Effects of Various pH Conditions on the Carbon Sequestration of *Carex nudata*

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Abstract

Carex nudata, a riparian sedge, functions as a key determinant in wetland health by providing nutrient cycling, carbon sequestration, and establishing prime conditions for other wetland species propagation as an early successional species[1] – plant species that tend to have greater rates of photosynthesis and respiration as well as nutrient uptake[2]. Specifically, *C. nudata* provides valuable substrate for other wetland plants while also protecting others from insect larvae and deer grazing. At times, wetland restoration projects do not reach successful integration due to a lack of understanding of the propagated species and the abiotic conditions that are present in the area. Plant performance is important and so are its effects on other wetland species it interacts with[1]. Examining the effect of pH on the species' ability to sequester carbon gives us insight into possible wetland health and restoration success indications. As restoration efforts aim to repair ecologically damaged sites, oftentimes as a result of human development and resource extraction, chosen plants must be able to survive atypical abiotic conditions such as high acidity from abandoned mining sites and areas where exposed minerals alter the neutral pH of water sources. Here, same-maturity Torrent Sedge plugs sourced from Wetland Nursery in Richmond, California, have been grown in a controlled environment and closely monitored for the effects of pH on growth, carbon sequestration, and biomass. Throughout the experiment, other abiotic conditions such as light exposure, nitrate levels, dissolved oxygen levels, phosphorus levels, and potassium levels were held constant for the duration of the study. The exposed pH conditions: low pH (5), neutral pH (7), and high pH (9) were carefully maintained every five days. After a 14-day growth period, weight measurements were taken of biomass and organic carbon in plant subjects. *C. nudata* subjects performed better in neutral pH conditions while demonstrating higher susceptibility to fungal diseases in more acidic and basic conditions. Over the course of 2 months, observations and measurements tracked the growth of eighteen plant subjects, correlating data on how pH affects different wetland species. The observations in this study may hopefully provide insight into wetland restoration efforts, environmental impact foresight, and wetland health indication.

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Introduction

Carex nudata is a winter deciduous grass native to Northern California marshes, streams, and river banks. The sedge is cespitose and habitually grows in "large, raised, dense clumps, not connected in rhizomes".[3] In restoration, its use is found commonly in bogs and ponds as a deer-resistant monocot, thriving in conditions ranging from slow to fast-draining soils as long as water is available. *C. nudata* is often found below the high water mark in wet biomes, although they can be found in non-wetland biomes.[4] As a likely host plant, the California native grass promotes the larvae and pupae stages of the Umber Skipper Butterfly (*Poanes melane*), Large Heath Butterfly (*Coenonympha tullia*), Dun Skipper Butterfly (*Euphyes vestris*), American Ear Moth (*Amphipoea americana*), Girdler Moth (*Dargida procinctus*), *Elachista cucullata,* and the Lesser Wainscot Moth (*Mythimna oxygala*).[4]

In wetland ecosystems, oftentimes plants will establish prime conditions for other plants; in some stages, wetland species can provide shaded areas, collect soil in stoloniferous plants to create wet flats (spaces where other plants may establish themselves), and provide soil available nutrients through a unique microbiome culture in roots. These relationships and services are not a well-studied area in regards to specific and complex native plant species interactions. More targeted research of plant interaction and abiotic conditional effects on specific species will improve the tools and information available for wetland restoration projects. As the artificial creation of wetland biomes aims to mimic the natural establishment of robust wetland habitats, how these natural biomes operate must also be understood.

Further research is needed to understand species-specific relationships within wetland fauna and flora, as well as inter-species interactions and behaviors based on conditions such as topography, nitrogen-phosphorus-potassium (NPK) availability in water and soil stores, acidity or basicity of water conditions, and other stressors that can change the community composition in a stream. Frequently shifting pH levels, or pH levels constantly outside ideal ranges, cause physiological stress that results in decreased growth and reproduction and increased risks of death and disease, which lead to reduced biodiversity in the ecosystem.[5] By understanding what pH level *Carex nudata* thrives in, restoration

projects can have higher rates of success. Choosing an appropriate plant species when restoring an area with naturally high amounts of pyrite-rich rocks that create more acidic abiotic conditions (when the pyrite oxidized into sulfuric acid) would better sustain a proposed habitat restoration.

