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Biodiversity of Testate Amoeba Communities in Panthertown, North Carolina

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Abstract

Testate amoeba (TA) are a diverse polyphyletic group of shelled protozoans that dominate *Sphagnum* peatlands. TA have been used as proxies for water quality, environmental acidity, and land use changes globally [1, 2] however little work has been done using these microorganisms in the southeastern U.S. Although the modern vegetation of this region is well documented, the TA communities are not. The primary objectives of this study were to describe patterns of community composition of TA in Panthertown's last remaining wetlands, to develop hypotheses to explain differences within the communities, and to determine if taxa occupy similar ecological niches with respect to substrate moisture, total elemental C, and pH. This research will provide valuable data to aid in the analysis of long term studies evaluating environmental and climatic changes in the wetland during the Holocene. *Sphagnum* peat moss and soil were sampled from hiking trail and non-trail sites within Panthertown valley of Nantahala forest during the fall of 2016. Non-trail sites represent pristine sites and trail sites represent disturbed sites determined by their respective qualitative level of trampling. The categories were compared to look for correlations between anthropogenic disturbance and the residential TA population assemblages. The methods include qualitative and quantitative analysis for each sample; moisture class, water table depth, pH, total elemental carbon/nitrogen content, as well as TA identification. TA processing followed standard wash and filtration preparation protocol. Statistical analysis included, Shannon's diversity index to determine the biodiversity of each sample, multivariate ordinations to compare species and environmental variables. Panthertown had high TA biodiversity and results indicated that populations were dynamic and changing. Biodiverse sites indicated that TA were most associated with depth to water table (DWT) out of all environmental variables considered. The Environmental stresses due to drought may have caused variation in species richness. These data aid in the long-term analysis of biodiversity of TA in western North Carolina.

1. Introduction

Historically the Southern Appalachian Mountains are highly diverse; however, the forests and fens have endured significant alterations due to anthropogenic disturbances following human settlements. Plant and animal communities have thus been impacted by the changing landscape. Rare plant communities are becoming increasingly threatened due to anthropogenic disturbance.

Testate amoebae (TA) are an abundant group of microorganisms associated with many rare plant communities within aquatic and terrestrial environments in the Southern Appalachians. Little work has been done in North America but, globally TA have been used as proxies for water quality, environmental acidity and land use changes[1,2] . Their high abundance and diversity allows them to respond fast to environmental changes and they are thus useful bioindicators for monitoring present and past ecosystem changes[1,2] . They are more sensitive to minor differences in hydrology and water chemistry than macroscopic plants and unlike vascular plants the TA respond only to conditions at the surface environment of the wetland[3]. TA are often model organisms because they produce shells that differ in composition, size and shape[4], and the secreted test can be identified to species level[5, 6, 7]. TA are a dominant group of polyphyletic protozoa[8] and are top predators in the microbial food web that contribute significantly to nutrient cycling in the ecosystem[9] .TA community patterns repeatedly have been shown to be explained by moisture and/or depth to the water table in mid- to high-latitude Sphagnum dominated peatlands[1] .TA are divided into two phylogenetically distinct groups Euglyphida (Rhizaria)[10] and Acellinida (Amoebazoa)[11] Morphological adaption to the soil (i.e. test compression and aperture in a ventral position) appeared independently in both TA groups[12,13] .TA favor habitat with moist soil conditions including a high-water table, thick layers of moss or litter and shading by trees. Soil moisture is determined by many environmental variables such as soil particle size distribution, organic matter content, vegetation structure, litter input, elevation and other ecological factors[1] .In Fournier et al the study sought to better understand TA as indicators of flood plain restoration success, their results suggested TA species traits relate to the test including the aperture, compression and test material origin were strongly correlated with litter variables and soil moisture environmental variables[4] . Their results supported a clear response for shell related traits, and there was also support of the origin of test origin materials having a strong correlation with the surrounding nonwoody and herbaceous vegetation. Out of the sampling sites the TA were found to prefer upland forest soils and restoration sites as opposed to the non-restored sites[4] The use of TA as environmental indicators should consider context specific protocols as many regions vary in their flora and fauna[4] .

