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Research Article

Carbon, Nitrogen, and pH Analysis in Bulk Soil and Rhizosphere Samples in CSUSM Wetlands

Stacey Saldana[1,*], Joseph Rocha[1], Elinne Becket[1], and George Vourlitis[1]

[1] Department of Biological Sciences, California State University San Marcos
* Correspondence: salda059@csusm.edu

Abstract: Differences in vegetation composition can significantly impact soil carbon (C), nitrogen (N), and pH levels. The purpose of the current study was to measure how C, N, and pH levels vary with bulk and rhizosphere wetland soil samples at California State University San Marcos. The procedure entailed randomly collecting samples from the upper 20 cm soil layer from the three main vegetation types in the wetland: lowland (L), riparian (R), and sedge (S). From each site, eight bulk samples were collected, and eight rhizospheric samples were extracted from the bulk soil (n = 24). All of the samples were then isolated, dried, ground to a fine powder, then analyzed using both a CHN analyzer via dry combustion, and a pH meter. Statistical analysis was conducted through Jamovi, and graph creation was utilized through Excel. Non-parametric tests were conducted on data samples where transformations did not accommodate for normality. Results of the study showed that our hypotheses were partially true: though we were correct that the rhizosphere did have a higher C% compared to bulk soil, significance of this difference was only found in L and S, but not for R. Similarly, though we found that the rhizosphere was more acidic compared to bulk soil, significance of this difference was only found in L and S, but not for R. Finally, our results showed that there was no significance in average N% between L, R, and S in either rhizosphere or bulk soils. These findings may help to better understand the processes involved for successful agricultural practices, namely if in the possible future, there could be technology utilized to manipulate carbon, nitrogen, and pH variations in soil.

Though accounting for less than 9% of the Earth's terrestrial surface, wetlands possess significant levels of biodiversity and provide a myriad of benefits to people, including soil erosion protection and regulating water purification (Meli et al. 2014). While occupying a relatively small portion of the Earth's surface, wetlands also wield a significant influence on global carbon cycling due to their carbon sequestration abilities in which they are highly effective at trapping and storing carbon in their soils (Schlesinger & Bernhardt 2013). Furthermore, though they only comprise approximately 7-15% of terrestrial productivity worldwide, wetlands serve as essential global carbon reservoirs, collectively possessing over half of the planet's soil carbon (Schlesinger & Bernhardt 2013). These statistics emphasize the critical role wetlands play in regulating global carbon balance, as well as their importance in climate change mitigation efforts.

Wetlands provide an optimal environment for denitrification by effectively reducing reactive nitrogen levels, and facilitating the retention of nitrogen within organic matter (Schlesinger & Bernhardt 2013). However, they also act as significant contributors of dissolved organic matter, including organic nutrients, to downstream and coastal ecosystems (Schlesinger & Bernhardt 2013). Despite the substantial denitrification potential in wetlands, accurate estimates of their contribution to global N2O emissions remain unknown (Schlesinger & Bernhardt 2013). Historically, however, it has been speculated that N2O production rates are substantially lower in wetlands compared to upland soils (Schlesinger & Bernhardt 2013).

The impact of pH on soil microbial communities cannot be understated- namely the influence pH has on the structure, diversity, and distribution of these systems- including

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the survival and growth of different microbial taxa, as well as nutrient availability (Lopes et al. 2021). However, there remains some gaps in understanding how soil pH specifically correlates with microbial habitats surrounding plant roots, like the rhizosphere (Lopes et al. 2021). In cases such as Yang et al. (2012), however, it was found that pH levels in rhizosphere soils were lower than that of non-rhizosphere soils when examining emergent-rooted wetland plants. In another study, pH measurements were conducted to assess variations around the lateral root of wetland plants, and it was observed that the pH near the surface of the lateral root was relatively acidic in contrast to the pH of the surrounding bulk solution (Bezbaruah & Zhang 2004). As the distance from the root surface increased radially outwards, there was a gradual elevation in pH values, indicating that pH values were higher in the rhizosphere compared to the bulk soil (Bezbaruah & Zhang 2004).

California State University San Marcos (CSUSM) wetlands have been studied in recent years: Maziarz et al. (2019) wanted to compare the function of CSUSM wetlands to other local wetlands in Southern California. This included analyzing carbon (C) and nitrogen (N) stocks for each respective wetland (Maziarz et al. 2019). The authors found that CSUSM wetlands had higher soil C and N pools than the local marshes they were being compared to (Maziarz et al. 2019). The authors also noted that when it comes to C and N storage, hydrology- which focuses on the movement, distribution, and properties of water and how it can impact soil systems- is often an important variable that can affect these systems (Maziarz et al. 2019). Finally, Maziarz et al. (2019) utilized sedge and riparian vegetation as references to compare to some of the same vegetation types found in CSUSM wetlands.

