Title: Microbial community structure corresponds to nutrient gradients and human impact within coastal wetlands of the Great Lakes.

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Originality-Significance Statement

This research is original in providing an initial, geographically wide-ranging characterization of microbial communities of the Great Lakes coastal wetlands, an understudied system of wetlands directly bordering the North American Laurentian Great Lakes. Relationships between microbial communities within coastal wetland soils and geochemistry highlighted that anthropogenic impacts may be significantly influencing microbial community structure, as well as unique subnetworks of microbial taxa. Negative anthropogenic impacts to these coastal wetland communities could influence natural biogeochemical cycles which occur within coastal wetland soils, and by extension would directly influence the Great Lakes themselves.

Summary

Microbial communities within the soil of Great Lakes coastal wetlands drive biogeochemical cycles and provide several other ecosystems services. However, there exists a lack of understanding of how microbial communities respond to nutrient gradients and human activity in these systems. This research sought to address this lack of understanding through exploration of microbial community diversity and networks among coastal wetlands throughout the Great Lakes. Significant differences in microbial community structure were illuminated between Lake Erie and all other wetlands, and chemical and biological structure did not vary within Lake Erie with increasing soil depth. Beyond this, alpha diversity levels were highest within Lake Erie coastal wetlands. These diversity differences coincided with higher nutrient levels within the Lake Erie region. Site-to-site variability existed within Lake Erie, East and North Saginaw Bay regions, suggesting site-scale heterogeneity may impact microbial community structure. Several subnetworks of microbial communities and individual OTUs were

related to chemical gradients among wetland regions, revealing several candidate indicator communities and taxa which may be useful for Great Lakes coastal wetland management. This research provides an initial characterization of microbial communities among Great Lakes coastal wetlands, and demonstrates that microbial communities could be negatively impacted by anthropogenic activities.

Introduction

The Laurentian Great Lakes of North America are one of the largest freshwater systems on Earth, and are critical in supporting biogeochemical cycles, freshwater resources, biodiversity, and economic viability of the surrounding region. Coastal wetlands bordering the Great Lakes are integral in sustaining the proper functioning of the Great Lakes themselves, making up nearly 200,000 ha of habitat between the United States and Canada despite reduction of habitat by approximately 50% since European colonization (Dahl, 1990; Hecnar, 2004; Sierszen *et al.*, 2012). In addition, Great Lakes coastal wetlands are vital in the retention of chemical pollutants (e.g., heavy metals), sediments, and excess nutrients (e.g., N and P) caused by anthropogenic activity, and provide critical habitat for countless biological species (Wang & Mitsch, 1998; Sierszen *et al.*, 2012). The economy of the Great Lakes is contingent on the existence and proper functioning of coastal wetlands. In providing ecosystem services and promoting biodiversity, these wetlands have an estimated annual worth of $69 billion USD; the value of recreational fishing alone is valued at $7.4 billion USD per year (Krantzberg & de Boer, 2008; Campbell *et al.*, 2015). Notably, the Great Lakes region has been impacted by negative anthropogenic pressure, with cumulative stress having a particular impact on the western basin of Lake Erie (Danz *et al.*, 2007; Uzarski *et al.*, 2017). These negative impacts extend to the coastal

wetlands, as pressure from agricultural runoff, atmospheric deposition, and urbanization influence water chemistry, thereby reducing water quality and impairing wetlands (Trebitz *et al.*, 2007; Morrice *et al.*, 2008). This has stoked a surge in research assessing biodiversity and anthropogenic pressure on coastal wetlands of the Great Lakes since the Great Lakes Water Quality Agreement (GLWQA) was established in 1972 (Hackett *et al.*, 2017). While much research on coastal wetlands has flourished in the wake of this international agreement, microbial communities within Great Lakes coastal wetlands remain almost entirely uncharacterized (Hackett *et al.*, 2017).

As ecological transition zones between upland and aquatic environments (Uzarski, 2009), wetlands host a unique suite of chemical cycles while providing ecosystem services to bordering environments. Most notably, carbon mineralization occurs within wetland soils via redox processes mediated by microbial communities, and these processes contribute to pollution mitigation and atmospheric greenhouse gas flux (Conrad, 1996; Reddy & DeLaune, 2008). Wetland soil often becomes chemically structured with increasing depth through sequential reduction of electron acceptors that decrease in metabolic favorability to microbes due to thermodynamic constraints (Conrad, 1996; Reddy & DeLaune, 2008; Kögel-Knabner *et al.*, 2010). As microbial community metabolism will change in concert with soil chemical profiles, microbial community structural shifts commonly result (Lüdemann *et al.*, 2000; Edlund *et al.*, 2008; Lipson *et al.*, 2015). However, concentration of carbon electron donors can influence the vertical stratification of redox processes (Achtnich *et al.*, 1995; Alewell *et al.*, 2008), and by extension, vertical microbial community structure (defined as relative proportions of microbial taxa within a community). As an example of how this may apply to natural environments, increased nutrient influx from anthropogenic activities (such as agricultural pressure) may

impact microbial community structure within coastal wetlands, which may extend to redox processes. While sequential reduction chains and vertical microbial community structuring can be predictable in lab experiments, complex heterogeneity within wetland soil can often influence microbial community processes and structure to a large degree (Alewell *et al.*, 2008; Lamers *et al.*, 2012). As such, it is relevant to understand relationships between microbial community structure and vertical soil depth *in situ* along a gradient of nutrient input, as microbial community beta diversity could be indirectly linked to carbon mineralization.

As the microbial communities within Great Lakes coastal wetlands have yet to be fundamentally described, it is of importance to gather baseline data on what microbes exist within these systems, elucidate how these microbes could be interacting, and determine to what extent microbial diversity may already be impacted by anthropogenic chemical disturbance. Additionally, if specific groups of microbial taxa are found to be predictive of environmental gradients, this could aid in understanding specific impacts of anthropogenic activity in generating these gradients and selecting for specific microbial networks or taxa (Sims *et al.*, 2013; Urakawa & Bernhard, 2017). This study sought to provide an initial characterization of microbial communities within soils of Great Lakes coastal wetlands bordering the western basin of Lake Erie, Saginaw Bay of Lake Huron, and northern Lake Michigan. Additionally, this study explored how environmental conditions of these coastal wetlands could act as potential drivers of microbial community structure among and within wetlands. It was predicted that microbial community structure would be related to environmental gradients among and within coastal wetland regions of the Great Lakes. Further, it was anticipated that high nutrient levels within wetlands would decouple the relationship between microbial community structure and soil depth, as has been suggested in previous studies (Achtnich *et al.*, 1995; Alewell *et al.*, 2008). Through

high-throughput sequencing of the 16S rRNA gene and network analyses, variations in key microbial taxa and subcommunities related to environmental gradients established by wetlands were identified.

Results

Chemical analyses

Significant correlations ($r > 0.7$, $p \leq 0.001$) were found among NH_4^+, OM, OC, and TN. Thus, downstream analysis combined these values into one parameter, "NUTR", represented by OM values as this variable was the most strongly correlated with each of the other variables. PC1 and PC2 explained 56.2% and 20.6% of the variation among samples, respectively (Fig. 1). MANOVA found significant differences in physicochemical profiles based on region ($F = 2.71$, $p \leq 0.001$) and depth ($F = 6.85$, $p \leq 0.01$). Ninety-five percent confidence intervals suggested that significant variation among regions was driven primarily by physicochemical distinctness of Lake Erie coastal wetlands. This separation was related to increased NUTR, NO_3^-, and S.

Increasing depth within cores showed a consistent shift in environmental variables, except those sites located in the western basin of Lake Erie (Supplemental Fig. 1). Specifically, OM, OC, and TN consistently decreased with increasing depths within all wetlands except Lake Erie sites. Similarly, C:N increased with depth in all wetlands except Lake Erie sites, where C:N ratio remained relatively low (~ 12) and stable with increasing soil depth. Within the Lake Erie wetland region, pH was more acidic in the overlying water with respect to all other wetland regions (Supplemental Table 1). However, pH was still relatively neutral within Lake Erie (average pH = 7.26, standard deviation = 0.24), where other wetland regions experienced slightly more basic pH, with averages ranging between 7.72 – 8.39.

Alpha Diversity

Sufficient depth of sampling was also reinforced by rarefaction curve analysis (Supplemental Fig. 2). Good's coverage reported averages ranging between 89.3 – 93.5% for each region at the subsampled value of 48,226 sequences. Chao 1 richness estimates varied significantly among wetland regions ($F = 8.38$, $p \leq 0.05$), as well as wetland sites ($F = 16.78$, $p \leq 0.001$). Pairwise comparisons found that the LE region had significantly higher ($p \leq 0.01$) Chao1 estimates than NSB and WSB regions (Fig. 2; Supplemental Table 2). Additionally, pairwise comparisons found a high degree of significant variability ($p \leq 0.01$) in Chao1 estimates among wetland sites (Supplemental Table 2). Further, Shannon diversity levels also significantly varied among wetland sites ($F = 4.57$, $p \leq 0.001$), with site LE_D having significantly higher ($p \leq 0.01$) Shannon diversity levels than sites ESBT_A and WSB_B (Supplemental Table 2). Soil depth was not found to be significant in influencing alpha diversity levels.

Shannon diversity and Chao1 both significantly correlated with measured environmental variables (Table 1). Specifically, Chao1 estimates increased with NO_3^-, P, and S concentrations ($p \leq 0.01$), and were weakly positively correlated ($p \leq 0.05$) with NUTR. Additionally, Shannon diversity levels increased alongside NUTR and S ($p \leq 0.001$), and were weakly positively correlated with NO_3^- ($p \leq 0.05$). There were no significant relationships between alpha diversity and C:N, and there were no negative relationships between alpha diversity and any of the measured environmental variables.