In some cases, species and functional-trait composition of TA communities are more strongly correlated with spatial than environmental variables[14] . For one study found that pH may have been the driving factor influencing the testate amoeba assemblages in certain habitats[15] . There are many variables to consider in ecological research of TA for different biogeographical regions that may correlate with species assemblages This research will 1) analyze the TA community assemblages across Panthertown wetland, 2) examine the diversity of the communities 3) to determine if the TA community correlates with environmental variables.

2. Methods

Sphagnum moss was sampled across part of the forest and wetland pools at hiking trail and non-trail sites. The TA inhabited *Sphagnum* samples sites were selected based upon meeting the category of either trail or non-trail. Non-trail sites represent more pristine sites with more vegetation cover and height, while trail sites represent disturbed sites with less vegetation cover, height and bare ground. This was determined by the trails respective qualitative level of trampling. Trampling can be defined as changes in vegetation cover, vegetation height, and bare ground cover[16]. At each sample site PT1-16, depth of water table (DWT) was measured, moisture class was assigned (i.e. submerged aquatic = 1, very dry = 9)[17] and a surface sample was collected at approximately 5 x 5 x 5 cm. Each sample was divided, and one part was used to calculate moisture content. The other sub sample was disaggregated in deionized water and tested for pH. The *Sphagnum* moss was filtered at 250µm, 100µm and 10µm, with the 10 µm > 250 µm retained, which followed standardized TA protocol. For TA identification approximately 100 TA per sample were counted and identified with the aid of several taxonomic guides [18,19] .

Several statistical models were employed to better understand both the environmental and TA data from Panthertown. Ordination methods were used in PCORD7 to examine the general structure of TA data and to test the correlations between the TA and environmental variables. Detrended Correspondence Analysis (DCA) was utilized to determine the general structure of the TA data. Principal components analyses (PCA) were applied to both sites and TA species data. To look at species and environmental variables a redundancy analysis (RDA) was used to examine the relationship. These variables include depth of water table (DWT), pH, and total carbon content (C). DWT was collected using a soil auger, water present was measured after a half hour. A SevenEasy pH Mettler-Toledo AG 8603 electronic meter was used to test the pH. Total carbon was tested at North Carolina State University Environmental and Agricultural Testing Services (NCSU-EATS) with a CHN elemental, model Perkin Elmer's 2400CHNO/S Elemental Analyzer, series II. TA data were logarithmically transformed and samples with insufficient data were excluded. Shannon Diversity Index, Simpson's Index, and species richness (N) were used to better understand the diversity of each sampling site.

2.1 Sites

Panthertown is a high elevation Southern Appalachian valley with a forest fen complex and a unique vegetation community, that includes a diverse *Sphagnum* community. Panthertown wetland is approximately 1130m above sea level. It is distinguished by a highly diverse moderate canopy cover, shrub layer and an extensive herb dominated wetland. The canopy consists primarily of deciduous species such as *Quercus* spp., *Acer* spp., *Morus* spp. The understory contained many herbaceous plants and shrubs, as well as *Sphagnum* mosses, which are a flora of mid- to high- latitude peatlands, and are present at Panthertown Valley's wetland. Two of the mosses sampled that were present in the fen were identified as *S. Palustre*, and *S. Magellanicum* and were abundant in Panthertown. The wetland is partially within a valley, and is crossed by Panthertown creek.

3. Results

3.1 Present testate amoeba communities

A total of 42 testate amoeba taxa were identified; including 15 genera, 7 families and 30 species (Table 1). The three most abundant genera in this study were *Hyalosophenia* (29.7% at 537 individuals), *Nebela* (19.7% at 356 individuals) and *Euglypha* (14.14% at 256 individuals). Together they accounted for 63.5% of the taxa counted. Most species were associated with sites PT1, PT2, PT6, PT11 and PT12. The rest of the sites were associated with only a few taxa comparatively.