Knowing that fish have adverse reactions to changes in pH in their environment, I questioned to what extent pH had an effect on the flora in an area and whether or not there were pH ranges that certain wetland plant species would thrive in. I hypothesized that the plant species would sequester more carbon in neutral pH conditions while more acidic and basic pH levels would impair plant growth during the two trials.

In a study of passive restoration efforts on cattle grazing land in the Middle Fork of the John Day River in Oregon, the University of Oregon and Oregon State University observed *C. nudata*'s ability to establish patches, reduce erosion and impact the geomorphology of the channels into multiple pathways for the river.[6] *C. nudata* was transplanted into the South Fork Eel River in northern California by an ecologist hoping to understand its role in providing a critical substrate for other species to survive winter floods and herbivory from deer and insect larvae. During the growing season, it reduced the biomass of five other native species by 50% and reduced their ability to reproduce by 60% in a competitive manner; however, *Mimulus guttatus and Epipactis gigantea* that grew on the *C. nudata* tussocks were protected from herbivory by more than 75% during the growing season, thereby researchers concluded that its "associational defense" was equal to its negative effects of competition in the ecosystem.[7] Experiments conducted by the University of California - Berkeley used the tussocks formed by *C. nudata* to explore how seed dispersal in a waterway interacted with its environment. The study found that an increase in biological invasions and plant diversity downstream occurred, which can inform methods of restoration projects to include additions of seeds into established tussocks of *C. nudata*.[8] The observations in this study may hopefully be used as a reference when restoration efforts consider the use of *C. nudata* as a

native perennial wetland species onsite. Creating a more detailed plant profile of native species will help restorationists make greater informed decisions on what to plant based on site conditions.

This investigation into the effects on biomass, or carbon sequestration, of *C. nudata* in high, low or neutral pH water conditions is intended to mimic the anthropogenic effects of dumping from a mine site into a body of water that would increase acidity through or agricultural lime runoff that would increase basicity. Most aquatic species have an optimal pH range of 6.5 - 8.[5] Finding whether *C. nudata* thrives better in low, high, or neutral pH will inform the restoration and conservation efforts. When a plant's ability to sequester carbon dioxide is affected by subpar abiotic conditions, the entirety of the ecosystem may be affected. The sustainability of native plants is critical as it encourages native fauna while providing erosion control, and protection of less-sturdy native species from associational defense. Animals that rely on the *C. nudata* as a food source will find less available biomass and energy, resulting in a reduced carrying capacity in the ecosystem. Wetlands are an important form of carbon sink to reduce the amount of greenhouse gasses in the atmosphere because they represent a vast underground carbon stock relative to their smaller expanse.[9] By deducing carbon sequestration through measuring biomass, this investigation aims to find if pH conditions compromise the effectiveness of sequestering carbon in the *C. nudata* sedge, and which pH conditions the species performs well in.

Methodology

Materials:

