

# Effects of Environmental pH Change on Wetland Microbial Communities

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## Introduction

The Waco Wetlands serves as a filtration system for the North Bosque water that flows through. The water upstream of the wetlands is contaminated with phosphates and nitrates that arise from animal waste and fertilizers; therefore, as this contaminated water flows slowly through the wetland system, these nitrates are taken up by the microbial ecosystems in the soil. This process, called bioremediation, results in more purified water as it travels downstream. Many factors, such as pH, contribute to the level of activity microbes exhibit determined by the uptake of nitrates in the water. Bacterial proteins and enzymes perform optimally in certain pH conditions and “a very common environmental modification is a change of the environmental pH;” therefore it is important that the effects are tested [1]. If the pH conditions of the wetlands are altered, then microbes may not perform as optimally as in unaltered conditions. However, there is also a possibility that microbial ecosystems may perform better. The primary objective of this was to discover the effects of altering the environmental pH of wetland microbial ecosystems and determine whether which adjustment (higher or lower) positively or negatively affected nitrate uptake in the wetland water. “We find experimentally that these pH changes create feedback loops that can determine the fate of bacterial populations; they can either facilitate or inhibit growth, and in extreme cases will cause extinction of the bacterial population” [1]. Research in this area can contribute to more efficient bioremediation by microbial ecosystems and a better understanding of how changes in pH from environmental factors can affect the quality of Waco water.

## Materials and Methods

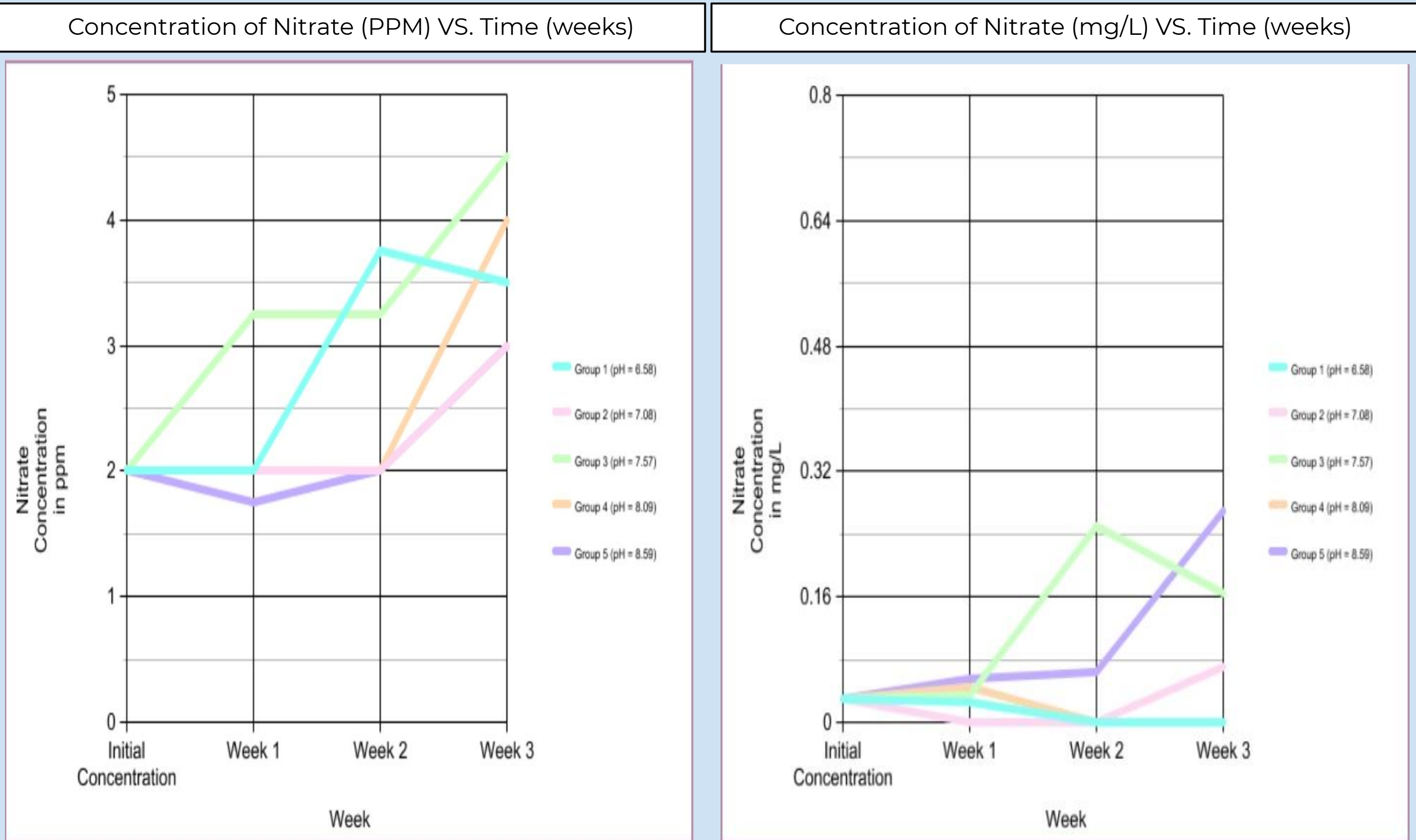
- Water and soil samples were collected in a five-gallon bucket from an infall at the Waco Wetlands
- Five different environments with varying pH were created by using 15% KOH solution and 15% H<sub>3</sub>PO<sub>4</sub>. Environments began with a pH of ~ 6.5, 7, 7.5 (control), 8, and 8.5
  - KOH and H<sub>3</sub>PO<sub>4</sub> were used because they do not directly alter existing nitrate levels.
- 2 samples were created for each pH level
- 1 liter of wetland water and 300g of soil were added to each sample bucket and the initial concentrations of nitrate and nitrite in the wetland water were recorded (in ppm) using Hach Aquachek Nitrate and Nitrite Testing Strips and Hach Colorimeter.
- The nitrate concentrations of each bucket were taken weekly for 3 weeks using the Hach Aquachek Nitrate and Nitrite Testing Strips and Hach Colorimeter.