Beta Diversity

Beta diversity among regions

NMDS demonstrated separation of microbial communities based on wetland site, region, and soil depth (Fig. 3). perMANOVA demonstrated that differences in microbial community structure were highly significantly related to wetland site (pseudo-F = 2.57, $p \leq 0.001$), region (pseudo-F = 5.91, $p \leq 0.001$), and soil depth (pseudo-F = 6.35; $p \leq 0.001$). Post-hoc pairwise perMANOVA found that community structure within the LE region was significantly distinct ($p \leq 0.01$) from all other wetland regions (Table 2). No significant differences in community structure were found between any other wetland regions compared. Additionally, microbial community beta diversity was distinct ($p \leq 0.003$) between the top soil depth and the middle and bottom soil depths. However, no significant differences in microbial community structure were found between the middle and bottom soil depths (Table 2). Variation in microbial community structure was strongly significantly correlated ($p \leq 0.001$) to depth ($r = 0.41$), NO_3^- ($r = 0.20$), NUTR ($r = 0.60$), and S ($r = 0.41$), and weakly correlated ($p \leq 0.016$) with C:N ($r = 0.11$) and P ($r = 0.14$) (Supplemental Table 3).

Beta dispersion tests suggested weakly significant variation in structural variance among regions ($p \leq 0.05$), however, Tukey's HSD test using adjusted p-values for multiple comparisons did not find any significance ($p > 0.05$) between pairwise comparisons of regional groups. There were no differences in community structural dispersion among soil depths.

Beta diversity within regions

Individual NMDS plots of each region identified a high degree of variability of significant correlations ($p \leq 0.05$) between microbial community structure and several environmental variables, as well as depth, dependent upon the wetland region explored (Fig. 4; Supplemental Table 3). Depth was significantly correlated with microbial community structure

in all wetland regions except NSB and LE. However, microbial community structure was more strongly related to depth in NSB ($r = 0.35$, $p = 0.071$) than LE ($r = 0.19$, $p = 0.40$). NUTR was significantly correlated ($p \leq 0.01$) with community structure within regions BA ($r = 0.82$), ESBT ($r = 0.51$), and LE ($r = 0.66$). C:N was significantly correlated ($p \leq 0.01$) with community structure within regions within Saginaw Bay (i.e., ESBT [$r = 0.65$], NSB [$r = 0.58$], and WSB [$r = 0.58$]). Beta diversity was not significantly associated with concentrations of NO_3^- in any region.

To test for significant differences in microbial beta diversity within regions, perMANOVA was used to evaluate differences in microbial community structure among soil depths and sites within wetland regions (Supplemental Table 3). Depth did not significantly explain microbial community structure within the region LE (pseudo-F = 0.87; $p = 0.65$), however, it did explain differences in microbial community structure within the other wetland regions. Specifically, BA (pseudo-F = 2.12; $p = 0.006$), ESBT (pseudo-F = 2.49; $p = 0.001$), NSB (pseudo-F = 1.24; $p = 0.093$), and WSB (pseudo-F = 1.53; $p = 0.014$). Significant differences in microbial community structure were found among different wetland sites within regions ESBT (pseudo-F = 3.11; $p = 0.001$), LE (pseudo-F = 2.89; $p = 0.004$), and NSB (pseudo-F = 2.16; $p = 0.003$). As only one site was sampled within the BA region, testing for differences among wetland sites within the BA region could not be accomplished.

Network Analyses

Weighted Correlation Network Analysis (WGCNA) was used to explore strong relationships between subcommunities and individual OTUs with environmental parameters within Great Lakes coastal wetlands. After removal of OTUs which did not have at least two

representative sequences in at least 10% of samples, a total of 7,562 OTUs remained for WGCNA. In determining scale-free topology of the OTU network, a soft power threshold of 4 was reached, and an R^2 of 0.87 was established as linear fit from the regression of the frequency distribution of node connectivity against node connectivity (Supplemental Fig. 3). Of the 33 constructed subnetworks, the same one (subnetwork "orange") was found to be most strongly correlated to both NUTR ($r = 0.94$) and NO_3^- ($r = 0.55$) (Supplemental Fig. 4). A separate subnetwork ("pink") was strongly correlated ($r = 0.74$) to C:N. All correlations of subnetworks to environmental variables were significant ($p \leq 0.001$). OTU VIP values ≤ 1 were removed due to the large amount of OTUs within subnetworks correlated with C:N for visualization purposes.

For subnetwork relationships to NUTR (including OM, OC, NH_4^+, and TN), partial least square analysis (PLS) found that 69 OTUs were 93.8% predictive of variance in NUTR (Supplemental Fig. 5). OTU co-correlation networks were constructed using an OTU co-correlation threshold of 0.25, with strong correlations ($r > 0.59$) between all OTUs and NUTR (Fig. 5). Of the top 15 OTUs contributing to PLS regression by VIP score, seven were related to *Betaproteobacteria*, five were related to *Anaerolineaceae* (within *Chloroflexi*), and one representative OTU was related to each of *Bellilinea* (*Chloroflexi*), *Desulfobacterales* (*Deltaproteobacteria*), and *Rhizobiales* (*Alphaproteobacteria*).

For subnetwork relationships to C:N, PLS found that 144 OTUs were 59.0% predictive of variance in C:N (Supplemental Fig. 6). Networks were constructed using an OTU co-correlation threshold of 0.1, within positive or negative correlations ($r > +/- 0.2$) between OTUs (VIP > 1) and C:N (Fig. 6). Of the top 15 OTUs by VIP score within the network, two OTUs related to *Bacteroidetes* were negatively correlated with C:N. Other top OTUs were positively related to

C:N, including seven OTUs related to *Anaerolineaceae*, four OTUs which were unclassified *Bacteria*, and one representative OTU related to each of *Bacillus* (*Firmicutes*) and *Chloroflexi*.

While a correlation strength of r = 0.55 was found between a subnetwork and NO_3^- concentrations, PLS did not find a strong relationship between predicted values and actual values of NO_3^- (Supplemental Fig. 7). As such, individual OTUs were correlated to NO_3^- (Fig. 7) and were further explored through this method rather than WGCNA. Correlation strength to NO_3^- ranged from +/-.37 to +/-.55 for positive and negative correlations, respectively. OTUs related to *Anaerolineaceae* simultaneously were most positively and most negatively related to NO_3^- concentration. OTUs related to *Acidobacteria*, *Chlorobi*, *Bacteroidetes*, *Proteobacteria*, OP11, and *Verrucomicrobia* were predominantly positively related to NO_3^-. OTUs related to *Actinomycetales* (*Actinobacteria*) and *Euryarchaeota* were negatively related to NO_3^-.

Discussion

Great Lakes coastal wetlands are biological diversity hotspots that mediate fluxes of biogeochemical cycles, as well as buffer the Great Lakes themselves from terrestrial pollutants. Despite the importance of coastal wetland microbial communities in mediating many of these ecosystem services, a lack of fundamental information on these communities has hindered full understanding of these critical environments (Hackett *et al.*, 2017). Previous research on microbial communities in Great Lakes coastal wetlands has focused on the use of microbial enzymatic assays as a tool to explore decomposition rates and nutrient limitation in wetlands (Jackson *et al.*, 1995; Hill *et al.*, 2006). This study is the first to deeply characterize microbial communities among multiple regions across the Laurentian Great Lakes basin, while simultaneously delineating the importance of environmental conditions in structuring these

microbial communities. Additionally, this study highlights important keystone subcommunities of potentially interacting OTUs, which may serve as indicators of ecosystem health.

Microbial diversity driven by chemistry within Great Lakes coastal wetlands

Taxonomic groups such as plants, birds, fish, and invertebrates within the Great Lakes coastal wetlands have been impacted by human practices within the Great Lakes watershed (Howe *et al.*, 2007; Tulbure *et al.*, 2007; Uzarski *et al.*, 2009; Cooper *et al.*, 2012; Uzarski *et al.*, 2017). This study is the first to establish that these patterns appear consistent with microbial communities in these ecosystems as well. Microbial community structure was significantly dissimilar between LE and all other wetland regions. It is therefore likely that anthropogenic stressors related to nutrient loading (and potentially other pollutants) are driving these trends, as nutrient levels were substantially elevated within LE sites. Likewise, Lake Erie costal wetland sites had distinct microbial communities. Previous research has found that nutrient levels (e.g., C, N, P, etc.), to varying degrees, can influence microbial community composition and structure (Hartman *et al.*, 2008; Peralta *et al.*, 2013; Ligi *et al.*, 2014; Arroyo *et al.*, 2015). Indeed, PCA and MANOVA found that Lake Erie coastal wetlands were chemically distinct from all other wetland regions, primarily driven by elevated nutrient (C, N, P), NO_3^-, and S concentrations within the soil. Additionally, Lake Erie coastal wetlands (and the watershed which drains into them) have been historically impacted by anthropogenic pollution and agricultural practices, particularly in comparison to other coastal wetlands within the Laurentian Great Lakes region. This has been demonstrated by multiple ecological indices (e.g., Cvetkovic & Chow-Fraser, 2011; Uzarski *et al.*, 2017) and physicochemical uniqueness (increased levels of nutrients and particulate matter) within the western basin of Lake Erie (Danz *et al.*, 2007; Trebitz *et al.*, 2007;

Cvetkovic & Chow-Fraser, 2011; Uzarski *et al.*, 2017). Data presented in this study corroborate this historical evidence of human impact and nutrient loading in the western basin of Lake Erie.

High nutrient influx could also be influencing chemical and microbial vertical structure within coastal wetland soils. Vertical microbial community structure was not evident within the first 6 cm of soil of Lake Erie coastal wetlands, unlike other coastal wetlands regions. Also unique to Lake Erie, vertical chemical structure was not evident for nutrient levels (C, N, P) or C:N. These data provide evidence that microbial community structural shifts corresponding to wetland vertical profiles are related to concentrations of organic carbon and other nutrients (e.g., NO_3^-, NUTR, P, S). This is meaningful, as it has been previously postulated that concentrations of carbon electron donors may influence redox gradients within wetland soils (Achtnich *et al.*, 1995). Redox gradients are uniquely tied to carbon cycling rates, and as microbial community structure corresponds with redox gradients (Lüdemann *et al.*, 2000; Edlund *et al.*, 2008; Lipson *et al.*, 2015), distinct patterns of vertical community structure within the soil may be indicative of biogeochemical processes being affected within Great Lakes coastal wetlands. Connections between microbial community metabolic shifts with soil depth and levels of dissolved organic carbon *in situ* were incompletely resolved (Alewell *et al.*, 2008). The results presented here suggest that a connection between microbial community metabolism and organic carbon concentration may exist within Great Lakes coastal wetlands, however, it is necessary to better link microbial community diversity, microbial activity, and chemical cycles to establish this connection. As a caveat, it is possible that chemical and microbial structuring still existed within Lake Erie wetlands, yet they were not evident within the first 6 cm of soil or at the spatial scale measured. Nevertheless, microbial communities within Lake Erie coastal wetlands did not follow the same pattern of vertical structure, either chemically or biologically, evident in other regions,

suggesting that the biological integrity of coastal wetland systems is susceptible to negative anthropogenic pressure. Furthermore, carbon and nutrient levels are stable with increasing soil depth in Lake Erie coastal wetlands, which could be indicative of low carbon cycling rates or elevated sedimentation rates.