3.2 Moisture class

Moisture class was compared by two sections: a drier section and a more wet section. The average moisture class of PT1, PT2, PT6, PT11, PT12, and PT16 was 6.42 as the drier section. The average moisture class of PT3, PT4, PT5, PT7, PT8, PT9, PT10, PT13, PT14 and PT15 was 3.7 as the more wet section.

3.3 Diversity

Shannon's Diversity Index (H) was high for Panthertown. 62.5% of the sites were over 1.5 out of 3. There was particularly high diversity in PT1, PT3, PT6, PT11 and PT16, while species richness (N) follows a similar trend to diversity. PT1 shows the highest diversity with a species richness of N=25 present (Figure 1). Environmental data showed a strong correlation between DWT and TA community assemblege and diversity. Simpson's Diversity Index was relatively high for all samples except for PT 4, 5, and 15 (Figure 2). Overall diversity was high for both Shannon's and Simpson's Diversity Index. Samples PT 4, 5 and 15 were the lowest in species evenness and richness (Figure 3).

3.4 Statistics

The TA data were logarithmically transformed for the ordination calculations, and samples with insufficient data were omitted. The data structure was determined with the use of detrended correspondence analysis (DCA) (Table 2**)**. The (DCA) gradient length of less than 3 SD indicates that species data can be described using linear analysis (SD= 2.23), such as principal component analysis (PCA) (Birks 1998; Payne et al. 2013). Principle component analysis (PCA) was utilized to compare sites sampled and species present. The Monte Carlo test and broken-stick Eigen value indicated significant variance correlation within the first 2 axes, axis 1 (P=0.008) and axis 2 (P=0.0030) (Table 3). Axis 1 describes 19.6%, and axis 2 explains an additional 14% of variance in sites. These high values could indicate that species and site are highly associated. PCA results show distinct differences in TA communities among sites for axis 1 (P=0.00800), and axis 2 (P=0.030). PCA indicated that of the 30 taxa, the majority were associated with sites PT1, PT6, PT11, PT2, and PT12. It also indicated that 10 taxa were associated with the remaining sites in PCA **(**Figure 4).

Redundancy analysis (RDA) was used to examine any correlations between TA and environmental variables. These environmental variables include depth of water table (DWT), pH, and carbon content (C) (Figure 5). Axis 1 associated with pH (P=.0020), this explains 23.8% of the variance shown in the data (Table 4).

Table 1. TA taxa present in Panthertown and associated code

Order	Family	Genus Species	Code
Euglyphida	Euglyphidae	*Assulina muscorum*	A5
Euglyphida	Euglyphidae	*Assulina seminulum*	A3
Euglyphida	Euglyphidae	*Assulina sp.*	A.sp
Arcellinida	Centropyxidae	*Centropyxis aerophila*	C2
Arcellinida	Centropyxidae	*Centropyxis aculeata*	C12
Arcellinida	Centropyxidae	*Centropyxis ecornis*	C9
Arcellinida	Centropyxidae	*Centropyxis sylvatica* v. *major*	C5
Arcellinida	Centropyxidae	*Centropyxis* sp.	C.sp
Arcellinida	Centropyxidae	*Centropyxis cassis*	C15
Arcellinida	Centropyxidae	*Centropyxis Elongata*	C16
Arcellinida	Centropyxidae	*Centropyxis discoides*	C17
Euglyphida	Trinematidae	*Corythion dubium*	Cor1
Arcellinida	Trigonopyxidae	*Cyclopyxis eurystoma*	Cy6
Arcellinida	Difflugiidae	*Difflugia* sp.	U11
Euglyphida	Euglyphidae	*Euglypha compressa*	E1
Euglyphida	Euglyphidae	*Euglypha ciliata*	E9
Euglyphida	Euglyphidae	*Euglypha cristata*	E11
Euglyphida	Euglyphidae	*Euglypha* sp	E14
Euglyphida	Euglyphidae	*Euglypha laevis*	E17
Arcellinida	Hyalospheniidae	*Heleopera* sp.	H2
Arcellinida	Hyalospheniidae	*Heleopera pertricola*	H3
Arcellinida	Hyalospheniidae	*Hyalosophenia papilio*	Hy1
Arcellinida	Hyalospheniidae	*Hyalosophenia elegans*	Hy2
Arcellinida	Hyalospheniidae	*Hyalosophenia subflava*	Hy3
Arcellinida	Hyalospheniidae	*Hyalosophenia sp*	Hy.sp
Arcellinida	Hyalospheniidae	*Nebela collaris*	N1
Arcellinida	Hyalospheniidae	*Nebela flabellulum*	N10
Arcellinida	Hyalospheniidae	*Nebela militaris*	N11
Arcellinida	Hyalospheniidae	*Nebela tincta*	N12
Arcellinida	Hyalospheniidae	*Nebela* sp.	N.sp
Euglyphida	Euglyphidae	*Placocista spinosa*	Pc1
Arcellinida	Arcellidae	*Plagiopyxis sp*	Pg.sp
Arcellinida	Hyalospheniidae	*Quadrulella symmetrica*	Q4
Arcellinida	Hyalospheniidae	*Quadrulella sp.*	Qsp
Euglyphida	Euglyphidae	*Sphenoderia lenta*	S1
Arcellinida	Trigonopyxidae	*Trigonopyxis arcula*	Tr1
Euglyphida	Trinematidae	*Trinema complanatum*	T1