Figure 1



The Hach Nitrate Powder Pillows, Hach Colorimeter, and Hach Aquachek Nitrate and Nitrite Testing Strips (pictured left to right) were used to test the nitrate concentration in the water of different buckets.

Figure 2



**Left:** Concentration of Nitrate (PPM) over a 3 week period using Hach Aquachek Nitrate and Nitrite Testing Strips

**Right:** Concentration of Nitrate (mg/L) over a 3 week period using Hach Colorimeter

Figure 3

Groups	1	2	3	4	5
Averaged pH (2 buckets in each group)	6.58	7.08	7.57 (Control)	8.09	8.59
Initial Nitrate Concentration (ppm)	2	2	2	2	2
Final Nitrate Concentration (ppm)	3.5	3	4.5	4	3
Initial Nitrate Concentration (mg/L)	0.03	0.03	0.03	0.03	0.03
Final Nitrate Concentration (mg/L)	0	0.06	0.17	0	0.27

Data table shows changes in nitrate concentration in mg/L and ppm of buckets containing various pH. The Hach Aquachek Nitrate Test Strips showed an increase in nitrate concentration (in ppm) from the initial value in all buckets. The Hack Colorimeter showed an increase in nitrate concentration (in mg/L) for groups 2, 3, and 5 and a decrease in nitrate concentration (in mg/L) for groups 1 and 4.

## References

[1] Modifying and reacting to the environmental pH can drive bacterial interactions Ratzke C, Gore J (2018) Modifying and reacting to the environmental pH can drive bacterial interactions. PLOS Biology 16(3)