- 1 quantity: IGGRO 2 pack plant scaffolding (https://www.amazon.com/your-orders/pop?ref=ppx_yo2ov_dt_b_pop&orderId=111-3620682-0487438&lineItemId=nonisrislmotony&shipmentId=Th78J9bDT&packageId=1&asin=B07MVYF9C9)
- 1 quantity: LaMotte Water Test Kit (https://www.amazon.com/your-orders/pop?ref=ppx_yo2ov_dt_b_pop&orderId=111-3620682-0487438&lineItemId=nonisrislmosqny&shipmentId=TWcdldMZT&packageId=1&asin=B00BWXI1L4)
- 2 quantity: Gooing Top LED grow light (https://www.amazon.com/gp/product/B085CDPSMR/ref=ppx_od_dt_b_asin_title_s00?ie=UTF8&psc=1)
- 1 quantity: Ruolan hydroponics digital pH meter pen ±0.01 accuracy (https://www.amazon.com/dp/B08HLXBBK4?psc=1&ref=ppx_pop_dt_b_asin_title)
- 18 quantity: Perennial "California black-flowering sedge" *Carex nudata* plugs/stubs from The Watershed Nursery at 601 A Canal Blvd., Richmond, CA 94804, purchased July 6, 2022 https://www.watershednursery.com/
- 1 quantity: Vigoro Weed Control Film (3x50ft) (https://www.amazon.com/Vigoro-Landscape-Installation-Flowerbeds-Planters/dp/B00IKVIPA8/ref=cm_cr_arp_d_product_top?ie=UTF8)
- 6 quantity: 1 gallon Good & Gather Distilled Water (https://www.target.com/p/distilled-water-1gal-good-gather-8482/-/A-54444818)
- 1 quantity: API pH Up solution (sodium carbonate) https://www.apifishcare.com/pdfs/products-us/ph-test-adjuster-kit/api-ph-up-safety-data-sheet.pdf
- 1 quantity: API pH Down solution (sulfuric acid) https://apifishcare.com/pdfs/products-us/ph-test-adjuster-kit/api-ph-down-safety-data-sheet.pdf
- 1 quantity: Excalibur 2400 4-tray Food Dehydrator https://excaliburdehydrator.com/products/2400-excalibur-4-tray-no-timer-black-solid-door?_pos=7&_sid=f328bbe49&_ss=r

- 4 quantity: EcoCraft 16” x 24” Unbleached Parchment Paper/Full Sheet Pan Liners https://bagcraft.com/2011/09/15/ecocraft-bake-n-reuse-pan-liner-unbleached/?pl=supermarket
- 1 quantity: Smart Weigh Digital Scale ±0.01 gram https://www.amazon.com/Smart-Weigh-kilograms-Platforms-Ingredients/dp/B01LXXBQWD

Methods:

Measurable biomass estimates. The sequestration of carbon was deduced by measuring plant subject biomass. Plants in this study were raised in batches of equal maturity. Regardless of size and weight, the initial biomass was calculated in order for future measurements to be compared to a baseline. To measure biomass without dehydrating the entire subject, plants were dissected vertically after being removed from nursery pots, thoroughly cleaned of dirt and debris, and pruned. The two parts were labeled with respect to their destination. The larger Group A plants were to be observed in the experimental conditions, while the Group B plants were to be dehydrated in order to determine biomass to wet weight ratio. To determine the biomass of Group B plants, the initial plant weight was measured, as well as the combined weight of a parchment paper bag and the plant. After labeling the plant bags, nine Group B sections from the first observed trial (referred to as Trial 1) were left untouched, sitting out to dry for 72 hours before being dried in an Excalibur 2400 food dehydrator at 145 °F for 48 hours. Cooked plant material was weighed on parchment paper to calculate organic matter. A ratio was generated comparing the final and initial weights of Group B plants. For each Group B plant, the related Group A plant’s biomass was estimated using the wet-to-dry mass ratio (Measured by dividing wet mass by dry mass weight) at the start of Trial 1.

Site preparation. In the home-lab site, cardboard and duct tape were used to block light sources including the sun, overhead LEDs, and blue light pulses from machinery. Consistent light cycles (8:10 am - 8:10 pm) were maintained by built-in automatic timer grow lights that were positioned at a 10-inch height above plant tubs. Tubs were fashioned from plant scaffolding and clear plastic bins. Three equally spaced tubs were designated to hold a single low pH, control pH, or high pH group where labeled Group A plants

were placed. Between tubs, opaque dividers were placed to reduce light pollution from adjacent grow lights.

Pest treatment (error). Treatment of an insect infection included neem oil wash, relocation, and a nutrient-charged daily watering plan. Plant health showed a steady increase and the return to green from yellowed leaves occurred over the next 14 days.