Similar to beta diversity, patterns in alpha diversity of microbial communities may also be related to soil chemical characteristics within the sampled freshwater coastal wetlands. Microbial community alpha diversity was highest within Lake Erie soils, which experienced the highest levels of nutrient concentrations of the sampled wetland regions. Significant correlations between alpha diversity and physicochemical properties (Table 1) suggest that the higher diversity in Lake Erie coastal wetlands could be driven by its distinct soil characteristics. It has been established in some cases that productivity and alpha diversity increase in a linear relationship on regional scales, but not at local scales (Chase & Leibold, 2002). This pattern of diversity is commonly the result of relatively few species existing within productive habitats on the local scale, while dissimilarity in beta diversity among productive habitat patches increases alpha diversity at larger spatial resolution. It is possible that this relationship exists within our data, with the Lake Erie region being the most OTU-diverse, coinciding with high nutrient concentrations. Habitat patches within Lake Erie wetlands may be smaller in spatial scale than was sampled within this study (where we might expect lower alpha diversity), and high dissimilarity in OTU composition among these hypothetical habitat patches may be responsible for higher alpha diversity in Lake Erie coastal wetlands. Substantiating this, taxa-area relationships have been established for microbial communities which suggest that microbial community turnover can occur at distances as short as millimeters to centimeters within soils and sediments (Grundmann & Debouzie, 2000; Ettema & Wardle, 2002; Horner-Devine *et al.*, 2004;

Martiny *et al.*, 2011). Microbial diversity has also been found to have a strong relationship with pH within forest and wetland soils (Fierer & Jackson, 2005; Hartman *et al.*, 2008; Lauber *et al.*, 2009). The water directly above the soil in the LE region had a pH that was relatively more acidic (although circumneutral) with respect to other sampled wetland regions. This may suggest that more OC was being oxidized as CO_2 reacts with water to form carbonic acid. This idea would then suggest that LE sites had a higher deposition rate replacing OC as oxidation occurred. While previous research has recognized links between pH and microbial community diversity within wetland soils, these relationships have been highly variable, ranging from a lack of relationship to a strong predictive relationship (Hartman *et al.*, 2008; Peralta *et al.*, 2013; Ligi *et al.*, 2014; Arroyo *et al.*, 2015). To date, research has been variable with respect to environments studied and methods by which pH was measured; this may partly explain the wide range of estimates of relationships between wetland soil pH and microbial community structure. As such, more research is needed to appreciate the influence of pH on microbial communities within wetland soils. Standardization of methodology for assessing environmental characteristics such as pH may also be required.

Relationships between microbial subnetworks and environmental gradients

Development of biological indices and establishment of indicator taxa have been suggested as necessary for microbial communities within wetlands, particularly through the use of high-throughput sequencing technologies which now allow for deep assessment of microbial community composition and structure within environmental samples (Sims *et al.*, 2013; Urakawa & Bernhard, 2017). In addition to their importance as biological signals for environmental health, indicator taxa may play prominent roles in bioremediation of excess nutrients and

pollutants found within anthropogenically impacted coastal wetlands. Through network analyses, we have delineated multiple subcommunities which were significantly related to environmental gradients (such as nutrients C, N, and P) measured by Uzarski *et al.* (2017) among coastal wetlands sampled in this study. Specifically, a subnetwork of 69 microbial taxa was 93.8% predictive of nutrient level variation among coastal wetland soils. Several microbial taxa within this subcommunity were individually predictive of nutrient levels to a high degree, including several OTUs related to *Anaerolineaceae*, one OTU related to genus *Anaerolinea*, and another related to genus *Bellilinea*. From the genus *Anaerolinea*, two thermophilic chemoorganotrophs (*Anaerolinea thermophila* and *Anaerolinea thermolimosa*) have been isolated (Sekiguchi *et al.*, 2003; Yamada *et al.*, 2006). Only one isolated member has been established within the genus *Bellilinea* (*Bellilinea caldifistulae*); it has been described as a thermophilic, fermentative, obligate anaerobe which thrives in co-culture with methanogens (Yamada *et al.*, 2007). It is unlikely that the OTUs found in our study are the same species as the isolated *Anaerolinea* and *Bellilinea* species, as coastal wetland soils are not high-temperature environments necessary for thermophilic species. Additionally, no OTUs related to methanogenic archaea were found within this subnetwork, suggesting that *Anaerolineacea* OTUs within coastal wetland soils may fluctuate independently of any specific methanogenic OTUs. It is possible that the *Bellilinea* OTU found within the subnetwork is related to nutrient level concentrations. This would support fermentative metabolism as noted within *Bellilinea caldifistulae*. It is important to note that several other studies have discovered OTUs related to *Anaerolineaceae* within wetland soils, with upwards of 90% relative abundance among *Chloroflexi* OTUs within these systems (Ansola *et al.*, 2014; Deng *et al.*, 2014; Hu *et al.*, 2016). This suggests that there are probable mesophilic

species yet to be isolated within this ubiquitous family of bacteria, which may be of high importance within wetland soils.

Betaproteobacteria were also found to significantly predict nutrient levels among coastal wetlands. Hu *et al.* (2016) found that both *Betaproteobacteria* and *Anaerolineae* were positively related to TN levels, which is consistent with the data presented here, and these two taxa were suggested to contribute to higher levels of heterotrophic activity. Further, *Anaerolineaceae* OTUs were consistently related to increasing C:N, suggesting that many taxa within this family have preference for recalcitrant carbon sources. As C:N also tends to increase with soil depth, it is also probable that the putatively obligate anaerobic *Anaerolineaceae* are coinciding with decreasing oxygen levels and/or changing metabolism requirements with increasing soil depth.

While there was no subnetwork of microbial taxa predictive of NO_3^- in the studied coastal wetland systems, there were several taxonomic groups that were either positively or negatively related to NO_3^- independently. Taxa spanning ten phyla were found to either positively or negatively correlate to NO_3^- gradients (Fig. 8). As nitrate reduction is a process which is undertaken by a wide breadth of phylogenetic groups (Reddy & DeLaune, 2008), it is possible that several taxa are capable of nitrate respiration within Great Lakes coastal wetlands. Notably, some taxa which positively related to NO_3^- are known nitrate reducers in other systems, such as members of *Deltaproteobacteria*, including *Myxococcales* and *Anaeromyxobacter* (Sanford *et al.*, 2002). Also noteworthy are the high number of OTUs within *Anaerolineaceae* which positively correlated with NO_3^- . However, several *Anaerolineaceae* OTUs were negatively related to NO_3^- gradients, suggesting that functional idiosyncrasy may exist among members of this family.

Conclusions

This study marks the first characterization of microbial communities within Great Lakes coastal wetlands. Coastal wetlands are integral in the proper functioning of biogeochemical cycles and environmental sustainability of the Great Lakes. While it has long been known that anthropogenic pressure can impact animal and plant communities within these coastal wetlands, this is the first evidence that these pressures may also be influencing microbial communities, and may be influencing biogeochemical cycles by extension. Alpha and beta diversity were both related to nutrient gradients among and within regions, suggesting that variability in microbial community structure is highly coupled to geochemistry within wetland soil. Further, this study provides insight on microbial community subnetworks and individual OTUs, which were predictive of chemical concentrations, and may be useful for future management of Great Lakes coastal wetland systems.

Beyond taxonomic assessments of microbial communities with relation to wetland monitoring, we propose that wetland microbial community structure can also potentially be used to assess a wetland for management purposes. As illustrated within this study, wetland microbial community structure and depth are decoupled within the wetlands experiencing the highest nutrient levels, likely originating from terrestrial inputs due to human activity. As such, multivariate statistics (as used in the methods of this study) may prove useful in examining relationships between wetland soil depth and microbial community structure alongside taxonomic analyses, which could provide indicators of nutrient loading stress on coastal wetland habitats.

Experimental Procedures

In the summer of 2014, wetland soil cores were collected within Laurentian Great Lakes coastal wetland ecosystems across several sites within several regions. Specifically, soil cores were collected from ten sites across five regions, including two sites in the western basin of Lake Erie (LE), three sites in eastern Saginaw Bay (ESBT), two sites in northern Saginaw Bay (NSB), two sites in western Saginaw Bay (WSB) in Lake Huron, and one site in the Beaver Island archipelago (BA) in Lake Michigan (Fig. 8). These sites were selected as they corresponded to environmental gradients, as well as a human impact gradients based upon SumRank scores as described in Uzarski *et al.* (2017) (Supplemental Fig. 8). Soil cores were collected by hand-driving plastic core tubes (~ 5 cm diameter) vertically into the soil. Among wetlands, samples were collected within the same vegetation zone (either dominated by cattails, genus *Typhus*, or bulrush, genus *Shoenoplectus*) as an attempt to control for collection bias across sites. Cores were sampled to a depth of at least 6 cm (except for one core which was sampled to a depth of 4 cm) and were immediately flash frozen in a dry ice ethanol bath. Samples were transported on dry ice to Central Michigan University wherein they were stored at -80 ˚C.

Triplicate cores were taken at five wetland sites while duplicate cores were taken at five other wetland sites. For sample extraction and sectioning, cores were extruded while still frozen via a custom-built core extruder. The edge of the core was warmed with a heat gun to allow the soil core to pass efficiently through the plastic container, however, the inner-core did not thaw during extrusion. Ice was applied to the plastic core liner to prevent accelerated thawing. Beginning from the top surface of soil, 2 cm sections were cut via an ethanol and flame-sterilized hacksaw blade and the sectioned core samples were placed into whirl pak bags and stored at -80 ˚C. The extruder face plate was sterilized between cuts of the same core with ethanol. The

extruder device was fully cleaned and sterilized between cores with physical scrubbing and ethanol sterilization.