Euglyphida	Trinematidae	*Trinema enchelys*	T2
Euglyphida	Trinematidae	*Trinema pernardi*	T11
Euglyphida	Trinematidae	*Trinema sp.*	T.sp

Table 2. DCA output for the general structure of the TA dataset.

Axes	1	2	3
Eigenvalue	0.42107	0.21453	0.08593
Gradient Length	2.779	2.240	1.733

Table 3. PCA output for TA dataset

Axes	1	2	3
Eigenvalue	7.847	5.672	4.930
Broken-stick Eigen value	4.279	3.279	2.779
% of variance	19.617	14.179	12.325
Cumulative % variance	19.617	33.796	46.122

Table 4. RDA output for TA dataset

Axes	1	2	3
Eigenvalue	6.115	2.583	1.320
Broken-stick Eigenvalue			
% of variance	23.8	10.1	5.1
Cumulative % variance	23.8	33.9	39.0

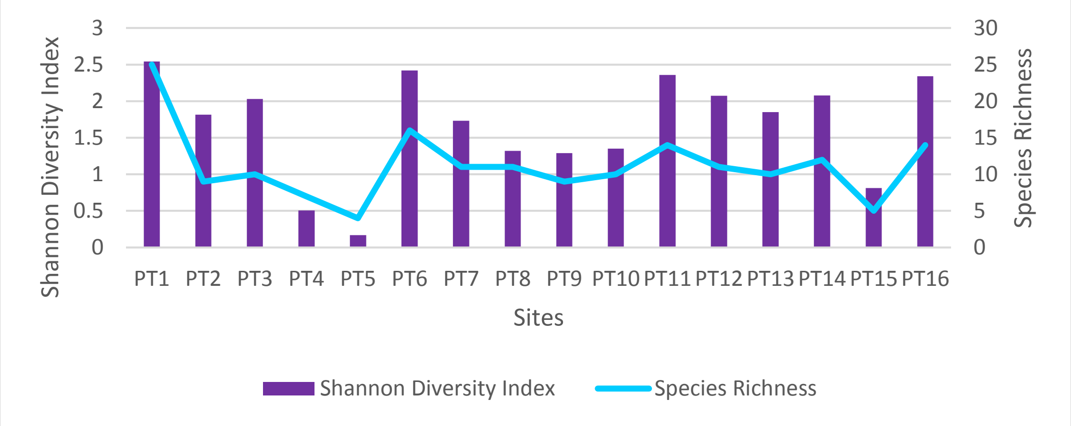

Figure 1. Shannon Diversity index and species richness with sites.