Water treatment and preparation. Each pH group's water was carefully measured and adjusted to create conditions with isolated variables. Using distilled water, the pH levels of three different gallon jugs were measured with a Ruolan hydroponics digital pH meter pen. After measuring, API pH Up and API pH Down were applied until measured levels were within ±0.05 of the target numbers. Target numbers were: low(pH 5), control(pH 7) and high(pH 9). 1000 mL of distilled water was used to fill levels up to two inches on all tubs. After seven days after the trial start, each plant group's water tub was replenished with fresh distilled water (containing matching pH levels) to counteract evaporation.

Progress and site observations. Over the course of the 14-day growth period, photos were taken at precisely 10:10 pm each day to record progress and monitor plant health (see Appendix A). Whenever any maintenance or adjustments were performed (for example watering, turning on lights, etc.), the change was written in a log book (see Appendix B).

Data analysis. All data was collected and presented in a Google spreadsheet. https://docs.google.com/spreadsheets/d/1BoJQifotyGq5M8bYWVBZu299Wz0hWNNkzAciTkAuRR4/edit#gid=0

Trial 2 alterations. Aspects of Trial 2 were adjusted based on results obtained from Trial 1. Instead of a water line, tubs were filled to the 3-inch mark with soil, and scaffolding was used as section dividers to separate different plant subjects. The water was replenished by 800 mL every seven days and the trial

length was extended to three weeks to provide a longer period for plants to increase their biomass and sequester carbon. All other methods not listed in the alterations are the same as Trial 1.

Data & Observations

Legend:	Low pH - L	Control pH - C	High pH - H	Weight - Wt

Table I

Pre-Trial Biomass Estimations								
Date	Label	A Section Wt (g)	B Section Wt Before Cook (g)	B Section w/ Bag Before Cook (g)	B Section w/ Bag After Cook (g)	B Section **Bag Only** After Cook (g)	B Section Biomass to Wt Ratio (g)	A Section Biomass (g)
7/11/2022	C1	8.34	2.1	5	3.42	2.86	0.266	2.224
7/11/2022	C2	3.71	1.8	4.68	3.34	2.91	0.238	0.886
7/11/2022	C3	4.7	1.02	4.24	3.48	3.23	0.245	1.151
7/11/2022	L1	9.55	1.17	3.56	2.74	2.37	0.316	3.020
7/11/2022	L2	8.95	1.34	4.1	3.1	2.72	0.283	2.538
7/11/2022	L3	10.89	0.93	3.55	2.8	2.57	0.247	2.693
7/11/2022	H1	7.93	1.03	3.7	2.88	2.66	0.213	1.693
7/11/2022	H2	10.45	1	3.73	3.01	2.74	0.27	2.821
7/11/2022	H3	6.61	0.89	3.56	2.87	2.62	0.280	1.856
8/3/2022	C4	1.43	0.12	2.72	2.57	2.55	0.166	0.238
8/3/2022	C5	18.49	1.5	4.14	2.88	2.59	0.193	3.574
8/3/2022	C6	14.22	2.38	4.97	2.99	2.55	0.184	2.628
8/3/2022	L4	20.3	2.19	4.81	2.96	2.57	0.178	3.615
8/3/2022	L5	5.66	2.66	5.02	3.11	2.51	0.225	1.276
8/3/2022	L6	9.28	1.86	4.43	2.87	2.51	0.193	1.796
8/3/2022	H4	14.1	3.58	6.11	3.41	2.55	0.240	3.387
8/3/2022	H5	7.89	2.35	4.95	3.03	2.57	0.195	1.544

