0.175	4	8.55	9.12
	0.175	5	8.55	9.19
	0.175	6	8.55	9.23
3	0.350	1	9.07	9.03
	0.350	2	9.07	8.94
	0.350	3	9.07	8.99
	0.350	4	9.07	8.90
	0.350	5	9.07	8.91
	0.350	6	9.07	9.02
	0.525	1	9.56	9.37
	0.525	2	9.56	8.78

4	0.525	3	9.56	8.82
	0.525	4	9.56	9.25
	0.525	5	9.56	9.18
	0.525	6	9.56	9.12
5	0.700	1	10.14	8.86
	0.700	2	10.14	9.26
	0.700	3	10.14	9.20
	0.700	4	10.14	9.60
	0.700	5	10.14	9.40
	0.700	6	10.14	9.41

Table 1. Initial and Final Dissolved Oxygen Concentrations

6.2 Data Processing

Raw dissolved oxygen values were used to calculate the average net oxygen accumulation rate for each trial. The average net photosynthetic rate is approximated using the change in dissolved oxygen concentration over time and is calculated with:

$$\Delta DO = DO_{15} - DO_0: \text{ mg } L^{-1}$$

$$\text{Average net rate} = \frac{\Delta DO}{15} \text{ (mg } L^{-1} \text{ min}^{-1}\text{)}$$

By using this equation it is possible to calculate average net change in dissolved oxygen concentration (mg L^{-1} min^{-1}). The equation divides the net change in dissolved oxygen accumulation into the total time Elodea canadensis were put under constant light.

Example calculation (0.00mM Trial 1)

DO_0 = 8.44 mg L^{-1}

DO_{15} = 9.39mg L^{-1}

ΔDO = 9.39 - 8.44 = 0.95 mg L^{-1}

Average net rate = $\frac{0.95}{15}$ = 0.0633 mg L^{-1} min^{-1}

Using the equation above, the following average net oxygen accumulation rates were obtained for each trial as it can be seen in the table underneath.

Trial	0.00 mM	0.175 mM	0.350 mM	0.525 mM	0.700 mM
1	0.0633	0.0720	-0.0027	-0.0127	-0.0853
2	0.0640	0.0387	-0.0087	-0.0520	-0.0587
3	0.0600	0.0367	-0.0053	-0.0493	-0.0627
4	0.0393	0.0380	-0.0113	-0.0207	-0.0360
5	0.0440	0.0427	-0.0107	-0.0253	-0.0493
6	0.0080	0.0453	-0.0033	-0.0293	-0.0487

Table 2. Average Net Oxygen Accumulation Rates

Standard deviation (SD) lets each $NaNO_3$ concentration to quantify the spread of trial rates around the mean and therefore the repeatability of the measurements and calculating it is important because between each individual measurement the small differences like probe stabilization can cause trial-to-trial variation even when conditions are controlled and analyzes of measurements and calculations can be meaningless and inaccurate.

SD value for each concentration will be calculated by using the sample standard deviation equation as it can be seen underneath.

$$SD = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$$

where,

x_i = the average net oxygen accumulation rate for trial i,

$\bar{x}$ = mean rate for the selected concentration,

n= number of trials (6)

The term n-1 in the denominator was used because SD is calculated from a sample instead of population.

Example SD calculation (0.350 mM)