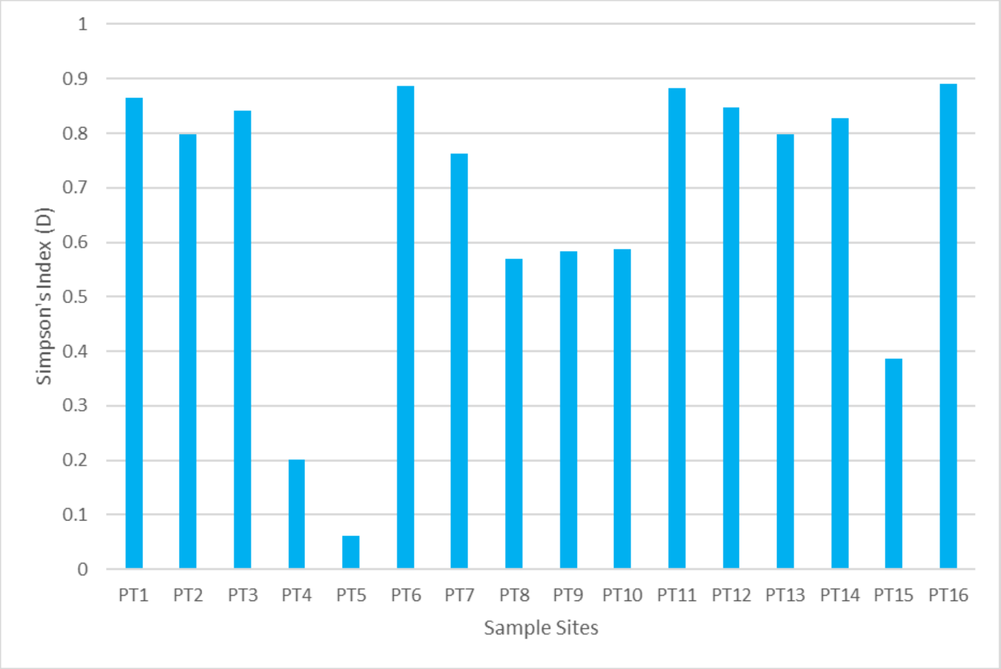

Figure 2. Simpson’s Diversity Index and sample sites.

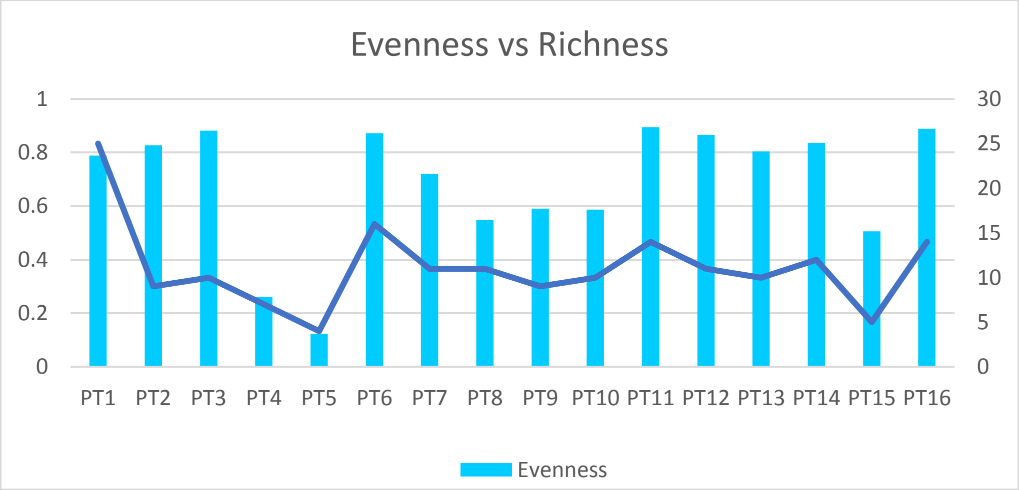

Figure 3. Species Evenness vs. Richness.