8/3/2022	H6	16.51	2.65	4.6	3.16	2.59	0.215	3.551

Table II

Post-Trial Biomass Calculations					
Date	Label	A Section w/ Bag After Cook (g)	A Section **Bag Only** After Cook (g)	A Section Biomass (g)	Net biomass gains (g)
7/30/2022	C1	5.01	2.77	2.24	0.016
7/30/2022	C2	3.64	2.81	0.83	-0.056
7/30/2022	C3	4.17	3.14	1.03	-0.121
7/30/2022	L1	4.58	2.31	2.27	-0.750
7/30/2022	L2	4.47	2.63	1.84	-0.698
7/30/2022	L3	4.87	2.49	2.38	-0.313
7/30/2022	H1	4.07	2.56	1.51	-0.183
7/30/2022	H2	4.94	2.65	2.29	-0.531
7/30/2022	H3	3.86	2.56	1.3	-0.556
8/27/2022	C4	3.05	2.86	0.19	-0.048
8/27/2022	C5	7.16	3.2	3.96	0.385
8/27/2022	C6	5.49	2.75	2.74	0.111
8/27/2022	L4	6.46	2.38	4.08	0.464
8/27/2022	L5	4.08	2.7	1.38	0.103
8/27/2022	L6	3.98	2.54	1.44	-0.356
8/27/2022	H4	5.4	2.6	2.8	-0.587
8/27/2022	H5	4.05	2.63	1.42	-0.124
8/27/2022	H6	5.87	2.72	3.15	-0.401

Table III

			sum	avg
C1	0.016			
C2	-0.056	Trial 1: Control	-0.162	-0.054
C3	-0.121			
L1	-0.750			
L2	-0.698	Trial 1: Low	-1.761	-0.587
L3	-0.313			
H1	-0.183			
H2	-0.531	Trial 1: High	-1.272	-0.424
H3	-0.556			
C4	-0.048			
C5	0.385	Trial 2: Control	0.448	0.149
C6	0.111			
L4	0.464			
L5	0.103	Trial 2: Low	0.212	0.070
L6	-0.356			
H4	-0.587			
H5	-0.124	Trial 2: High	-1.112	-0.370
H6	-0.401			

*math in tables may not add up due to rounding to nearest three sig figs

Discussion

Before beginning Trial 1, the plant subjects were infected by an unidentified pest due to an increase in plant stress as a result of the compounding conditions of low sunlight exposure, unsatisfactory watering, and nutrient deficiencies. The treatment was non-invasive and eliminated the insects without harming the plant. The plant subjects were given three weeks to make a full recovery. Pre-trial root pruning was suspected as a cause of lower net growth in Trial 1, and as the trial progressed, signs of plant stress from days 2 to 7. Yellowing tips are a sign of plant stress; I aimed to adjust the study to yield stronger results in Trial 2. In Trial 1, the control pH group produced the greatest average net biomass values, and (C1) was the only subject in the trial to yield positive net gains. While both the Low and High pH groups had lower net gains, the acidic pH groups performed the worst of all subjects in Trial 1. Toward day 12, unidentified mold-like fungi appeared at the bases of subjects (L2) and (L3), as well as the entire high pH group, while the control group exhibited no such signs of stress. Fungi signals reduce plant immune strength and can be the result of increased plant stress and unsuitable abiotic conditions.[10] The low and high pH groups showed greater susceptibility to fungal diseases during Trial 1. Taking into consideration the end-of-trial plant health, adjustments were made to the study to allow for a longer growth period, greater root health, and more space for root growth to increase net values at the end of Trial 2.

While setting up Trial 2, three adjustments were made to the experiment: pre-trial plant treatment, trial soil conditions, and trial length. During plant care, minimal root pruning was done to reduce possible plant stress and susceptibility to fungal infections and diseases during the trial. Soil was filled to the three inch mark to maximize space for root growth and Trial 2's duration was extended by one week to yield more distinct results. In Trial 2, both the control and low pH groups were recorded for possible net biomass gains in subjects (C5),(C6),(L4), & (L5). Although the control group had the greatest average net biomass gains, subject L4 from the low pH group had the greatest net biomass in Trial 2. Signs of stress were present in subjects (C4), (L6), (H4),(H5),(H6) as well as small amounts of fungus. The high pH

group during Trial 2 had an especially high amount of fungus growth on the stem base, a possible sign of compromised immunity and/or stressful conditions.[10]