$\bar{x}$ = -0.007 mg L^{-1} min^{-1}

$\sum(x_i - \bar{x})^2 \approx 0.00007$

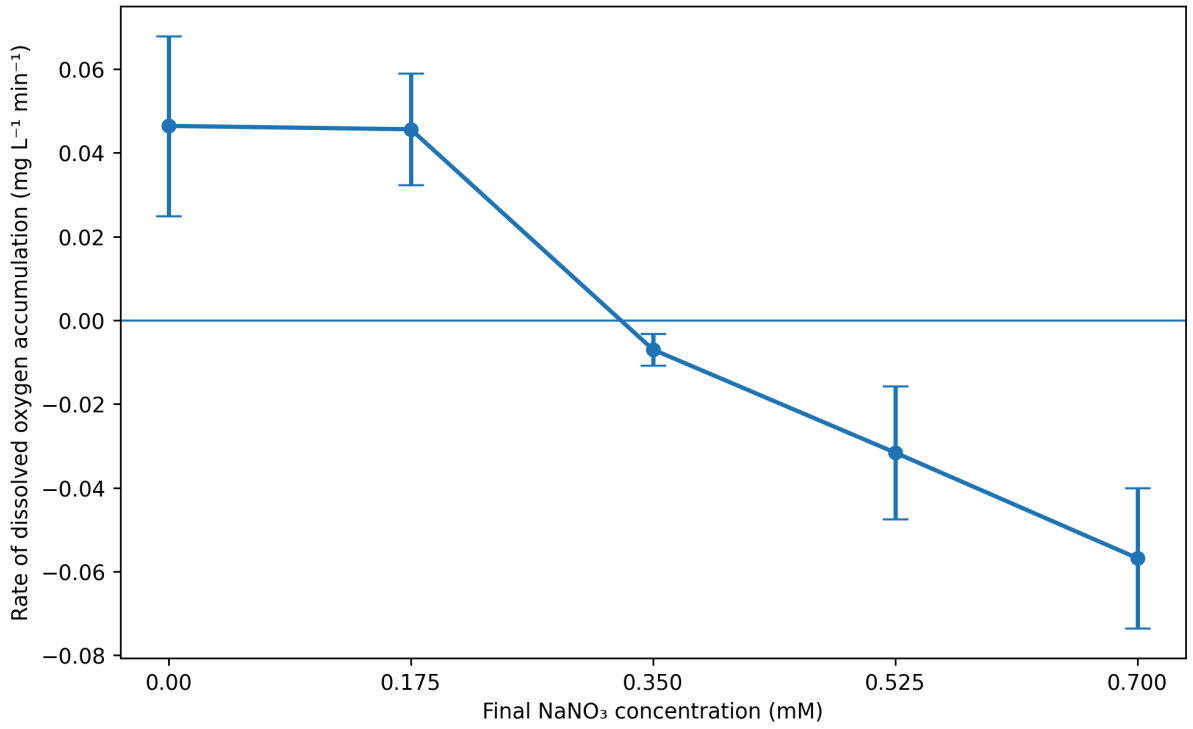
$SD = \sqrt{\frac{0.00007}{5}} \approx 0.00374$ mg L^{-1} min^{-1}

This relatively small SD indicates that the trial rates at $NaNO_3$ concentration of 0.350 mM were closely clustered around the mean, showing high consistency between repeated measurements.

$NaNO_3$ concentration (mM)	Mean rate (mg L^{-1} min^{-1})	Standard deviation, (SD)
0.00	0.0464	0.02150
0.175	0.0456	0.01335
0.350	-0.0070	0.00374
0.525	-0.0316	0.01583
0.700	-0.0568	0.01677

Table 3. Processed Data Table

Standard deviation (SD) was calculated from six trials per concentration to quantify trial-to-trial variability in the average net oxygen accumulation rate.



Graph 1. Mean rate of dissolved oxygen accumulation (mg L^{-1} min^{-1}) vs final $NaNO_3$ concentration (mM), with ±SD error bars

The graph shows a clear negative relationship between final $NaNO_3$ concentration and the mean rate of dissolved oxygen accumulation. Rates are positive at 0.00–0.175 mM, cross zero near 0.350 mM, and become increasingly negative at 0.525–0.700 mM, with ±SD error bars.