Microbial community analysis

Each soil sample was analyzed independently for microbial community analyses. DNA was extracted from ~ 0.25 g of soil using a MoBio PowerSoil DNA Isolation Kit (Mo Bio, Carlsbad, CA) following the standard manufacturer's protocol. Concentrations of extracted DNA were assessed using a Qubit® 2.0 fluorometer (Life Technologies, Carlsbad, CA) to ensure successful DNA extraction and quantification for sequence library preparation. DNA samples were sent to Michigan State University (East Lansing, MI) for library preparation and sequence analysis at the Research Technology Support Facility. The V4 region of the 16S rRNA gene was amplified for downstream sequencing with commonly used primers 16Sf-V4 (515f) and 16Sr-V4 (806r) and a previously developed protocol (Caporaso *et al.*, 2012; Kozich *et al.*, 2013). Paired-end 250 bp sequencing was accomplished via a MiSeq high-throughput sequencer (Illumina, San Diego, CA). Acquired DNA sequences were filtered for quality and analyzed using MOTHUR v 1.35.1 (Schloss *et al.*, 2009) following the MiSeq SOP (available at https://www.mothur.org/) with modifications. Scripts used for sequence processing can be found at the GitHub repository associated with this study (https://github.com/horto2dj/GLCW/). Briefly, paired end sequences were combined into single contigs. Sequences that contained homopolymers > 8 bases, and those less than 251 or greater than 254 bp were removed. Sequences were aligned against the Silva (v. 119) rRNA gene reference database (Quast *et al.*, 2012). Sequences which did not align with the V4 region were also subsequently removed from analysis. Chimeric DNA was searched for and removed via UCHIME (Edgar *et al.*, 2011). Sequences were classified via the Ribosomal

Database Project (training set v. 9; Cole *et al.*, 2013) using the 'wang' method with a confidence threshold of 80. Sequences classified as chloroplast, mitochondria, eukaryotic, or unknown were removed. Remaining sequences were clustered into Operational Taxonomic Units (OTUs) at 0.03 sequence dissimilarity using the opticlust clustering algorithm. Sequence data associated with this research have been submitted to the GenBank database under accession numbers SRR6261304 – SRR6261377.

Chemical analysis

Each soil layer (top, middle, and bottom) was analyzed separately for local chemistry at each site. Within each site, soil samples of the same depth (i.e., top, middle, and bottom soil samples) among duplicate/triplicate cores were combined and homogenized to obtain enough soil for chemical analyses. Soil samples were analyzed for percent total N ("TN"), total P ("TP", ppm), total S ("TS", ppm), NO_3^- (ppm), NH_4^+ (ppm), percent organic matter ("OM"), percent organic carbon ("OC"), and C:N at the Michigan State University Soil & Plant Nutrient Lab (East Lansing, MI). A YSI multiprobe (YSI Inc., Yellow Springs, OH) was used to measure pH of the water residing directly above each collected soil core.

Statistical analyses

Statistical analyses were completed using R statistical software version 3.2.2 (R Core Team, 2015) unless otherwise stated. Code used for statistical analyses (and bioinformatic workflow) in this study can be found in the associated GitHub repository (https://github.com/horto2dj/GLCW/).

Physicochemical Analysis

Differences in chemical profiles between samples within and among wetland regions were visualized using Principal Component Analysis (PCA). Prior to PCA, percentages were arcsin square root transformed and ratios were log transformed. Additionally, Pearson correlation analyses were performed to search for significant correlations between chemical variables. Collinearity in the dataset was addressed by combining highly correlated environmental variables ($r > 0.7$, $p \leq 0.001$). Only one of the correlated variables was included in PCA to remove exaggeration of correlated variables in PCA structure. MANOVA was used to determine the influences of region and soil depth on physicochemical composition of samples, and 95% confidence intervals were established to compare differences among groups. Chemical depth profiles were also visualized for each wetland site to understand shifts in measured environmental variables with increasing soil depth.

Alpha-diversity Analysis

Alpha diversity analyses were performed to explore variation in OTU richness and evenness among wetland sites, regions, and soil depths, as well as to determine whether observed trends were driven by environmental variables. Prior to alpha diversity analyses, sequence abundance for each sample was subsampled to the lowest sequence abundance for any one sample ($n = 48{,}226$ sequences). Singletons were maintained within the sequence dataset for alpha diversity analyses, as alpha diversity indices can be reliant on the presence of singletons for proper estimation. Alpha diversity was calculated for each site using MOTHUR, including Chao 1 richness, non-parametric Shannon diversity, and Good's coverage indices. Linear mixed-effect models and ANOVAs were used to test influences of wetland site, region, and soil depth

on alpha diversity, controlling for wetland site as a random effect. Linear models and ANOVAs were used to test for variation in alpha diversity among wetland sites. If significant variation was found within an ANOVA result, post-hoc comparisons were implemented between sample groups using Tukey's Honest Significant Differences (HSD) tests with Bonferroni adjustments (p-values obtained by number of comparisons) for pairwise comparisons.

Beta diversity Analysis

Beta diversity analyses were used to evaluate variation in microbial community structure among wetland sites, regions, and soil depths, and to assess the extent to which observed variation was explained by environmental conditions. Singletons and doubletons were removed from the dataset for beta diversity analyses. All sequence data were maintained for beta diversity analyses and transformed using the *DeSeq2* (Love *et al.*, 2014) package, which normalized OTU abundances among samples using a variance stabilizing transformation (VST) (McMurdie & Holmes, 2014). The *phyloseq* (McMurdie & Holmes, 2013) and *Vegan* (Oksanen *et al.*, 2007) packages were used to compare beta diversity among samples. Dissimilarity in microbial community structure among samples within and among sites was visualized using Non-metric Multidimensional Scaling (NMDS) plots based on pairwise Bray-Curtis dissimilarity estimates. The function *envfit* of the Vegan package was used to evaluate correlation between chemical parameters and microbial community structure among samples according to NMDS. "Depth" was also implemented as a dummy variable to test correlation between depth and microbial community structure.

To test for significant differences in beta diversity among wetland sites, regions, and soil depth, Permutational Multivariate Analysis of Variance (perMANOVA) (Anderson, 2001) were

implemented. Specifically, these tests evaluate significant variation among within group and between group means (Clarke, 1993; Anderson, 2001; Anderson & Walsh, 2013). If perMANOVA found significant differences among groups at the global level, pairwise perMANOVA tests between groups were implemented with Bonferroni significance adjustments to control for multiple pairwise comparisons. Anderson's permutation of dispersions test (PERMDISP; Anderson, 2006; Anderson *et al.*, 2006) was used to test for differences in variance of community structure among sample groups (i.e. sites, regions, soil depths). Tukey's Honest Significant Difference (HSD) tests were implemented with adjusted p-values for multiple pairwise comparisons if significant differences in dispersion were found among groups.

To explore relationships between regional microbial community structure and environmental variables, NMDS plots were generated for each individual region. Applying NMDS to each region also allowed for the assessment of the correlational relationship between community structure and soil depth (as a dummy variable) and other environmental variables (using the *envfit* function) within individual regions. To test for differences in microbial community structure between/among sites within a region, as well as among depths within a region, perMANOVA was implemented individually for each region.

Network and OTU Correlation Analyses

To explore relationships between microbial sub-communities and OTUs to environmental variables, Weighted Correlation Network Analysis (WGCNA) was implemented on OTU relative abundances using the *WGCNA* package (Langfelder & Horvath, 2008; Langfelder & Horvath, 2012), executed as previously described (Guidi *et al.*, 2016; Henson *et al.*, 2016) with modifications. OTUs which did not possess at least 2 sequences across 10% of samples were

removed from network analyses. These OTUs were removed to eliminate OTUs with potentially spurious correlations to environmental variables or other OTUs, as well as to reduce computational stress of analyses. Remaining OTU abundances across samples were normalized using variance stabilizing transformation (VST) performed as described previously for beta diversity analyses. To ensure scale-free topology of the network, the dissimilarity matrix generated through VST was transformed to an adjacency matrix by raising this dissimilarity matrix to a soft threshold power. A threshold power of $p = 4$ was chosen to meet scale-free topology assumptions based upon criterion established by Zhang & Horvath (2005). Scale-free topology of network relationships was further ensured through regression of the frequency distribution of node connectivity against node connectivity; a network is scale-free if an approximate linear fit of this regression is evident (see Zhang & Horvath, [2005] for more in-depth explanation). A topological overlap matrix (TOM) was generated using the adjacency matrix, and subnetworks of highly connected and correlated OTUs were delineated with the TOM and hierarchical clustering. Representative eigenvalues of each subnetwork (i.e., the first principal component) were correlated (Pearson) with values of measured environmental variables to identify the subnetworks most related to said environmental variables. The subnetworks with the highest positive correlations to environmental variables of interest (e.g., NO_3^-, C:N, etc.) were selected for further analyses of relationships among subnetwork structure, individual OTUs, and environmental variables. Partial Least Square regression (PLS) was used to test predictive ability of subnetworks in estimating variability of environmental parameters, which allowed for delineation of potential indicator subnetworks and OTUs. Pearson correlations were calculated between response variables and leave-one-out cross-validation (LOOCV) predicted values. If PLS found that regression between actual and predicted values was below minimum

threshold of $R^2 = 0.3$, WGCNA analysis was halted for that network, as the network was deemed to lack predictive ability of that environmental variable. Variable Importance in Projection (VIP) (Chong & Jun, 2005) analysis was used to determine the influence of individual OTUs in PLS. A high VIP value for an OTU indicates high importance in prediction of the environmental response variable for that OTU. For network construction and visualization purposes, the minimum correlation value required between two OTUs to constitute an "edge" between them was delineated at different *r* values for each network related to an individual environmental variable (ranging between 0.1 – 0.25), as co-correlations between OTUs within some networks were stronger than others. The number of co-correlations an OTU has with other OTUs within a network is defined as "node centrality" (as described by Henson *et al.*, 2016).

If an environmental variable of interest was not found to have a network strongly associated with it, Spearman's correlations were implemented between individual OTUs and that environmental variable. Significance was only considered for OTUs with strong relationships ($r > 0.3$, $p \leq 0.001$) to environmental variables to limit the impact of potentially spurious correlations. Correlations to OTUs with unknown taxonomic identification were excluded from correlational analysis.