With little research available on comparing the chemical differences between bulk and rhizosphere soils, the purpose of this study was to measure how C, N, and pH levels vary between bulk and rhizosphere wetland soil samples at CSUSM. These values would also be compared between three different CSUSM wetland vegetation types: lowland (L), sedge (S), and riparian (R). Based on results from the aforementioned studies, we hypothesized that the rhizosphere samples were going to have higher C% and N% levels compared to the bulk soil samples. We also hypothesized that the pH would be more acidic in the rhizosphere samples compared to the bulk soil samples.

Methods

Site Description

The CSUSM wetland is located in the northwest corner of the university: it measures 325 m long and approximately 30-50 m wide, and has 0.42 ha of coastal sage in the perimeter (Joshi & Walsh 2001). The construction of the wetland began in 2001 and was completed three years later in 2004 (Joshi & Walsh 2001). CSUSM wetland vegetation is dominated by *Iva heyesiana, Juncus acutus, Salix lasiolepis, Baccharis salicifolia,* and *Leymus condensatus* (Maziarz et al. 2019). Following the completion of the wetland, a five-year maintenance period was set to maintain it, and in 2006, though the irrigation to the wetland stopped, monitoring continued until 2009 (Maziarz et al. 2019). The composition of the first 10 cm of the soil present in this wetland is sand, silt, and clay, and has a bulk density of 1.43 g/cm^3 in the 20 cm soil layer (Maziarz et al. 2019)

Sample Design

Soil samples were collected using a stratified-random sampling design from the CSUSM wetland habitat. The samples were collected from the first 20 cm of the soil layer of three main vegetation types in the wetland, and eight samples were collected per vegetation type (n = 8 samples per site). The samples were collected from each site- lowland (L), riparian (R), and sedge (S)- using a 5 cm PVC soil Corer with a volume of 393 cm^3. The striated random sampling was conducted by splitting the wetland into eight, 40 m long sections and randomly selected sites for collection within the sections. The stratification was by vegetation type L, R, and S.

Sample Processing

The soil samples were sealed in Ziplock bags and taken to the laboratory for processing. The samples were then sieved through a 2 mm sieve for dry samples and through 4 mm sieves for wet samples to homogenize the soil sample composition. Bulk soil samples were stored, and samples containing roots were isolated for rhizosphere sample types. Soil samples consisted of 24 bulk soil samples and 24 rhizosphere samples. Samples were air-dried to remove moisture, and bulk soil samples were ground into a fine powder using mortar and pestle. The rhizosphere samples were further sieved after air-drying to extract soil from roots. The collected soil from each respective rhizosphere sample was then ground into a fine powder for further analysis.

pH processing

pH analysis was conducted using deionized water (DI water). The ratio that the sample was measured in was 2:1 DI water to sample. From the 24 bulk soil samples, 15 g were collected and 30 mL of DI water were added to each sample. The soil solution was mixed homogeneously and set to rest for 30 minutes. While incubating the sample for 30 minutes, the pH meter was calibrated using a neutral solution of pH 7. Once the 30 minutes had passed, these samples were mixed homogeneously once more and their pH was measured. The same process was done with the rhizosphere samples using the same ratio for the DI water to the sample. pH analysis for rhizosphere samples less than 1 g followed the 2:1 ratio for the DI water: sample.

Total C:N

Total nitrogen and carbon were measured with a carbon, hydrogen, and nitrogen elemental analyzer (CHN). The rhizosphere and bulk soil samples were isolated and air dried. Afterwards, the rhizosphere samples were sieved using a 2 mm sieve to remove inorganic rocks and roots and then ground to a fine powder with the use of a mortar and pestle. The bulk soil samples were sieved using a 2 mm sieve, and also ground to a fine powder via mortar and pestle. Once both samples were dried, sieved, and homogenized, they were prepared by weighing a range within 10-18 mg in a weighed tin sample cup. As mentioned previously, the bulk soil samples were prepared for the CHN analyzer. Once the adequate mass was collected, the tin cup was secured for CHN analysis.