ANOVA Test

Why ANOVA Test Was Chosen

Once the trial rates were calculated, and the variability was summarised with standard deviation of each final concentration of $NaNO_3$ (n = 6), an inferential test was required to test whether the differences in net oxygen accumulation rates with concentration were likely to be a real treatment effect and not due to random trial-to-trial variation. The use of a one-way ANOVA was due to the presence of one categorical independent variable ($NaNO_3$ concentration with five levels: 0.000, 0.175, 0.350, 0.525, 0.700 mM) and one continuous dependent variable (average net oxygen accumulation rate, mg L^{-1} min^{-1}). ANOVA is also suitable in this case since it compares all the group means at once rather than inflated Type I error that would arise in case many t-tests were applied.

Source	SS	df	MS	F	p-value
Between Groups	0.05	4	0.0125	53.7739	6.4×10^{-12}
Within Groups	0.01	25	0.0004	N/A	N/A
Total	0.06	29	N/A	N/A	N/A

Table 4. ANOVA Summary

A one-way ANOVA showed a statistically significant effect of $NaNO_3$ concentration on average net oxygen accumulation rate is: $F(4,25) = 53.77$, $p \approx 6.4 \times 10^{-12}$. This means that at least one concentration produced a mean rate that differs significantly from the others, supporting that the concentration-dependent pattern in the processed data is not due to random variation alone.

Also the effect size $\eta^2 = 0.896$ was very large suggesting that 89.6% of the variance in rates is explained by differences between nitrate concentration groups (within the limits of the design).

Post-hoc Test (Tukey HSD)

Since ANOVA can only indicate the occurrence of significant difference between at least one of the means of the nitrate concentrations, the post-hoc test will be required to reveal which particular nitrate concentrations differ among themselves. Thus, Tukey HSD post-hoc test was selected since it contrasts all of the pair-wise combinations of the five concentration groups and regulates the overall Type I error rate ($\alpha = 0.05$).

Tukey HSD results (α = 0.05)
The Tukey HSD test showed the following key comparisons (adjusted p-values):

Comparison	Difference	SE	Q	p-value	Significant (a=0.05)
Group 1 vs Group 2	0.0009	0.0063	0.1379	0.999978	No
Group 1 vs Group 3	0.0534	0.0063	8.5005	0.000026	Yes
Group 1 vs Group 4	0.0780	0.0063	12.4061	0.000000	Yes
Group 1 vs Group 5	0.1032	0.0063	16.4204	0.000000	Yes
Group 2 vs Group 3	0.0526	0.0063	8.3627	0.000033	Yes
Group 2 vs Group 4	0.0771	0.0063	12.2683	0.000000	Yes
Group 2 vs Group 5	0.1024	0.0063	16.2825	0.000000	Yes
Group 3 vs Group 4	0.0245	0.0063	3.9056	0.072553	No
Group 3 vs Group 5	0.0498	0.0063	7.9199	0.000073	Yes
Group 4 vs Group 5	0.0252	0.0063	4.0143	0.061733	No

Table 5. Tukey HSD Results

The Tukey HSD post hoc test makes it clear as to what concentrations of nitrates vary in the mean net oxygen accumulation rate. The concentrations of the two lowest levels (0.000 and 0.175 mM) are not significantly different ($p \approx 1.00$) meaning that the net oxygen accumulated in low nitrate is similar. Conversely, both low-nitrate groups do differ significantly at concentrations of 0.350 and higher ($p<0.001$) and there is a threshold-like transition in net oxygen accumulation between 0.175 and 0.350 mM. Two higher concentration comparisons of 0.350 vs 0.525 and 0.525 vs 0.700 are not significant ($p = 0.0726$ and 0.0617 respectively) whereas the highest order of separation is observed between low nitrate and highest nitrate condition (0.350 vs 0.700).