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Tables

Table 1. Correlations between alpha diversity metrics and measured environmental variables. Asterisks represent significance values where $p \leq 0.001$ (***), $p \leq 0.01$ (**), and $p \leq 0.05$ (*).

	Chao 1	Shannon
P	0.31**	-0.09
S	0.42***	0.45***
NO3	0.42***	0.24*
C:N	-0.03	-0.2
NUTR	0.24*	0.41***

Table 2. Pairwise perMANOVA results comparing pairwise differences between wetland regions and differences between wetland soil depths. Asterisks represent significance values where $p \leq 0.001$ (***), $p \leq 0.01$ (**), $p \leq 0.05$ (*), and *n.s.* representing 'not significant' below p-value thresholds.

Region	BA	ESBT	LE	NSB	WSB
BA	-				
ESBT	*n.s.*	-			
LE	**	**	-		
NSB	*n.s.*	*n.s.*	**	-	
WSB	*n.s.*	*n.s.*	**	*n.s.*	-

Depth	Top	Middle	Bottom
Top	-		
Middle	**	-	
Bottom	**	*n.s.*	-

Figures

Figure 1. Principal Component Analysis (PCA) illustrating separation of samples based upon soil geochemistry. Shapes and colors correspond to different wetland depths and regions, respectively, as listed in the legend. Percentages on axes represent explained variance of that principal component. Vectors represent impact of specific environmental variables on sample distribution. NUTR represents OM values, which correlated significantly ($p \leq .01$, $r > 0.56$) to NO_3^-, OC, OM, S, and TN. Ellipses represent 95% confidence intervals of region groupings.

Figure 2. Boxplot diagram comparing Chao1 diversity among wetland regions. Boxes with the same letter are not significantly different, while those with no common letters are significantly different ($p \leq 0.01$). Lines within boxes represent the median, hinges represent +/- 25% quartiles, whiskers represent up to 1.5x the interquartile range. Colors represent wetland region.

Figure 3. Nonmetric Multidimensional Scaling (NMDS) plot illustrating separation of samples based upon differences in microbial community structure. Shapes and colors correspond to

different depths and wetland regions, respectively, as listed in the legend. Vectors represent correlations of environmental variables to the distribution of the microbial communities represented in the plot.

Figure 4. NMDS plots of each wetland region demonstrating separation of samples based upon differences in microbial community structure, including a. BA, b. ESBT, c. LE, d. NSB, and e. WSB. Shapes and colors correspond to different depths and wetland sites, respectively, as listed in the legends. Vectors represent correlations of environmental variables to the distribution of microbial communities represented in the plots.

Figure 5. Network visualization and results of partial least squares analysis on the subnetwork most correlated with NUTR. The y-axis represents correlation of OTU to OC values, whereas the x-axis represents the node centrality. Points represent OTUs, and the color of points corresponds to the phylum to which an OTU belongs. Point size corresponds to VIP score of that OTU. The top 15 OTUs are labeled within the graph with corresponding lowest taxonomic identification possible, and the level of that classification. D = Domain; P = Phylum, C = Class, O = Order, F = Family, G = Genus.

Figure 6. Network visualization and results of partial least squares analysis on the subnetwork most correlated with C:N. The y-axis represents correlation of OTU to C:N, whereas the x-axis represents the node centrality. Points represent OTUs, and the color of points corresponds to the phylum to which an OTU belongs. Point size corresponds to VIP score of that OTU. Only OTUs with a VIP score > 1 were displayed for visualization purposes. The top 15 OTUs are labeled

within the graph with corresponding lowest taxonomic identification possible, and the level of that classification. D = Domain; P = Phylum, C = Class, O = Order, F = Family, G = Genus.

Figure 7. Significant ($p \leq 0.001$) positive and negative correlations between microbial taxonomic groups and NO_3^-. Abundance of OTUs corresponding to taxonomic groups is plotted on the y-axis. Positive correlations are plotted above the "0" line, while negative correlations are plotted below the "0" line. Colors of bars correspond to associated phylum of the taxonomic group on the x-axis.

Figure 8. Geographic map displaying location of sites sampled within this study. Colors of points correspond to region sampled.

Supplemental Material

Supplemental Figure 1. Depth profiles demonstrating trends in measured environmental variables with increasing depth among wetland regions and sites. Colors represent wetland regions, whereas point shapes represent distinct wetland sites.

Supplemental Figure 2. Rarefaction curve analysis demonstrating sequencing depth for alpha diversity analyses. Different colored lines represent different samples. The vertical black line represents the sequencing depth used to standardize all samples for alpha diversity analysis. The dashed line represents the 1:1 slope.

Supplemental Figure 3. Plots demonstrating regression of the frequency distribution of node connectivity against node connectivity.

Supplemental Figure 4. Individual correlations of established subnetworks with environmental parameters.

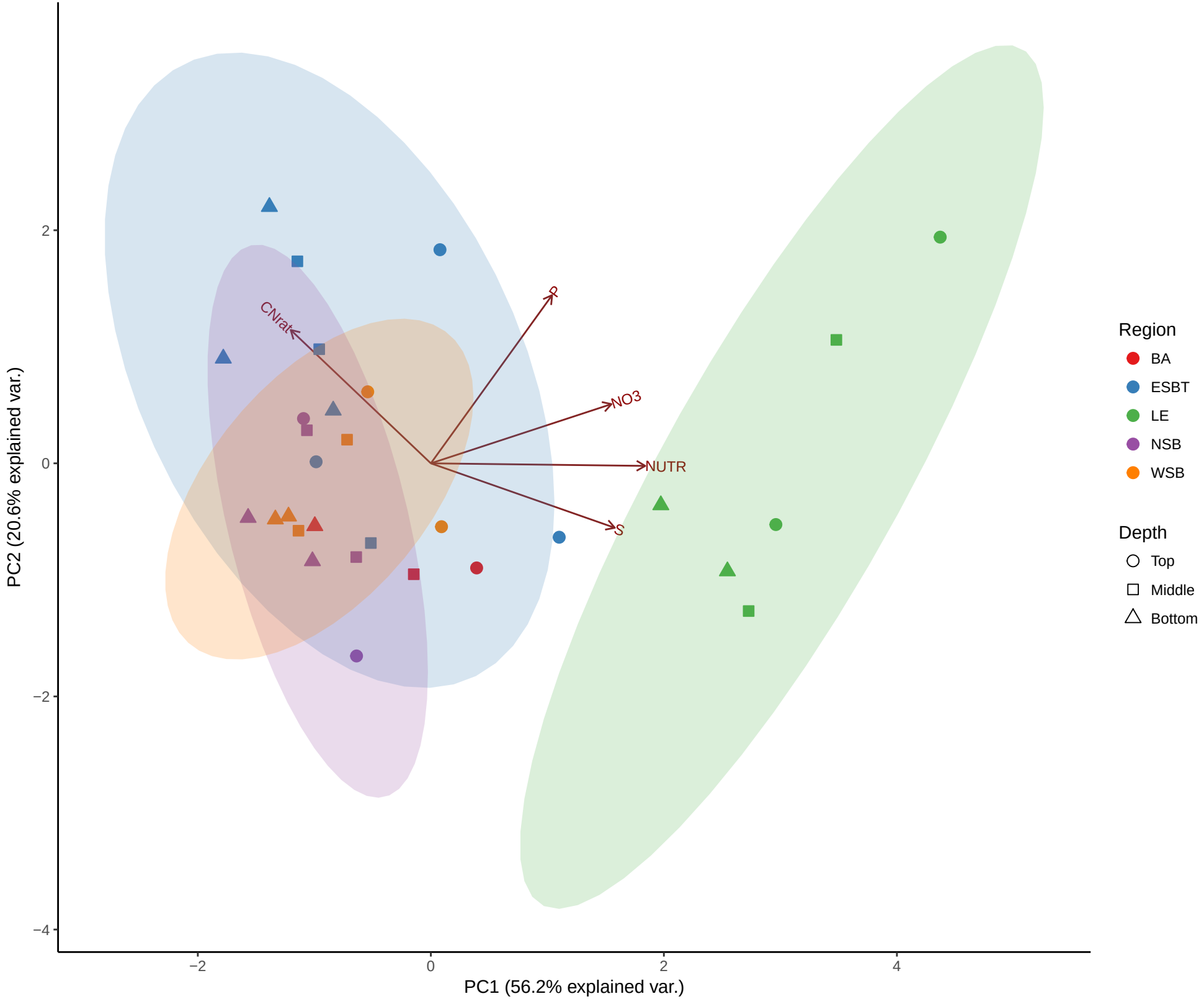
Supplemental Figure 5. Partial least squares analysis results predicting OC (NUTR) values using relative abundance values of OTUs within the module most connected to OC (NUTR).