Data Analysis

All data collected were analyzed using the program Jamovi for statistical analysis, and Excel for visualization. An independent samples t-test was utilized with an α: 0.05 on the sites for data analysis. Prior to this, the data was checked for normality using Shapiro-Wilkes (SW) test for normality and Levene's test for homogeneity of variance (HOV). Once Levene's test was conducted on the C:N data, L-site nitrogen % data for bulk soil was found to violate SW ($p = 0.002$). To accommodate for the violations of normality, log transformations were performed, violations were still present, and then non-parametric Welch's t-tests were conducted. Carbon and nitrogen rhizosphere sites L, R, and S did not violate SW ($p > 0.05$). Site L carbon bulk soil along with R and S bulk soil samples passed SW ($p > 0.05$). All sites reported passing SW normality check in pH analysis ($p > 0.05$). The mean and standard error were calculated as well to visually represent data. In summary, the L-site nitrogen % violation of normality was accommodated by non-parametric Welch's t-test after the log transformation failed to remediate the normality.

Results

C:N

For C%, site L and S independent t-tests reported statistical significance between the bulk and rhizosphere in each site except for R (**Fig. 1**). L-site reported t-stat = -2.208, df = 11.4, and $p = 0.049$ (with a mean difference of – 0.539). L-site rhizosphere data showed 2.092 ± 0.211 %; mean ± SE, and 1.552 ± 0.123 % for bulk soil. R-site independent sample

t-test reported t-stat = -0.812, df = 14, and p = 0.431 (with a mean difference of -0.425). R-site mean and SE reported 2.489 ± 0.356 % for rhizosphere, and 2.063 ± 0.384 % for bulk soil data. The S-site reported t-stat = -2.16, df = 11.8, and p = 0.052 (with a mean difference of – 0.590). S-site rhizosphere data showed 2.223 ± 0.230 %, and 1.632 ± 0.145 % for bulk soil data.

In N%, no sites reported statistical significance in the independent samples t-tests. Site L reported t-stat = 0.431, df = 10.7, and p = 0.675 (with a mean difference of 0.0123) (**Fig. 2**). Rhizosphere data showed 0.108 ± 0.135 %, and 0.121 ± 0.0250 % for bulk data. Site R t-test reported t-stat = -1.416, df = 14, and p = 0.179 (with a mean difference of – 0. 0606). Rhizosphere data showed 0.209 ± 0.0358 %, and 0.148 ± 0.0234 % for the bulk data. S-site t-test reported t-stat = 1.43, df = 14, and p = 0.174 (with a mean difference of 0.0232). Rhizosphere data showed 0.0725 ± 0.0117 %, and 0.956 ± 0.111 % for bulk data.

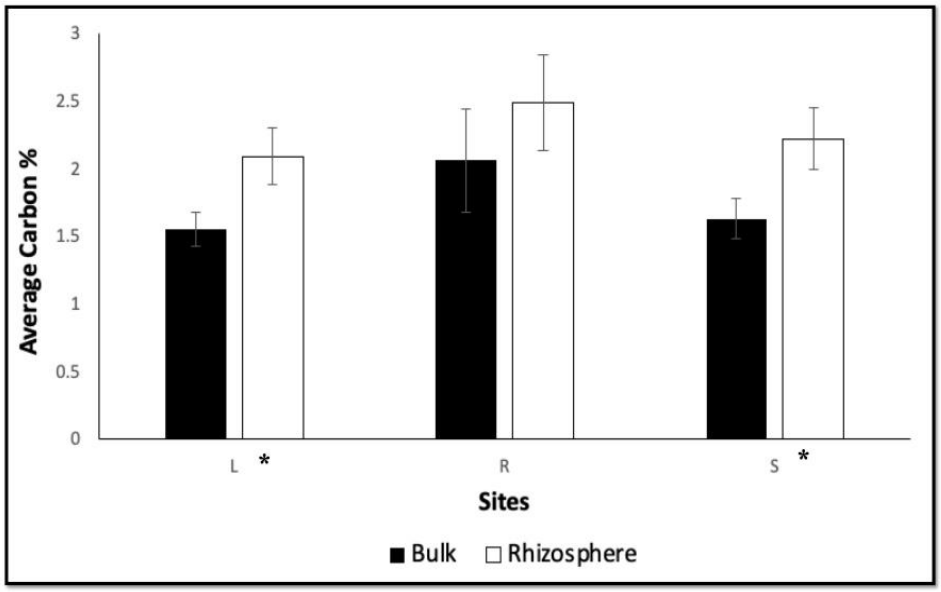

Figure 1. Figure 1 above depicts the average C% from bulk and rhizosphere soil types (mean ± standard error) for different sites L, R, and S. Significant sites were noted for sites L and S (n = 8 for each site).