7. Discussion

The maximum mean rates were recorded at 0.000 mM and 0.175 mM which was established to imply that the photosynthetic rate was higher than the rate of oxygen consumption by respiration under light. Beyond 0.350 mM the mean rates had negative values, implying that the oxygen consumption (by plant tissues and microorganisms) was greater than the oxygen production, and this provided an overall oxygen loss. Sign difference is important because such a change is qualitative (not just a minor

drop in photosynthesis) in the behaviour of the system. This can be justified by the results of the Tukey HSD: the two groups of the lowest nitrate concentrations (0.000 and 0.175 mM) are statistically equal, whereas the two are significantly different than the 0.350 and above concentrations, which aligns with a threshold-like shift occurring between the highest and lowest nitrate concentrations of 0.175 and 0.350 mM.

The system is also reflected on the pattern of standard deviation. The smallest SD is at 0.350 mM that is, there was not much trial to trial variation at that point that net oxygen accumulation is closest to zero. This is helpful in the fact that the system was continuously approaching equilibrium at this concentration; at which production of photosynthetic oxygen, and respiratory oxygen consumption were approximately equal. On the other hand, SD increases gradually at 0.525 mM which may indicate a transition region where subtle fluctuations in micro-conditions can cause larger changes to the trial-to-trial variation in net oxygen dynamics.

The one probable biological solution is that it was not nitrate that served as the key constraining factor in the experiment. The inorganic carbon (CO_2/HCO_3^-) often limits photosynthesis. Due to the absence of additional inorganic carbon addition there was also the possibility carbon fixation in Calvin cycle would restrict oxygen production so that no additional oxygen would be produced as nitrate was raised. In addition, the assimilation of nitrates requires energy and reducing power and can increase metabolic rate and can increase respiratory oxygen uptake. The technique measures net oxygen change (photosynthesis - respiration) thus the photosynthesis would not indicate any large increase in photosynthesis but would increase respiration leading to a loss in net oxygen accumulation and may be negative at high nitrate concentrations. In this way the adverse tendency could be considered as the evidence that under these conditions the nitrate enrichment did not trigger the net photosynthetic production of oxygen during the short periods and might have caused the higher consumption of oxygen in comparison with the production of oxygen.

8. Evaluation

The principal shortcomings include the fact that the oxygen probe, is the measurement of the net oxygen accumulation, rather than gross photosynthesis. Therefore the negative tendency recorded cannot be attributed solely to a reduction in the photosynthetic oxygen formation, it can also be attributed to high respiratory demand associated with uptake/assimilation of nitrates.

The other limitation is non-intensive standardisation of availability of inorganic carbon. As no source of bicarbonate/CO_2 was employed, the likely limiting factor that produced the most significant effect could have been inorganic carbon, and this would mask a potential effect of nitrate enrichment caused on photosynthetic oxygen production. A stronger design would retain inorganic carbon non-limiting with a constant concentration of sodium bicarbonate and constant pH such that the impacts of nitrates may be more directly examined.

Without continuous mixing, oxygen gradients may develop near leaves and the probe; although swirling was standardised, incomplete mixing could increase trial-to-trial variability, so a low-rpm magnetic stirrer would reduce this uncertainty.

Finally, the measuring time (15 minutes) was short. The nitrate impact on the chlorophyll concentration and the abundance of enzymes is long-term, therefore the short-run alteration of the net oxygen concentration can be regulated by the instant constraining factors (inorganic carbon in specific) and respiration. It is perhaps more convenient to increase the period of light, or introduce a

period of controlled acclimation to distinguish between the short-term effects of the metabolismic processes and long-term variations in photosynthetic capacity.

9. Conclusion

A general reduction in average net oxygen accumulation rate of Elodea canadensis was vividly recorded with replacement of the initial 0.000 mM to final 0.700 mM $NaNO_3$ concentration in 15 minutes. The positive value of the net oxygen accumulation at 0.000- 0.175 mM, and negative at 0.350 mM, and in higher positions showed that there was a change in net oxygen accumulation, that is, net oxygen loss during the process of illumination. One can then observe that such differences are not random, but systematic not only in the ANOVA results, but also in the HSD Tukey results. Broadly, it was not the nitrate which was likely to restrict the key, and it is most likely that it will be the inorganic carbon constriction, or some other requirement of respiration.

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