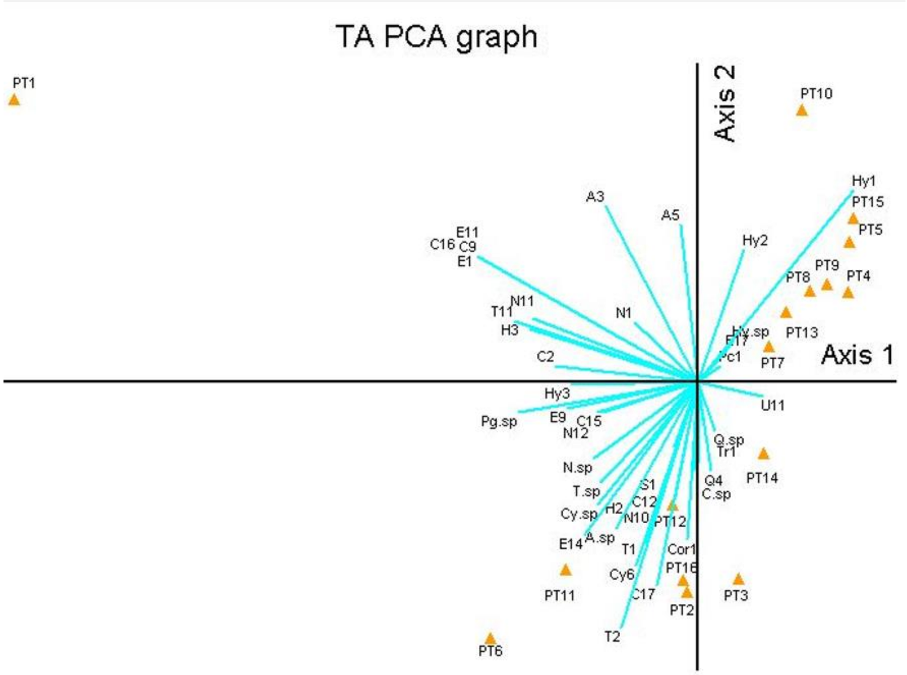

Figure 4. PCA site data and TA species by code.

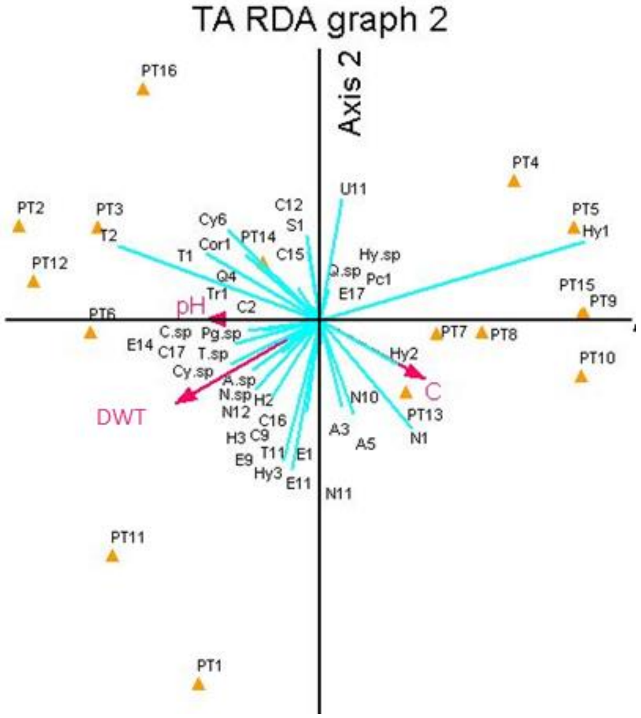

Figure 5. RDA for TA communities represented by code, site data and environmental variables including Carbon (C), DWT (Depth of water table), and pH.

4. Discussion

Knowledge of microorganism communities within Panthertown has applicable uses for land management practices of *Sphagnum* dominated fens in the Southern Appalachians. Most of the TA documented in this study are common and do occur throughout South America, North America and Europe. Low moisture class and less disturbance was hypothesized to produce higher diversity. Therefor drier sites were expected to be less diverse in the TA community for Panthertown. However, the data showed that as biodiversity increased, moisture class increased as well. The goals this section will address are (1) to analyze the TA community assemblages across Panthertown Valley wetland (2) to examine the diversity of the communities in Panthertown and to (3) determine if the TA community correlated with environmental variables.