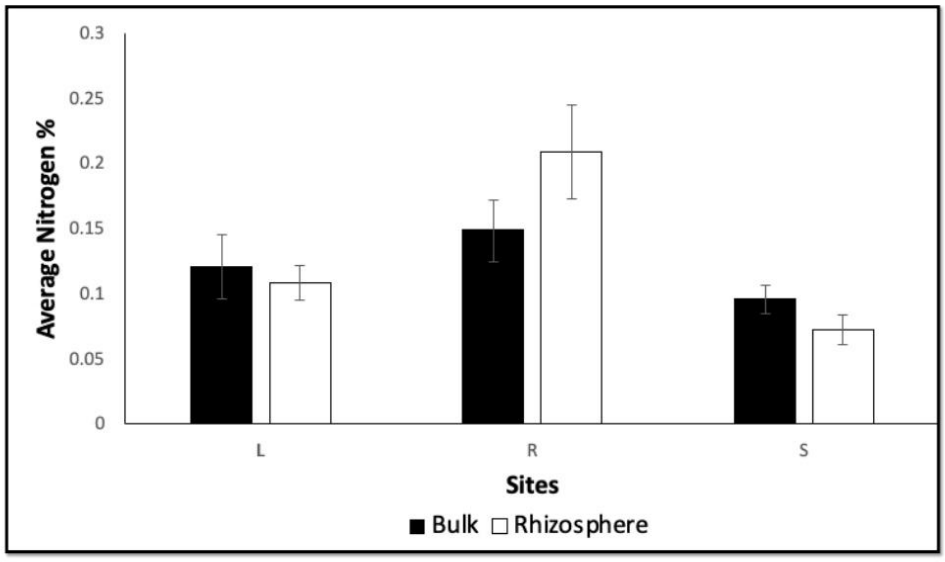

Figure 2. Figure 2 above depicts the average N% from the bulk and rhizosphere soil types (mean ± standard error) for different sites L, R, and S (n = 8 for each site). No significant sites were found here.

pH

For pH, site L and S reported statistical significance with independent samples t-test, but no significance was reported for site R (**Fig. 3**). Site L t-test reported t-stat = 6.25, df = 14, and p <0.001 (with a mean difference of 0.558). L-site Rhizosphere data showed 7.69 ± 0.0620, and 8.24 ± 0.0641 for bulk soil data. R-site reported t-stat = 1.0, df = 8.0, and p = 0.346 (with a mean difference of 0.343). R-site rhizosphere data showed an average of 7.21 ± 0.0887, and 7.56 ± 0.331 for bulk soil data. S-site t-test reported t-stat = 2.19, df = 14, and p = 0.046 (with a mean difference of 0.483). Rhizosphere S-site data showed 7.61 ± 0.134, with 8.09 ± 0.175 for bulk soil data.

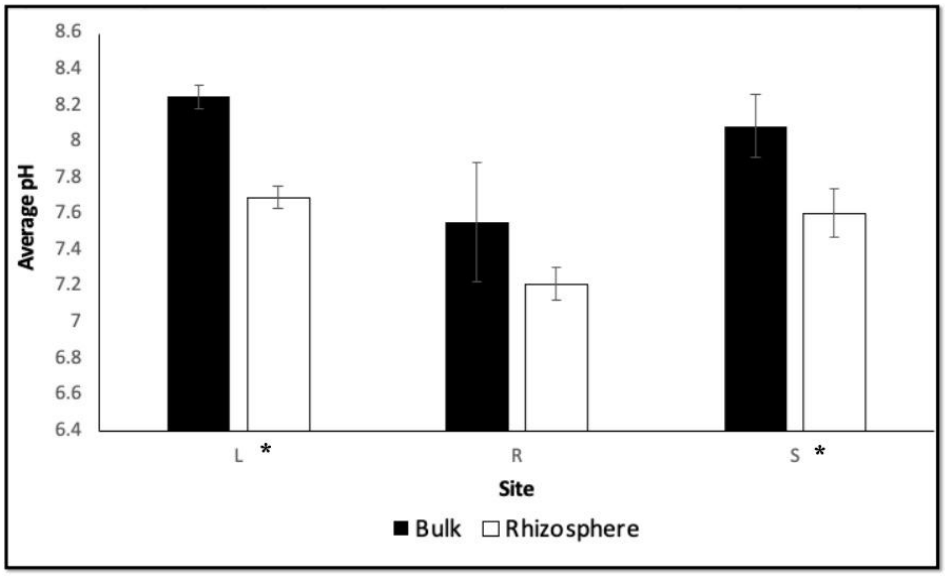

Figure 3. Figure 3 above depicts the average pH from bulk and rhizosphere soil types (mean ± standard error) for different sites L, R, and S. Significant sites were noted for sites L and S (n = 8 for each site).

