WHAT ENVIRONMENTAL CONDITIONS DO TESTATE AMOEBAE PREFER IN THE PERUVIAN PEATLANDS?

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

What Environmental Conditions do Testate Amoebae Prefer in the Peruvian Peatlands?

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Testate amoebae are a group of protozoa that live in aquatic environments such as peatlands, estuaries, and lakes, and their shells are often well-preserved in the sediments of these environments. These organisms are useful for reconstructing change in nutrient status and hydrology due to their sensitivity to pH, soil moisture, and water table depth (WTD). However, this method has seldom been applied to non *Sphagnum*-dominated peatland environments such as mountain fens. Here we present the first training set and transfer function from high Peruvian Andes cushion peatlands. These ecosystems are dominated by *Distichia muscoides,* a robust cushion-forming plant from the Juncaceae family. Fifty surface samples of *Distichia* peat were collected from six sites in the Cordillera Vilcanota, southeastern Peru, in summer 2017. Water table depth, conductivity, and pH were measured during sample collection; peat moisture was calculated in the lab using the difference between fresh and dry peat weights. After analyzing 20 of the 50 samples, a transfer function was generated on the basis of statistical relationships between each testate amoeba taxon and its environmental preferences. Preliminary results suggest that WTD is the main factor controlling testate amoebae communities at our sites. Our transfer function, which connects each testate amoeba taxon with its preferred WTD will be

applied to reconstruct past hydrological changes across the region and provide a new means to better understand past climate change.

ACKNOWLEDGMENTS

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CHAPTER I

INTRODUCTION

Peatlands

A peatland is a type of water-saturated wetland ecosystem with little to no oxygen that accumulates peat layers over millennia. These peat layers, which are typically several meters thick, are rich in organic matter because they are made of decomposed plant residues (Rydin and Jeglum 2006). Peat accumulation occurs because long-term peat accumulation outpaces peat decomposition. Peatlands are sensitive to changes in hydrology and temperature. For example, when temperature increases and water table levels decrease, plant matter decomposes more quickly, which may lead to slower accumulation rates. For this reason, peatlands are important paleoenvironmental and paleoclimate archives and have been used as such for many decades. In Peru, peat-accumulating wetland ecosystems are known as 'bofedales'.

Bofedales in the Peruvian Andes

Bofedales in the high Andes of South America are defined by the presence of peat or organic soil, and are dominated by *Distichia muscoides*, a plant from the Juncaceae family that forms hard cushions (Cooper et al. 2015). Bofedales receive their water from rivers, lakes, groundwater, and melting glaciers. They are important sources of water for local residents; they also support biodiversity, which varies depending on factors like altitude, location, livestock populations, topography, and moisture. Bofedales are threatened by heavy grazing from livestock across the region, which mainly consists of llamas, alpaca, and sheep.

The Peruvian Andes contain around 70% of the world's tropical glaciers and the world's largest tropical ice cap, including the Quelccaya ice cap, which is located 5670m above sea level (Thompson, 2006). These tropical glaciers are at high risk of disappearing due to global warming. Many tropical glaciers in the Andes are retreating, endangering bofedales by depleting them of meltwater during the dry summer season.

Bofedales are typically found between 3000 and 5000m in the Peruvian Andes (Squeo 2006). The vegetation of the bofedales is typically greener than the surrounding areas, forming a contrast (Figure 1). Vegetation is determined by water and moisture levels, water pH, humidity and temperature, and altitude. The water pH is close to neutral and can be alkaline, ranging from 6.82 to 9.71 based on our own field measurements. The preserved peat deposits in these bofedales can provide an insight into the age of the landscape as well as the paleoclimate and ecological development of the area. Overall, bofedales are very important carbon sinks regionally, but are threatened by anthropogenic activities (grazing) and climate change (glacier loss and change in hydrology).