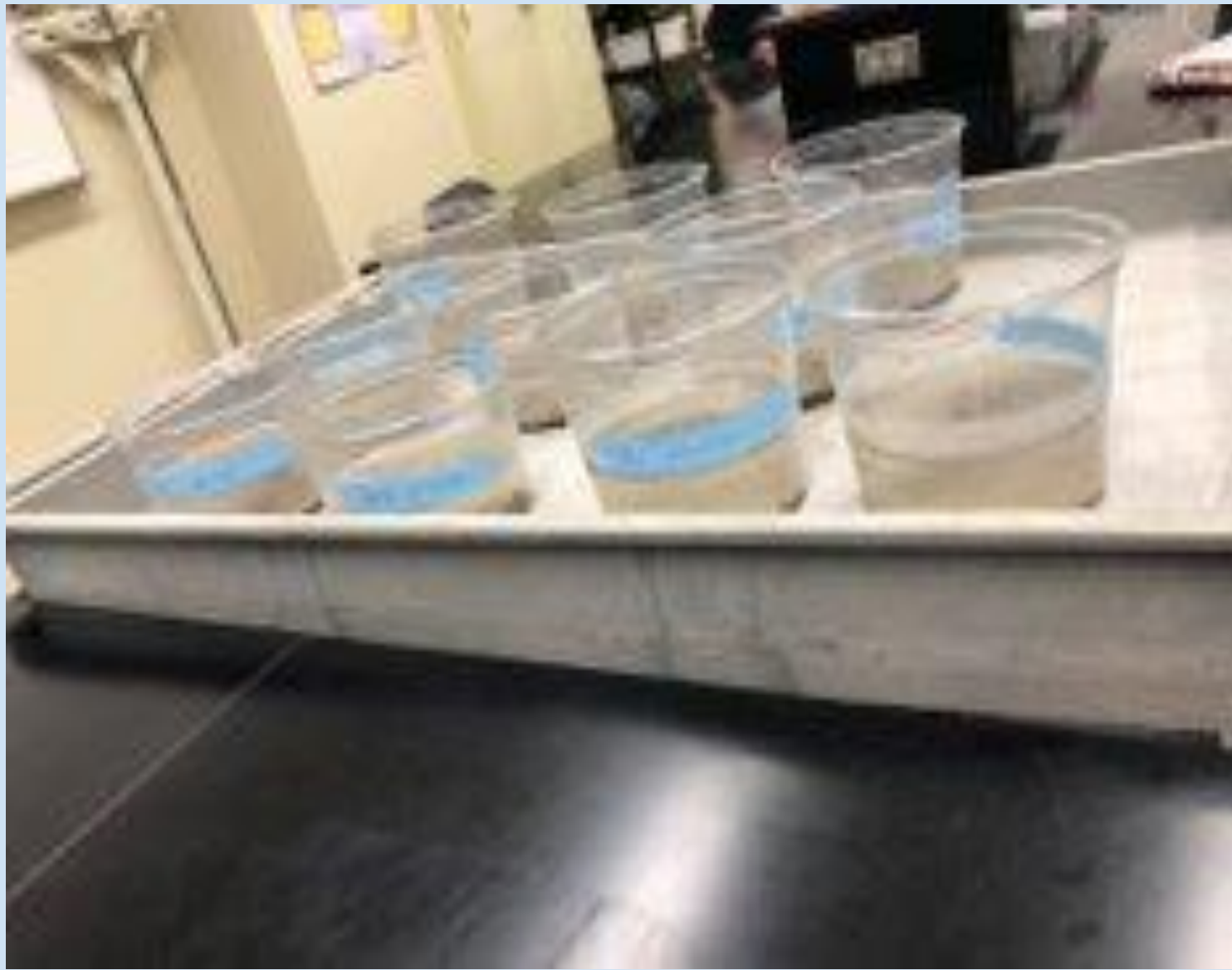
[2] Yu Jiang, Kai Yang, Yu Shang, Huining Zhang, Li Wei and Hongyu Wang, Response and recovery of aerobic granular sludge to pH shock for simultaneous removal of aniline and nitrogen, Chemosphere, 10.1016/j.chemosphere.2018.12.207, (2019).

Figure 4



Water samples were taken from Wetland infall water that came directly from the North Bosque River.

Figure 5



Ten buckets each hold a wetland microbial community; two buckets per pH level: 6.5, 7, 7.5, 8, and 8.5.

## Results

- P-value for the ANOVA test for the Test Strips data (rows 3 and 4 in *Figure 3*) is 0.0722. This is larger than the significance level of 0.05.
- P-value for the ANOVA test for the Colorimeter data (rows 5 and 6 in *Figure 3*) is 0.64692.
- Both ANOVA tests show no statistical significant difference in final nitrate concentration between the five groups

## Discussion and Conclusion

- Based on preliminary results, a difference in the Wetlands environmental pH of +/- 1 will not have a statistically significant impact on the microbial ecosystem’s ability to bioremediate.
- This finding supports the idea of microbial adaptability. However, further experimentation is needed to verify this perception.
- The initial hypothesis was incorrect. Nitrate concentration increased in microbial ecosystems regardless of environment (explained in Figure 4), and this may be attributed to water loss through evaporation. A decrease in solution caused an increase the nitrate concentration.
- Lastly, accuracy of measurements was influenced by the precision capabilities of available equipment which may have influenced statistical significance of final results.

## Acknowledgments

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