

# The Effect of Nitrate Levels on Algae Growth in the Soil



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## **Abstract**

Algae are photosynthetic organisms that, like their evolutionary descendants the plants, require large amounts of the nutrient nitrogen to produce the critical organic molecules they use to capture light energy. The most easily absorbed form of nitrogen that algae can use for this purpose is nitrate. The E.S.S.R.E. Biota Survey 2014 revealed that Site 4 had statistically lower algae levels than any of the other 3 microclimate research sites. Since this site is a partial wetland, we hypothesized there must be a direct correlation between the unusually low nitrate levels in this site that the Biota Survey also revealed low algae levels as well. Five locations in each of the four plots had soil samples removed for analysis, and the data collected confirmed the proposed correlation between algae and nitrate levels. We would next examine the density of earthworms in this plot to see if they might also account for the low algae levels there.

## Introduction

Algae are photosynthetic organisms that, like their evolutionary descendants the plants, require large amounts of the nutrient nitrogen to produce the critical organic molecules they use to capture light energy (Crawford 1995). Nitrate is the most easily absorbed form of nitrogen that algae can use for this purpose, but the majority of nitrogen is in the atmosphere, where it is not readily available. So algae that live in the soil must rely on the bacteria living there to convert nitrogen from the atmosphere into forms algae can use through the process of nitrogen fixation in which the soil bacteria convert nitrogen gas first into ammonia and then later into nitrite and eventually into the nitrate that plants, algae, and other organisms up the food chain depend upon (New Mexico State University 2013).

Due to the dependent relationship between algae and bacteria, we were puzzled when the E.S.S.R.E. 2014 Biota Survey revealed statistically significantly high densities of bacteria in E.S.S.R.E. Site 4 in conjunction with statistically significantly low algae levels (E.S.S.R.E. 2014). Site 4 contains partial wetlands and given the high density of bacteria found there this year along with the fact that algae are nonvascular and cannot maintain their hydration, requiring moist locations to thrive (Pennsylvania State, 2009), we expected to find high quantities of algae in its soil. When we found the opposite, we examined the biota survey data for a possible explanation and found that the nitrate levels in the soils throughout the different E.S.S.R.E. Microclimates had a direct correlation with algae growth: Site 3 had the highest nitrate (20.42 ppm) and algae ( $1.07/\text{mm}^2$ ) levels this year; while Site 4 had the lowest nitrate (5.6 ppm) and algae ( $0.43/\text{mm}^2$ ) levels. Since low amounts of algae in an ecosystem may be indicative of low nitrate levels (Fried, Mackie, Nothwehr 2003), we hypothesized that the lower nitrate levels found in E.S.S.R.E. Site 4 this year is the cause of the lower levels of algae observed as well.

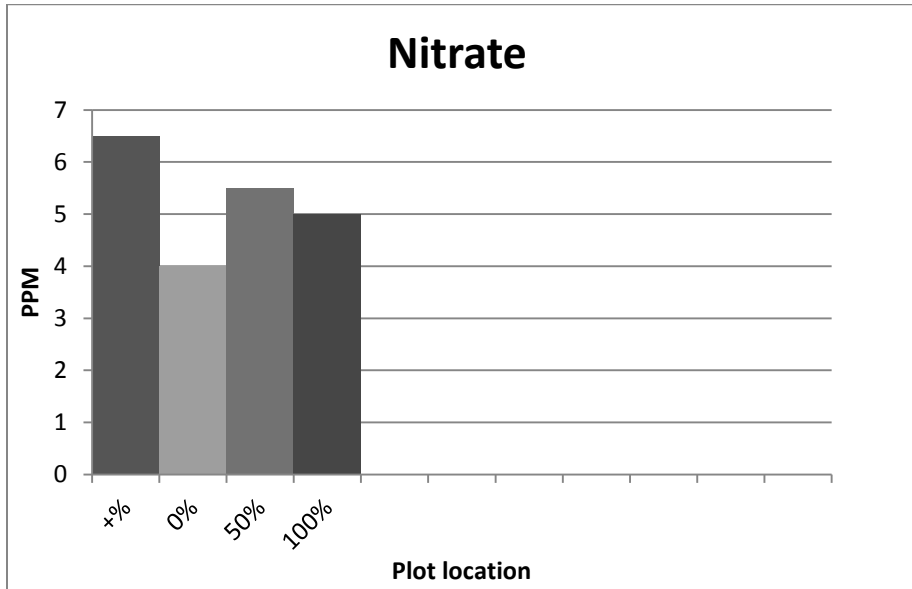
## Methods

Four ½-meter by ½-meter plots were chosen in E.S.S.R.E. Site 4 (E.S.S.R.E, 2001) based on the amount of *Leersia virginica* present; plot 1 contained no plant life and served as the positive control; plot 2 contained plant life but 0% *Leersia virginica*; plot 3 contained 50% *Leersia virginica*; and the plot 4 contained 100% *Leersia virginica*. Five standard sized glass microscope slides were inserted 6cm into each soil plot, one in each corner and one in the middle of the plot to test for algae (Pipe and Cullimore, 1980). A 15cm deep by 2cm in diameter sample of soil was extracted directly next to each microscope slide, and all soil samples were immediately tested for nitrate levels (ppm) using the LaMotte Model STH-14 Test Kit.