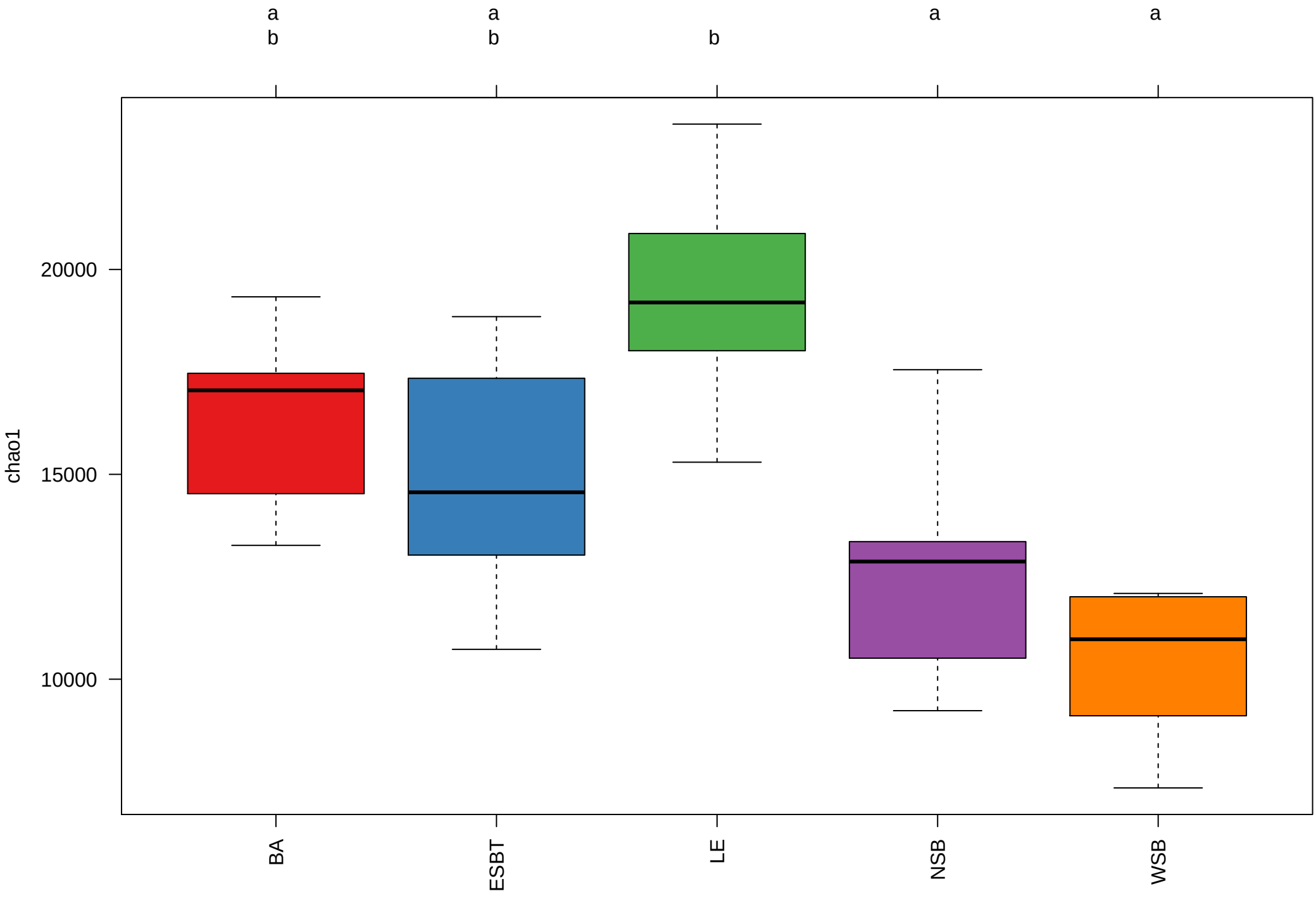
Supplemental Figure 6. Partial least squares analysis results predicting C:N values using relative abundance values of OTUs within the module most connected to C:N.

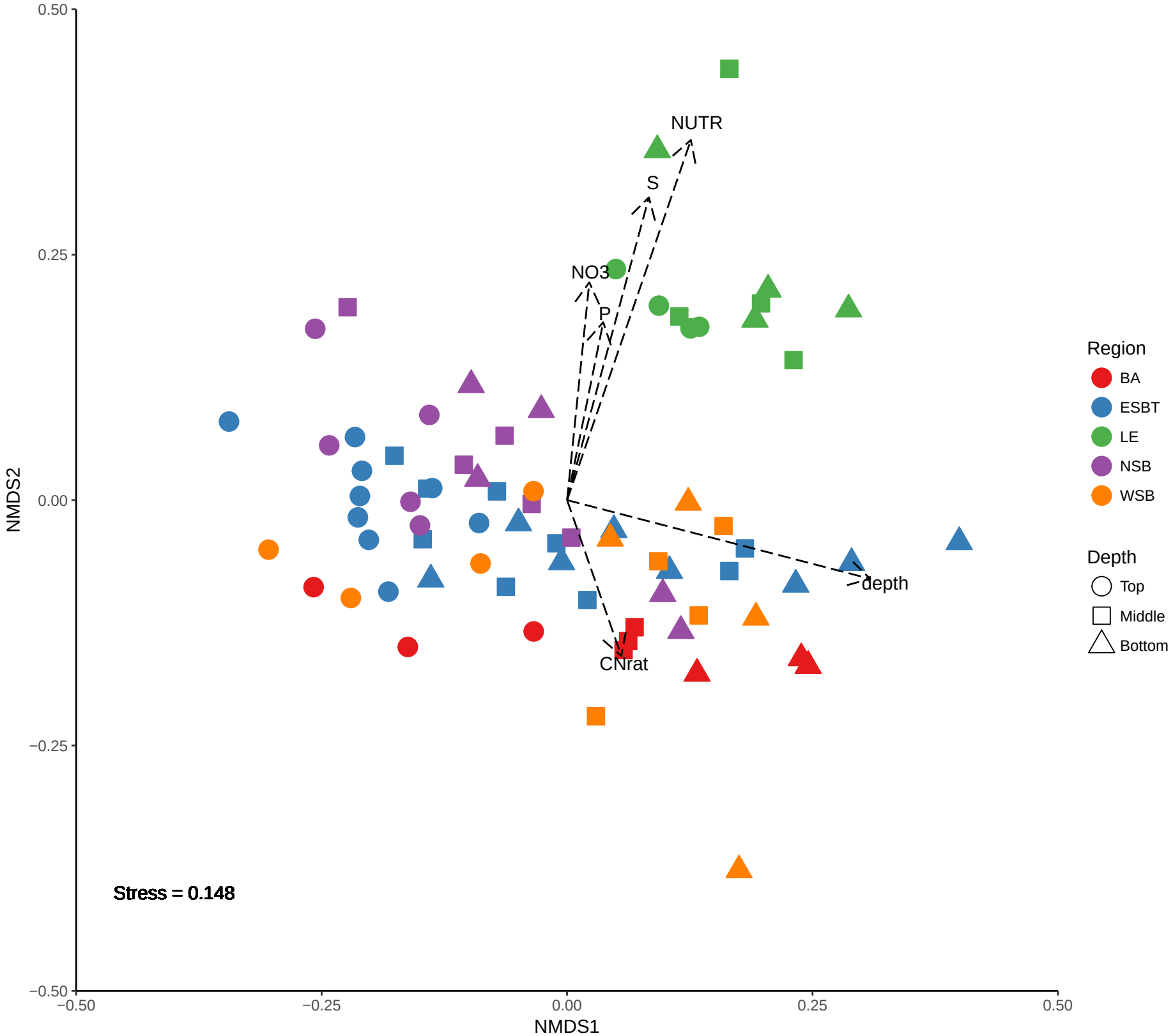
Supplemental Figure 7. Partial least squares analysis results predicting NO_3^- values using relative abundance values of OTUs within the module most connected to NO_3^-.

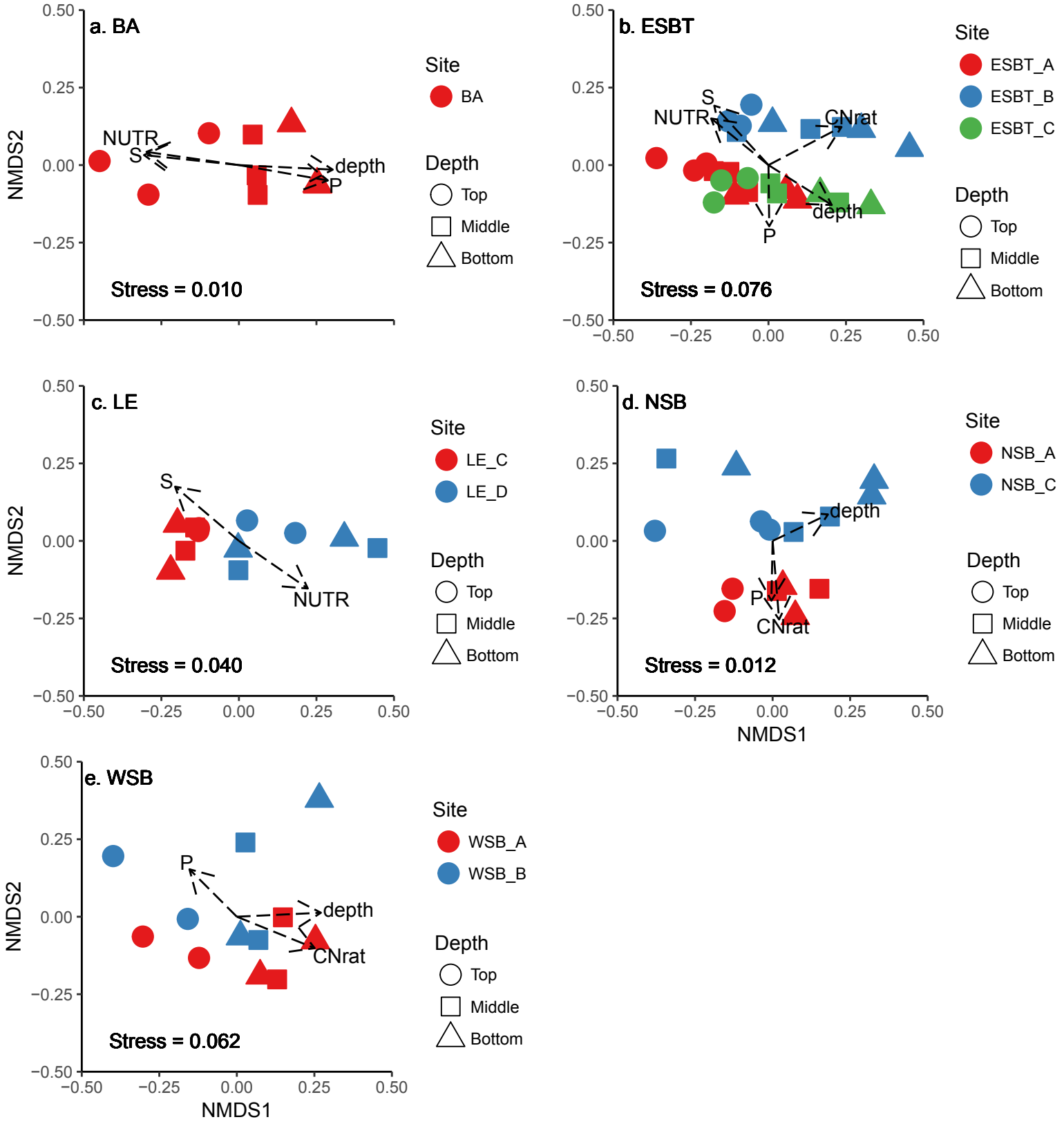
Supplemental Figure 8. Principal Components Analysis demonstrating separation of coastal wetland sampling locations by site water quality data. Points are color-coded by region. Percentages on axes represent explained variance of that principal component. Vectors represent impact of specific environmental variables on sample distribution. Choloro.a = chlorophyll A, DO = dissolved oxygen, Temp = water temperature, Turb = water turbidity. SumRank represents SumRank values calculated for each wetland site as outlined by Uzarski et al. (2017). DO also represents redox potential values, which correlated significantly ($r > 0.7$, $p \leq .001$). Temp also

