**Methods**

*Data prep*

Total percent Nitrogen was square root transformed to improve normalization of the feature prior to further analysis. Additional transformations were attempted on non-normal features (Density and Phosphorous ppm) but were reversed as normality did not improve. Distributions were visualized using the hist.data.frame function from the HMISC package (Harrell Jr 2019).

*Analysis*

Non-metric multidimensional scaling analysis was carried out using the metaMDS function from the Vegan library (Oksanen 2022) with Bray-Curtis dissimilarity as the distance metric, for 1 through 10 dimensions. Stress was assessed for each NMDS analysis, and the 2-dimension NMDS was selected for further analysis and visualization.

*Plots*

Ordination plots were produced using the ordiplot function from the vegan library (Oksanen 2022). One plot was produced for each of the response variables (depth and contour), each colored by the response category. 95% confidence ellipses were then added to the plots using the ordiellipse function from the vegan library (Oksanen 2022).

**Results**

*Transformations*

Percent Nitrogen was square root transformed to improve normality (Figure 1). Two other features were not normally distributed, but no transformation appeared to normalize the data.



Figure 1: % Nitrogen before and after square root transformation

*NMDS*

1-dimension NMDS using Bray-Curtis dissimilarity had a stress of 0.0758. Adding a second dimension decreased this stress level to 0.0481 (Figure 2). 2 dimensions was chosen for further analysis because it had ~36% lower stress than 1 dimension, and because a stress level under 0.005 is typically seen as very good.



Figure 2: Stress by number of dimensions in NMDS (using Bray-Curtis dissimilarity as the distance metric).

*Ordination Plots*

The ordination plot colored by contour shows no discernable differentiation between the 95% confidence ellipses for any of the soil sample contour groups (Figure 3). The ordination plot for soil sample depth shows clear differentiation between 95% confidence ellipses for soil depths that are not adjacent in order. However adjacent depth levels cannot be differentiated with 95% confidence (Figure 4). The stress level of 0.0481 means we can be relatively confident in this result.



Figure 3: Ordination plot of NMDS results, colored by contour gradient of the soil sample. 95% confidence ellipses are shown around each contour group. No contour group can be distinguished from the others with 95% confidence.



Figure 4: Ordination plot of NMDS results, colored by the soil sample collection depth. 95% confidence ellipses are shown around each depth level. Adjacent depth levels cannot be distinguished with 95% confidence, but non-adjacent depth levels can clearly be distinguished.

**Citations**

1. Harrell Jr., F., & Dupont, Ch. (2019). Hmisc: Harrell Miscellaneous. R Package Version 4.2-0. https://CRAN.R-project.org/package=Hmisc
2. Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022). \_vegan: Community Ecology Package\_. R package version 2.6-4, <https://CRAN.R-project.org/package=vegan>.