The assemblege consisted largely of genera that are common globally such as *Hyalosophenia*, *Nebela*, and *Euglypha*. Shannon's Diversity Index was relatively high, over half of the sample sites were 1.5 out of 3. Some TA genera did correlate with environmental variables. Several *Nebela, Assulina* and *Hyalosophenia* species were strongly associated with carbon. The data showed that DWT had a strong association with many TA taxa and is associated with the assemblege more closely than moisture class (Figure 5)

Shannon Diversity Index shows particularly high diversity in PT1, PT3, PT6, PT11 and PT16 while species richness follows a similar trend to diversity, shown in blue (Figure 1). PT1 shows the highest diversity with N=25 species present. Shannon's Diversity Index was high for sites like PT1, which were hypothesized to have low diversity because of high moisture class. As moisture class increased, the species richness increased. The most diverse sites had an average moisture class of 6.42 (semi dry), and the least diverse was 3.7 (wet to very wet). Moist sites should have been more diverse, with an increase in species richness (Figure 1). The PCA indicated that most species were associated with PT1 and similarly grouped sites (Figure 4). RDA reveals that carbon, pH and DWT variation is statistically explained within species assemblege. Data from RDA and PCA did show direct correlations between species and environmental variables: Most taxa were associated with DWT. These data show a decrease in species richness during a time of drought that would correspond with the idea that many taxa such as *Assulina*, *Nebela* and *Centropyxis* were associated with DWT. Sites PT1, PT6, PT11 were strongly associated with DWT and these were also the most diverse sites. The highest species richness (N) is associated with DWT. The DWT may be a vital resource that allows for more diversity within these associated sites, and may be a better association to TA assemblege than moisture class. Several *Nebela, Assulina* and *Hyalosophenia* species are associated with carbon (C) (Figure 5). Statistical analysis indicates that the populations are dynamic and changing, therefor we can reject the null hypothesis that the populations are unchanging or static and accept the alternative hypothesis that they are different and changing.

The data showed unexpected results for biodiversity because higher moisture class and therefor drier sites were more closely associated with TA assemblege than moisture class because DWT was the main association to TA diversity. PT1 had the highest diversity and was the most associated with a closed forest canopy when compared to the other sites. The results of high moisture class correlating with higher TA diversity in sites was unexpected because sites with low moisture class were hypothesized to be higher in diversity, and would increase in species richness. However, this hypothesis was not supported. Contributing factors to the results may have been environmental conditions such as the drought in the fall of 2016. The atypical environmental conditions in combination with increased light conditions for the wetland, and stressed vegetation communities, may have all correlated with the low species richness. Diversity at the few drier sites may be due to competition for minimal resources as TA biodiversity was most directly correlated with DWT. TA dispersal can occur in months and years, potentially explaining the unexpected assemblege in accordance with that times environmental conditions.

Additional research may include more methods to analyze the relationships between vegetation communities and TA communities' correlations. To provide more baseline ecological data on vegetation communities percent canopy cover for each sample could be utilized to give a better indication of the environmental variables. More samples sites would provide more data that may lead to more accurate and generalized results.