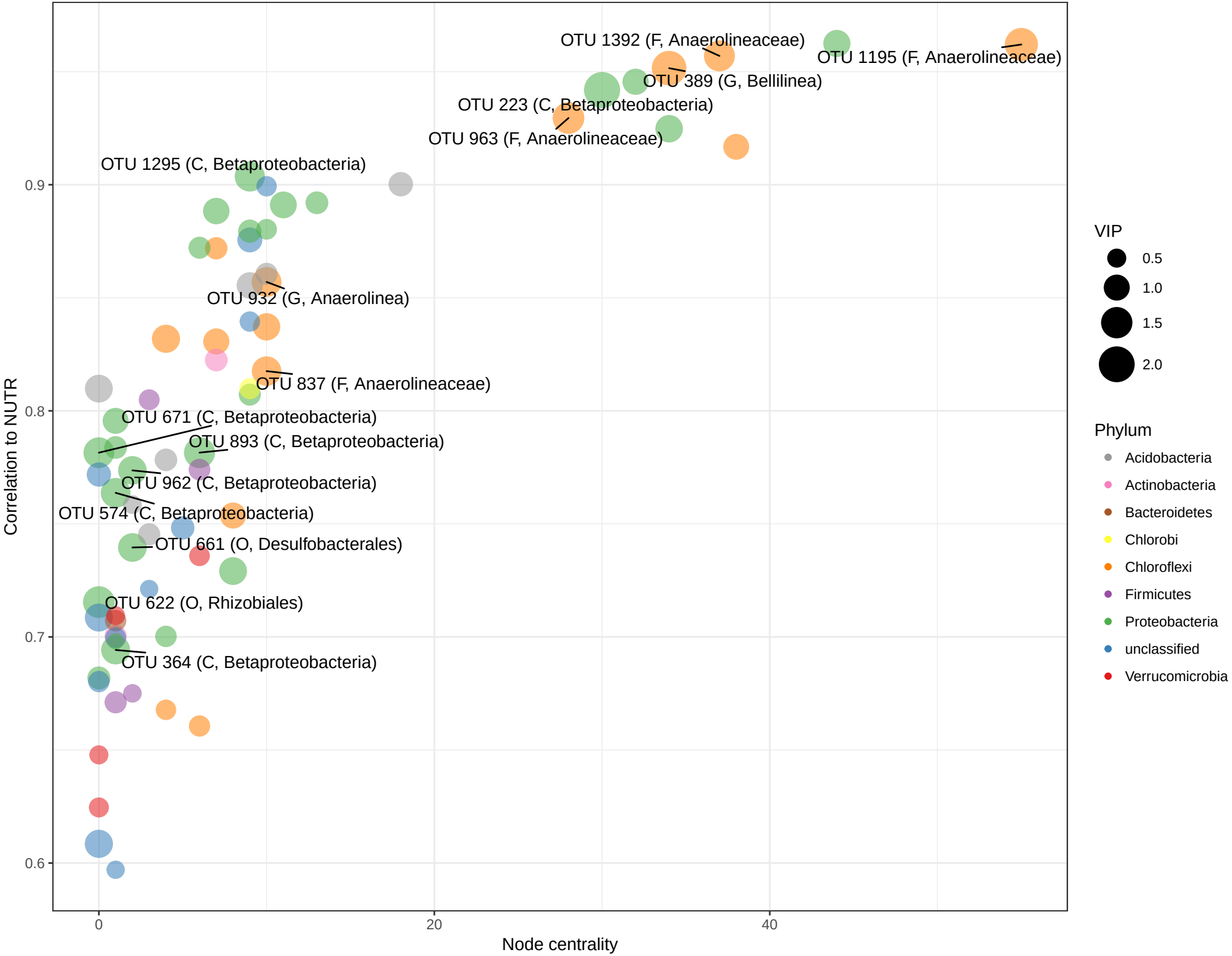
represents conductivity and total dissolved solids values, which correlated significantly ($r > 0.7$, $p \leq .001$).

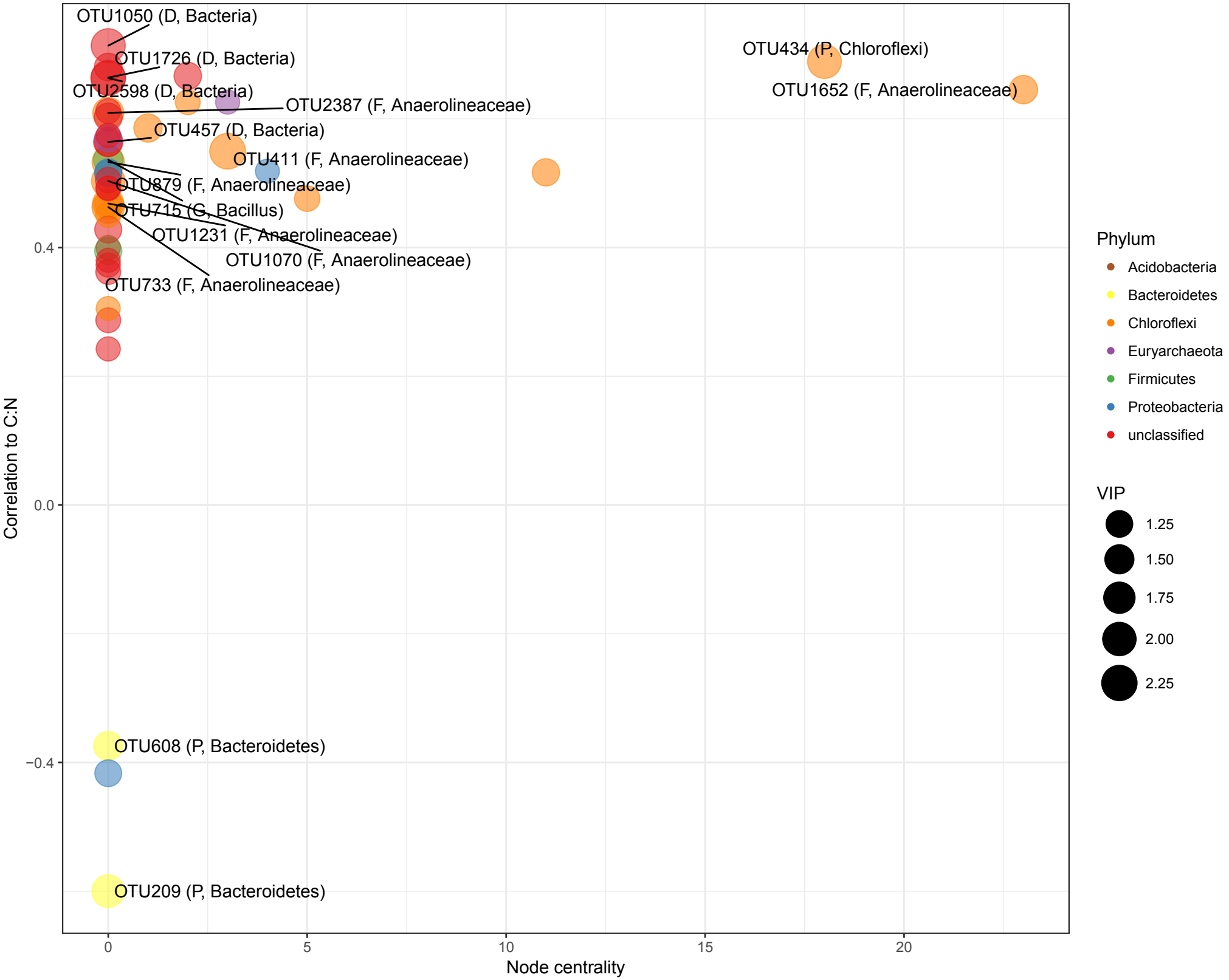

PC2 (20.6% explained var.)
PC1 (56.2% explained var.)
2
0
−2
−4
−2
0
2
4
CNrat
P
NO3
NUTR
S
Region
BA
ESBT
LE
NSB
WSB
Depth
Top
Middle
Bottom