Figure 1: Visual of bofedales and surrounding landscape. Above is a picture of alpacas grazing a patch of Peruvian peatlands. Notice the difference between the bofedales and the rest of the terrain. *Photo credit: Maryam Cheta*

Testate Amoebae

Testate amoebae are microorganisms found in aquatic environments such as peatlands, lakes, and estuaries. They have decay-resistant shells that are well-preserved in the sediments of these aquatic environments (Mitchell *et al.* 2008). Testate amoebae communities that live within the peat layers tend to be very sensitive to changes in water table depth (Charman, 2001). These cosmopolitan species**,** which are found everywhere on Earth and appear to have similar environmental preferences regardless of location, are useful to reconstruct past hydrological changes from the peat archive.

CHAPTER II

METHODOLOGY

Study Area

This study took place in the Cordillera Vilcanota in the Peruvian Andes, located around 100km from Cusco, Peru (Figure 2). The tallest peak in the Cordillera Vilcanota mountain range is Mount Ausangate at 6384 meters above sea level (asl). This mountain range includes several other peaks over 6000 meters. During the past glaciation, the lower valleys of this region were glaciated; a multitude of glaciers still remain at higher elevations. A large portion of these valleys are composed of bofedales, where Quechua locals reside and raise livestock such as alpaca and llamas.

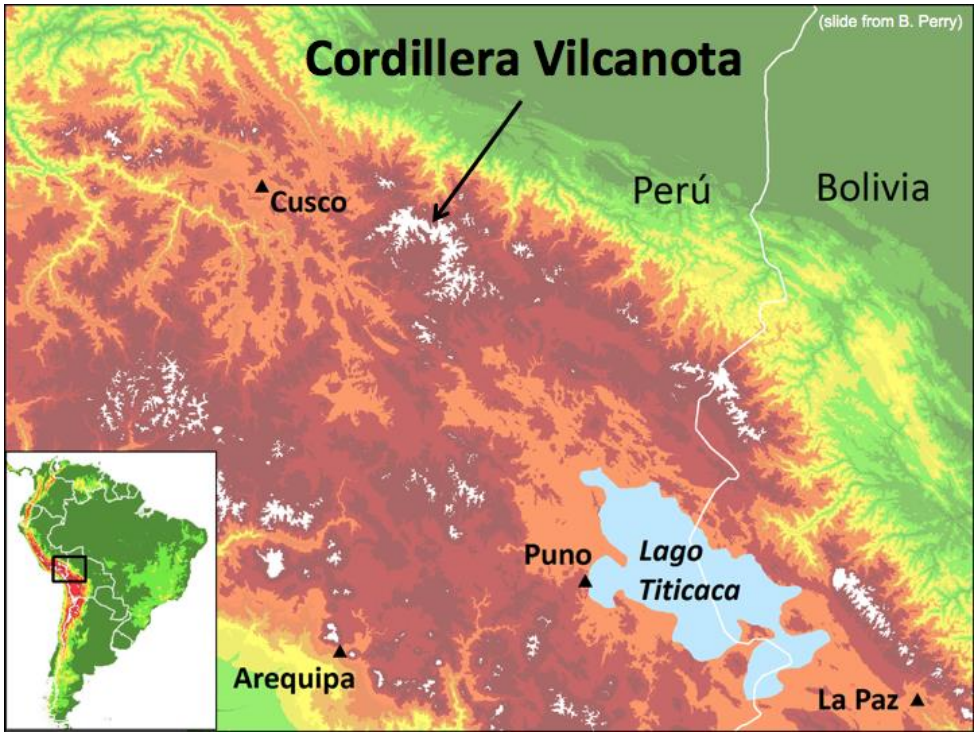

Figure 2: Study area map. Map courtesy of Dr. Baker Perry.

Field Sampling

Fifty soil surface samples were collected within two bofedales: Murmurani (4883 meters asl with coordinates 13°48’47.14” S 71°4’33.94” W) and Quilleta (4706 meters asl with coordinates 13°50’33.50” S 71°10’20.21” W) (Figure 3). These surface samples, which consist of approximately 5 x 5 x 10 cm cores, were extracted by cutting out a piece of the peatland with a large bread knife (Figure 4). The samples were collected following a hydrological gradient at each of the two sites, such that a broad range of WTD (from wet to dry) was sampled. Water samples were also bottled and brought back to our lab; water table level, pH, water temperature, and conductivity were measured on site. Each sample was also weighed at the time of sampling. In Quilleta, the samples were taken from quadrants that were 15.24 meters (50 feet) apart along a transect. In Murmurani, the samples were taken following the same methods as the previous site, but from random areas within the sampling site.