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6. References

1. E. Mitchell, D. Charman, B. Warner, "Testate amoeba analysis in ecological and paleoecological studies of wetlands: past, present and future," *Biodiversity and Conservation* 17, (2008): 2115-2137.
2. R. Payne, D.J. Charman, S. Matthews, W.J. Eastwood,"Testate amoebae as palaeohydrological proxies in Sürmene Ağaçbaşi Yaylasi Peatland (Northeast Turkey)," *Wetlands* 28 no. 2 (2013): 311-323.
3. E.A.D. Mitchell, A. Butler, P. Grosvernier, H. Rydin, C. Albinson, A. L. Greenup, M.M.P.D Heijmans, M. R. Hoosbeek, and T. Saarinen. "Relationships among testate amoebae (Protozoa), vegetation and water chemistry in five *Sphagnum*-dominated peatlands in Europe," *New Phytologist* 146 (2000): 95-106.
4. B. Fournier, E. Malysheva, Y. Mazei, M. Moretti, and E.A.D. Mitchell, "Toward the use of testate amoeba functional traits as indicator of floodplain restoration success," *European Journal of Soil Biology* 49 (2012): 85-91.
5. C. G. Ogden, and R. H. Hedley. *An atlas of freshwater Testate Amoeba*. (London and Oxford, UK: British Museum of Natural History an Oxford University press, 1980)
6. K. Tolonen, *Rhizopod analysis* (New York, NY, USA: John Wiley and Sons, 1986): 645-666
7. B. G. Warner, *Testate amoeba (Protozoa),* ed. B.G. Warner (St. John's Newfoundland, Canada: Geological Association of Canada, 1990), 65:74
8. D. Gilbert, C. Amblard, G. Bourdier, and A.J. Francez, "The microbial loop at the surface of a peatland: structure function, and impact of nutrient input," *Microbial Ecology* 35 (1998): 83-93
9. V.E.J. Jassey, C. Meyer, C. Dupuy, N. Bernard, E.A.D. Mitchell, M.L. Toussaint, M. Metian, A. Chatelain, and D. Gilbert, "To what extent do food preferences explain the trophic position of heterotrophic and mixotrophic microbial consumers in a *Sphagnum* peatland?" *Microbial Ecology* 66 (2013): 571-580.
10. R. Wylezich, S. Meisterfeld, M. Meisterfeld, M. Schlegel, "Phylogenetic analyses of small subunit ribosomal RBA coding regions reveal a monophyletic lineage of euglyphid testate amoeba (order Euglyphida)," *Journal of Eukaryotic Microbiology* (2002): 108-118.
11. S.I. Nikolaev, E.A.D. Mitchell, N.B. Petrov, C. Berney, J. Fahrbi, J. Pawlowski, "The testate lobose amoeba (order Arcellinida Kent, 1880) finally find their home within Amoebozoa," *Protist* no. 156 (2005): 191-202.
12. E. Lara, C. Berney, F. Ekelund, H. Harms, A. Chatzinotas, "Molecular comparison of cultivable protozoa from a pristine and a polycyclic aromatic hydrocarbon polluted site," *Soil Biology and Biochemistry* 39 (2007): 139-148.
13. E. Lara, T.J. Heger, F. Ekelund, M. Lamentowicz, E.A.D. Mitchell,"Ribosomal RBA genes challenge the monophyly of the hyalospheniidae (Amoebozoa: Arcellinida) *Protist* 156 (2008): 165-176.
14. B. Fournier, E.E.D. Coffey, W.O. Van der Knaap, L.D. Fernandez, Anatoly Bobrov and Edward A.D. Mitchell, "A legacy of human-induced ecosystem changes: spatial processes drive the taxonomic and functional diversities of testate amoebae in *Sphagnum* peatlands of the Galapagos," *Journal of Biogeography* (2015): 1-9.
15. K. Booth, "Ecology of Testate Amoeba (Protozoa) in two Lake Superior coastal wetlands: Implications for paleoecology and environmental monitoring," *Wetlands* 21 no. 4 (2001): 564-576.
16. D.N. Cole, and N.G. Bayfield, "Recreational trampling of vegetation: Standard experimental procedures," *Biological Conservation* 63 (1992): 209-215.
17. W. Jung, "Thecamöben ursprünglicher lebender deutscher Hochmoore," *Abh Landesmus Westif* 7 (1936): 1-87
18. D. Charman, D. Hendon, and W. Woodland, *The Identification of Testate Amoebae (Protozoa: Rhizopoda) from British Oligotrophic Peats*. (Cambridge: Quaternary Research Association, 2000).
19. K.J. Clarke, *Guide to the Identification of Soil Protozoa Testate Amoebae Special Publication* (Ambleside: Freshwater Biological Association 2015), 12.