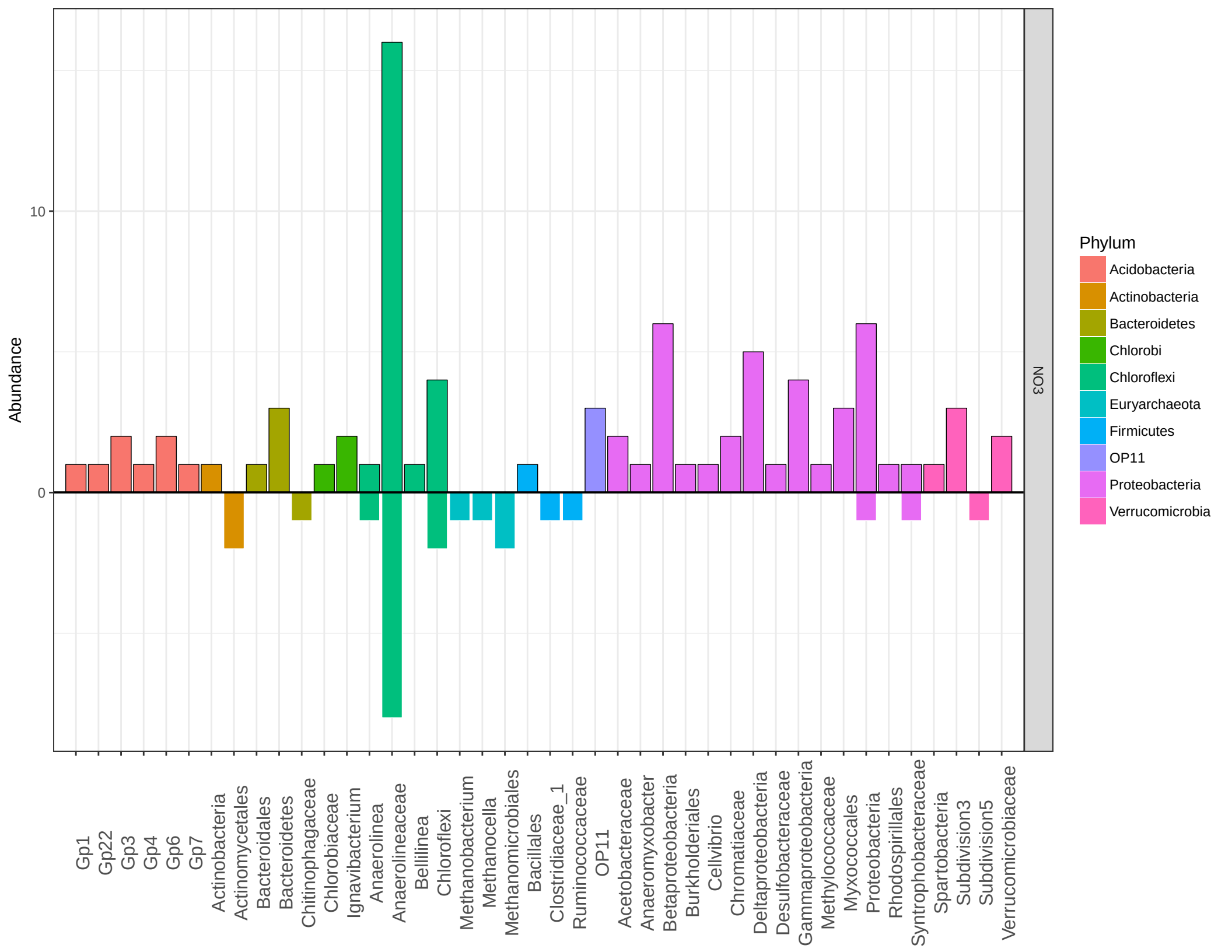

a
b
a
b
b
a
a
20000
15000
10000
chao1
BA
ESBT
LE
NSB
WSB

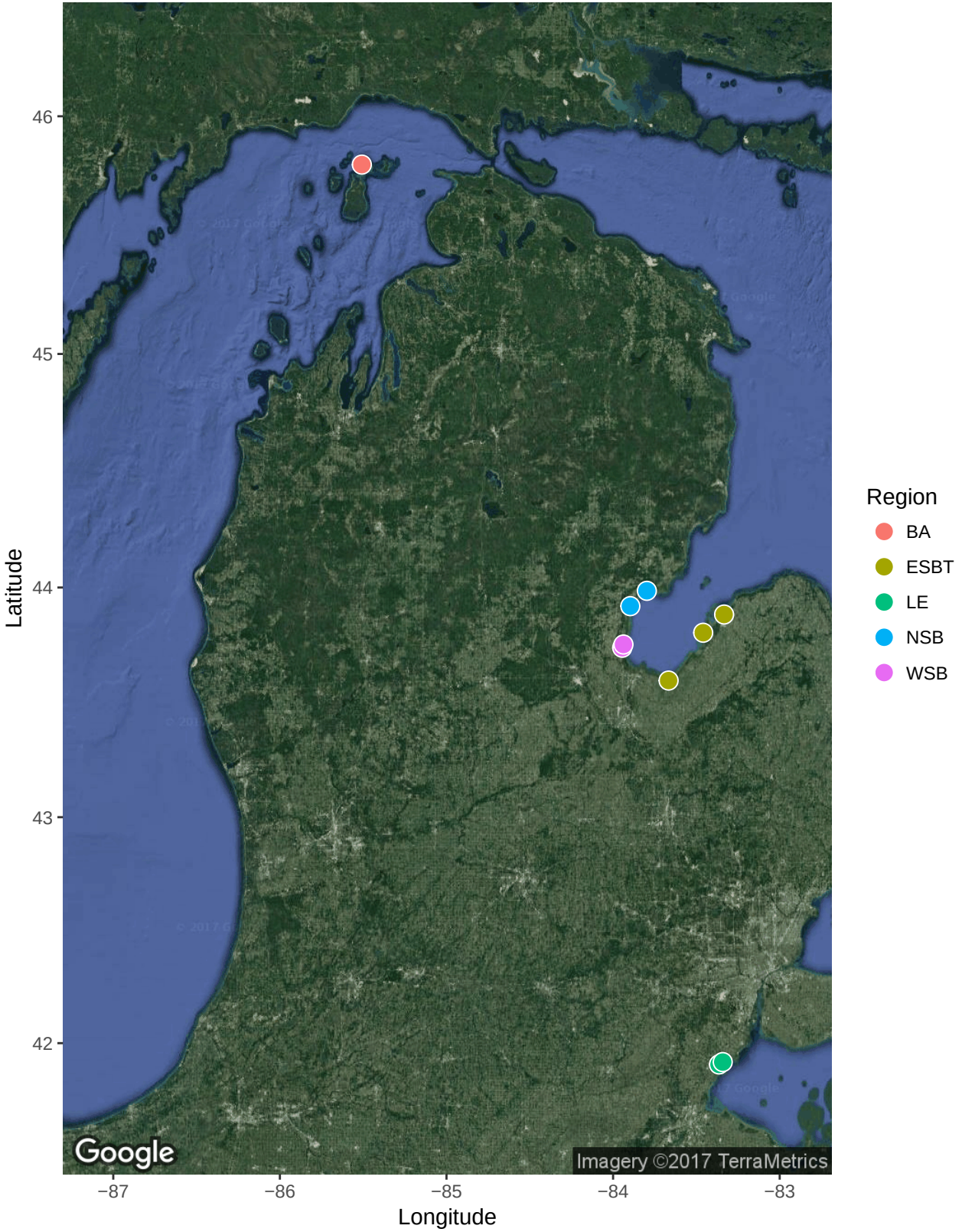

NUTR
S
NO3
P
depth
CNrat
Stress = 0.148
NMDS1
NMDS2
0.50
0.25
0.00
−0.25
−0.50
Region
BA
ESBT
LE
NSB
WSB
Depth
Top
Middle
Bottom


a. BA
NUTR
S
depth
P
Stress = 0.010
Site
BA
Depth
Top
Middle
Bottom
b. ESBT
S
NUTR
CNrat
depth
P
Stress = 0.076
Site
ESBT_A
ESBT_B
ESBT_C
Depth
Top
Middle
Bottom
c. LE
S
NUTR
Stress = 0.040
Site
LE_C
LE_D
Depth
Top
Middle
Bottom
d. NSB
depth
P
CNrat
Stress = 0.012
Site
NSB_A
NSB_C
Depth
Top
Middle
Bottom
e. WSB
P
depth
CNrat
Stress = 0.062
Site
WSB_A
WSB_B
Depth
Top
Middle
Bottom
NMDS1
NMDS2
0.50
0.25
0.00
−0.25
−0.50


OTU 1392 (F, Anaerolineaceae)
OTU 1195 (F, Anaerolineaceae)
OTU 389 (G, Bellilinea)
OTU 223 (C, Betaproteobacteria)
OTU 963 (F, Anaerolineaceae)
OTU 1295 (C, Betaproteobacteria)
OTU 932 (G, Anaerolinea)
OTU 837 (F, Anaerolineaceae)
OTU 671 (C, Betaproteobacteria)
OTU 893 (C, Betaproteobacteria)
OTU 962 (C, Betaproteobacteria)
OTU 574 (C, Betaproteobacteria)
OTU 661 (O, Desulfobacterales)
OTU 622 (O, Rhizobiales)
OTU 364 (C, Betaproteobacteria)
Correlation to NUTR
0.9
0.8
0.7
0.6
0
20
40
Node centrality
VIP
0.5
1.0
1.5
2.0
Phylum
Acidobacteria
Actinobacteria
Bacteroidetes
Chlorobi
Chloroflexi
Firmicutes
Proteobacteria
unclassified
Verrucomicrobia


OTU1050 (D, Bacteria)
OTU1726 (D, Bacteria)
OTU2598 (D, Bacteria)
OTU2387 (F, Anaerolineaceae)
OTU457 (D, Bacteria)
OTU411 (F, Anaerolineaceae)
OTU879 (F, Anaerolineaceae)
OTU715 (G, Bacillus)
OTU1231 (F, Anaerolineaceae)
OTU1070 (F, Anaerolineaceae)
OTU733 (F, Anaerolineaceae)
OTU434 (P, Chloroflexi)
OTU1652 (F, Anaerolineaceae)
OTU608 (P, Bacteroidetes)
OTU209 (P, Bacteroidetes)
Correlation to C:N
0.4
0.0
−0.4
0
5
10
15
20
Node centrality
Phylum
Acidobacteria
Bacteroidetes
Chloroflexi
Euryarchaeota
Firmicutes
Proteobacteria
unclassified
VIP
1.25
1.50
1.75
2.00
2.25


Abundance
10
0
NO3
Gp1
Gp22
Gp3
Gp4
Gp6
Gp7
Actinobacteria
Actinomycetales
Bacteroidales
Bacteroidetes
Chitinophagaceae
Chlorobiaceae
Ignavibacterium
Anaerolinea
Anaerolineaceae
Bellilinea
Chloroflexi
Methanobacterium
Methanocella
Methanomicrobiales
Bacillales
Clostridiaceae_1
Ruminococcaceae
OP11
Acetobacteraceae
Anaeromyxobacter
Betaproteobacteria
Burkholderiales
Cellvibrio
Chromatiaceae
Deltaproteobacteria
Desulfobacteraceae
Gammaproteobacteria
Methylococcaceae
Myxococcales
Proteobacteria
Rhodospirillales
Syntrophobacteraceae
Spartobacteria
Subdivision3
Subdivision5
Verrucomicrobiaceae
Phylum
Acidobacteria
Actinobacteria
Bacteroidetes
Chlorobi
Chloroflexi
Euryarchaeota
Firmicutes
OP11
Proteobacteria
Verrucomicrobia


46
45
44
43
42
Latitude
−87
−86
−85
−84
−83
Longitude
Google
Imagery ©2017 TerraMetrics
Region
BA
ESBT
LE
NSB
WSB