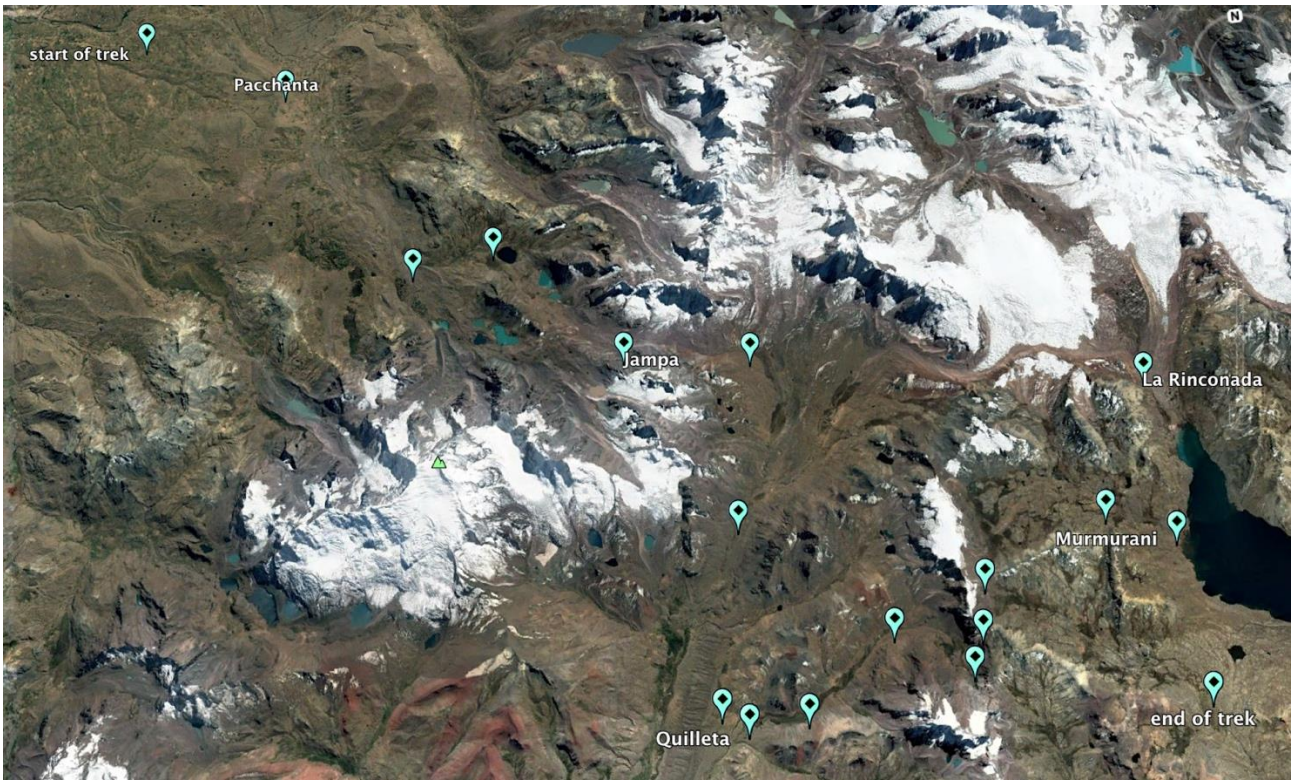

Figure 3: Field sampling map. Shown are the two sampling sites, Quilleta and Murmurani. *Data source: Dr. Julie Loisel*

Figure 4: Field sampling. The top left picture shows sample extraction using a large bread knife. The bottom left picture shows students flagging potential sample sites in Murmurani. The picture on the right shows an extracted soil sample from the sample site in Murmurani. *Photo credit: Dr. Julie Loisel*

Laboratory Methods

To determine peat moisture content, each soil sample was individually re-weighed upon our arrival in the lab at Texas A&M University. A subsample was then taken from each soil sample and individually weighted (fresh weight), placed in a drying oven over two days to evaporate all moisture content, and weighted once more (dry weight). The peat bulk density and its moisture content were calculated following the standard 'loss-on-ignition' procedure (Chambers et al. 2010).