Discussion

We hypothesized that the rhizosphere was going to have higher C% and N% levels compared to bulk soil, and that the pH would be more acidic in the rhizosphere compared to the bulk soil. Our results showed that these hypotheses were partially true: though we were correct that the rhizosphere did have a higher C% compared to bulk soil, significance of this difference was only found in L and S, but not for R (**Fig. 1**). Similarly, though we found that the rhizosphere was more acidic compared to bulk soil, significance of this difference was only found in L and S, but not for R (**Fig. 3**). It should be noted that although no significance was found for R for C% and pH between bulk and rhizosphere soils, all vegetation types were trending in the same direction. Finally, our results showed that there was no significance in average N% between L, R, and S in either rhizosphere or bulk soils (**Fig. 2**).

Similar results to the current study were found by Yang et al. (2012), who found that rhizosphere soils were more acidic than that of non-rhizosphere soils when examining emergent-rooted wetland plants. Moreover, Bezbaruah and Zhang (2004) found in their study of wetland plants that the distance from the root surface increased radially outwards, leading to a gradual elevation in pH values and showing that pH values were higher in the rhizosphere compared to the bulk soil. When studying CSUSM wetlands, Maziarz et al. (2019) found high soil C storage in the data collected for their research. However, they also found high N storage in their study, which we did not find when studying the same vegetation types in the same area (Maziarz et al. 2019).

One explanation as to why N% did not report significance may be due to the large amounts of interannual variation that was present (Maziarz et al. 2019). Another factor that could have impacted the results may have been the change in hydrology to the wetland. During the week of sample acquisition, an increase in rainfall patterns led to flooding in the CSUSM wetland. This rainfall continued during the day of sample collection as well. With the high amounts of water, saturation introduced to the soil could have had an impact on the carbon, nitrogen, and pH compositions. Leaching can occur because of this, which in turn causes the removal of nitrogen from the environment in the form of nitrate-nitrogen (Poiani et al. 1996). Leaching is when soluble materials are drained from the soil and, in this case, it is possible that the change in hydrology could have increased the leaching of the nitrogen from the soil, causing the nitrogen to leach below our 20 cm sample collection threshold. Additionally, it may be possible that runoff from higher lands may have deposited nitrogen from certain fertilizers.

One discrepancy that occurred with the R-site samples was that the riparian samples had small roots and extraction. This seems as expected, due to the vegetation composition of the riparian site being mainly *B. salicifolia* and *S. lasiolepis.* Both species' root depth would average 0.65 m for *B. salicifolia* perennial vegetation, and 3.1- 4 m for the *S. lasiolepis* (Stromberg 2013). This could explain and account for the lack of rhizosphere samples in the sampling pool for the riparian site due to our sample collection depth being 20 cm. Riparian sample turnout would be less than 1 g, causing difficulty in measuring pH properly.

Other discrepancies in our study pertain to our rhizosphere samples, namely the validity and availability of our samples. When working through the methods of our experiment, we noticed many of our rhizosphere samples had the appearance of sticks and arguably did not look to be roots. However, we decided to carry out this experiment regardless and trusted that the samples were indeed rhizospheres and not anything else. Additionally, the sample size of the rhizospheres was small (eight samples for each site; n = 24), which may have impacted our results. Future studies wanting to replicate this study may consider working with a larger sample size as a means of augmenting the validity of their results. Other considerations would be to make this study into a longitudinal one, where sample acquisition would be over a period of a year or more- instead of one day- to account for biotic and abiotic variations. Refinement of rhizosphere sample collection would also help benefit in increasing mass for analysis and account for the variability in vegetation type root depths.

These findings may help to better understand the processes involved for successful agricultural practices, namely if in the possible future, there could be technology utilized to manipulate carbon, nitrogen, and pH variations in soil. Conditions could be created to favor microorganisms to promote nitrogen fixation, carbon sequestration, and microbial diversity. Future endeavors could involve further investigating the correlation between microbial communities and abiotic factors, such as nitrogen, carbon, and pH composition, in wetland soils.

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