The results of both Trial 1 & 2 were slightly unexpected. While one subject (C1) of the Trial 1 control group produced a positive net gain in biomass, all eight other Trial 1 subjects showed negative net biomass gains. However, given the initial biomass measurements were based on estimations, measurements may have been slightly higher than the true biomass values due to incomplete evaporation and drying resulting in offset values. The time at dehydration, total time spent dehydrating, and air conditions when dehydrating were equal amongst all subjects and thus rules out differences in the rate of dehydration as a factor in higher estimations. A difference in humidity during the estimation period and the end-trial dehydration period may have played a role in the negative net biomass values and the incomplete dehydration of the initial trial estimations while completely dehydrating the end-of-trial subjects. When observing both trials, it is apparent that the average net biomass of the control pH group subjects was higher than both the lower and higher pH group subjects. In both trials, *C. nudata* performed better and sequestered more carbon in pH 7 conditions than in pH 5 & 9 conditions. Not only did the control group demonstrate greater rates of carbon sequestration, but they also demonstrated lower susceptibility to fungal diseases. It can then be inferred that *C. nudata* is less robust and efficient in wetland sites where abiotic conditions are more acidic or basic. From these results, I theorized two possible causes of the reduced sequestration were reductions in the plants' abilities to absorb nutrients and available energy to put toward growth.

Conclusion

Going into the study, I aimed to discover whether or not there was a relationship between wetland pH conditions and a versatile wetland plant species, *C. nudata*, a plant whose use has been established in wetland restoration projects. After observing eighteen subjects over the course of two months, I determined that low and high pH ranges reduce the plant species' ability to sequester carbon while increasing the likelihood of experiencing fungal infections. This is possibly a result of increased stress and/or reduced immune system strength. Study limitations include: small sample size, lack of study in a natural environment, pre-trial root pruning, bug infestations, and the affliction of fungal disease may have skewed the results. Although this study may shed some light on *Carex nudata's* performance in different pH conditions, more research is needed to study and learn about the interspecies and abiotic-biotic interactions in wetland ecosystems. I hypothesized that the acidic and basic conditions would impair growth or reduce the plants' ability to absorb nutrients, while subjects in neutral pH conditions would sequester the most carbon. The acidic and basic conditions could have resulted in more energy being used for survival in such conditions, therefore reducing the plant's ability to sequester carbon. Future research will hopefully support solutions for environments where conditions are evolving and becoming more acidic or basic than the baseline. Not only may future studies of wetland interactions allow for further understanding of interspecies and biotic-abiotic relationships, but also assist in the design of robust wetland ecosystems in more effective and sustainable ways.

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Appendix B

Experiment Water Log Trial 1		Experiment Water Log Trial 2	
Date	Details	Date	Details
7/13/2022	Measure & Tweak: Low pH: 4.97	8/4/2022	Measure & Tweak: Low pH: 5.00
7/13/2022	Measure & Tweak: Control pH: 6.99	8/4/2022	Measure & Tweak: Control pH: 7.00
7/13/2022	Measure & Tweak: High pH: 9.01	8/4/2022	Measure & Tweak: High pH: 8.95
(9:54pmPST) 7/13/2022	1000 mL water added to tubs	(10:00PST) 8/4/2022	800 mL added to tubs
(10:00pmPST) 7/13/2022	Trial 1 Start	(10:00PST) 8/4/2022	Trial 2 Start
7/20/2022	Measure & Tweak: Low pH: 4.99	8/11/2022	Measure & Tweak: Low pH: 4.97
7/20/2022	Measure & Tweak: Control pH: 6.97	8/11/2022	Measure & Tweak: Control pH: 7.03
7/20/2022	Measure & Tweak: High pH: 9.02	8/11/2022	Measure & Tweak: High pH: 8.97
(9:58pmPST) 7/20/2022	1000 mL water added to tubs	(10:09 PST) 8/11/2022	800mL added to tubs
(10:10pmPST) 7/27/2022	Trial 1 End	8/18/2022	Measure & Tweak: Low pH: 4.98
		8/18/2022	Measure & Tweak: Control pH: 6.96
		8/18/2022	Measure & Tweak: High pH: 9.00
		(10:08 PST) 8/18/2022	800mL added to tubs
		(10:10 PST) 8/25/2022	Trial 2 End