To prepare the testate amoebae for analysis, we follow standard procedures (Booth et al. 2010). The first step involved subsampling 1 cubic centimeter from each sample and placing it in a 100ml beaker. Each beaker was filled with approximately 30mL of distilled water; one tablet of *Lycopodium* spores was added. The *Lycopodium* spores serve as a marker to calculate the concentration of testate amoebae in our future analysis. The samples were placed on a hotplate and gently boiled for 10 minutes. After the samples had boiled, they were sieved using distilled water and two sieves, one of 15 microns and one of 300 microns. The leftover particulate matter and water caught between the two sieves was washed in a centrifuge tube of 50mL. The centrifuge tubes were run at 5,000 rpm for five minutes. Water was then decanted from the centrifuge tubes and the remaining residue was stained with Safranine. Safranine is a red dye that allows for easier distinction of testates in analysis. After the Safranine was added, water was again added to the centrifuge tube, which was run through the centrifuge for a second time. The remaining residue was transferred into a 5mL vial and stored in glycerol.

The prepared testate samples were transferred to the microscope room to conduct further research. Compound microscopes were used to identify and count the various testate amoebae taxa in 20 of the 50 samples. At least 50 testates were counted per sample to get a statistically representative community. After the samples were analyzed, the first training set to be formed for the high Andes was generated on the basis of statistical relationships between each testate amoeba taxon and its environmental preferences. A transfer function was then created by using the training set; this new paleo tool will be used to infer past environmental conditions down a peat core by analyzing the testate communities in the future.

CHAPTER III

RESULTS

Testate Amoebae Counts

Over the course of this analysis, 43 species of testate amoebae were identified (Figure 5). Three of the 43 species identified remain unknown and have been given names temporarily for identification purposes.

	Testate Species Identified		
Amphitrema wrightianum	*Centropyxis ecornis*	*Heleopera sylvatica*	*Placocista spinosa*
Apodera vas	*Centropyxis platystoma*	*Hyalosphenia elegans*	*Pseudodifflugia fulva*
Arcella artocrea	*Cyclopyxis arcelloides*	*Hyalosphenia hyanis*	***Quadrulella soccerball***
Arcella catinus	*Difflugia bacillariarum*	*Hyalosphenia insecta*	*Quadrulella symmetrica*
Arcella discoides	*Difflugia pulex*	*Hyalosphenia minuta*	*Spenoderia lenta*
Arcella gibbosa	*Difflugia rubescens*	*Hyalosphenia papilio*	*Trigonopyxis arcula*
Arcella vulgaris	***Euglypha noteeth***	*Lesquereusia modesta*	***Trigonopyxis huge***
Assulina muscorum	*Euglypha rotunda*	*Nebela collaris-bohemica*	*Trigonopyxis microstoma*
Assulina seminulum	*Euglypha tuberculata*	*Nebela militaris*	*Trinema lineare*
Centropyxis aculeata	*Heleopera petricola*	*Nebela tincta*	*Trinema/Corythion*
Centropyxis cassis	*Heleopera sphagni*	*Phryganella acropodia*	

Figure 5: List of testate amoebae. Above are all the species of testates identified in this study. Three testate species are still unknown to us and have been given names (those underlined and bolded).