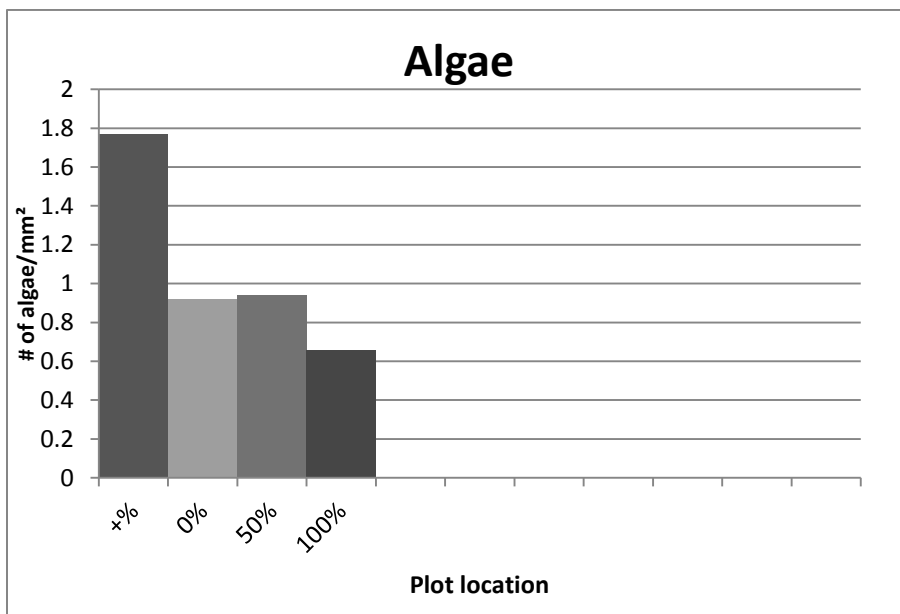
After 96 hours, all microscope slides were removed from the soil, gently rinsed with distilled water, and placed between 2 clean glass microscope slides for observation. Simultaneously, a 15cm deep by 2cm in diameter sample of soil was extracted directly from where each microscope slide had been implanted, and all the soil samples were immediately tested for nitrate levels (ppm) using the LaMotte Model STH-14 Test Kit. The microscope slides containing the algae were examined using a light microscope at 40x. Five fields of view were examined for each slide, and the number of algae in each field of view was counted. For each

slide, the 5 counts were averaged and divided by 17.3 to determine the number of algae per mm<sup>2</sup> of soil depth.

## Results



Graph 1: represents the correlation between the plot location and the amount of nitrate found in the soil located there.



Graph 2: represents the correlation between the plot location and the amount of algae found in the soil located there.

Table 1

Changes in Nitrate Levels Over the Course of the Experiment			
Slide #	Before (PPM)	After (PPM)	# of algae/mm <sup>2</sup>
1A	10	7.5	0.277
1B	5	7.5	4.439
1C	10	5	0.439
1D	5	7.5	2.879
2D	5	2.5	1.156
2E	5	2.5	0.636
3E	5	7.5	0.324

Table 2

Nitrate levels- p values					
		+%	0%	50%	100%
+%	P		0.364	0.378	0.25
0%	P			0.962	0.467
50%	P				0.482
100%	P				

Table 2: represents a statistical analysis of the nitrate data using t-testing.

Table 3

Algae levels- p values					
		+%	0%	50%	100%
+%	P		0.364	0.378	0.25
0%	P			0.962	0.467
50%	P				0.483
100%	P				

Table 3: represents a statistical analysis of the algae data using t-testing.

## Discussion

As Graph 1 shows, the average nitrate levels in the 4 research plots did vary from 4 ppm to 6.5 ppm, with a statistical significance between  $p=.364$  and  $p=.482$ . When we look at Graph 2, we see that the algae levels similarly varied in the 4 research plots from  $1.77 \text{ mm}^2$  to  $0.66 \text{ mm}^2$  with a statistical significance between  $p=.364$  and  $p=.483$ . Furthermore, when comparing Graphs 1 and 2 we see the expected corresponding relationship between nitrate and algae levels in the 4 research plots, therefore affirming our original hypothesis. In addition, when we examine Table 1, we see that for several of the samples taken (see highlighted values), as the nitrate levels changed from the start of our experiment to its end, a corresponding increase or decrease in algae count was observed which further supports our hypothesis.

However, the statistical analysis also reveals that with most of our p-values hovering around 0.40, we can only be 60% certain that the algae levels that we observed were due to the low amounts of nitrate found in this site. Therefore, we must consider other possible sources for the unexpectedly low algae levels observed in this year's biota survey (E.S.S.R.E 2014). Since the survey also revealed large quantities of the earthworms in Site 4 where our research plots

were located, we would next investigate the correlation between the density of this known predator of soil algae and the amount of algae in our research plots.

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