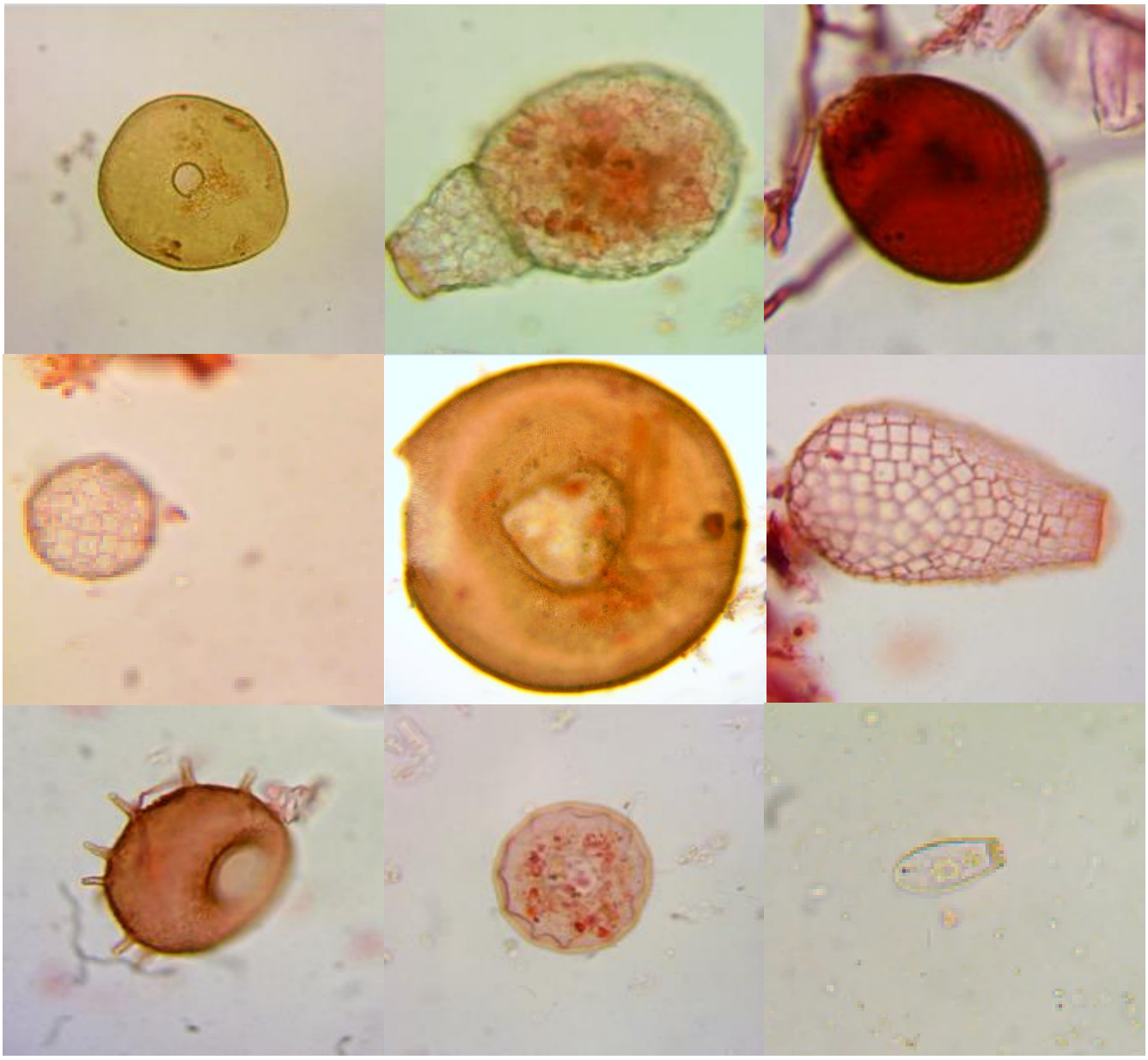

Figure 6: Testate amoebae. Above are a few of the testate amoebae found during out analysis (moving top left to right, then bottom left to right): *Arcella artocrea, Apodera vas, Assulina seminulum, Unknown testate 1 (referred to as Quadrulella soccerball), Arcella discoides, Quadrulella symmetrica, Centropyxis aculeata, Arcella gibbosa, and Euglypha rotunda*. *Photo credit: Marla Martinez*

There was a broad range of species per sample (Figure 6). In the majority of the samples, a large percentage of the testate *Centropyxis aculeata* was found (Figure 7). Upon analyzing the testate counts, an inverse relationship between the concentrations of *Centropyxis aculeata* and both *Assulina muscorum* and *Assulina seminulum* was observed. A weaker inverse relationship was also discovered between the concentrations of *Centropyxis aculeata* and *Euglypha rotunda*

and *Euglypha tuberculata*. One of our unknown samples, *Quadrulella soccerball*, was typically found in the same samples that *Quadrulella symmetrica* was found.

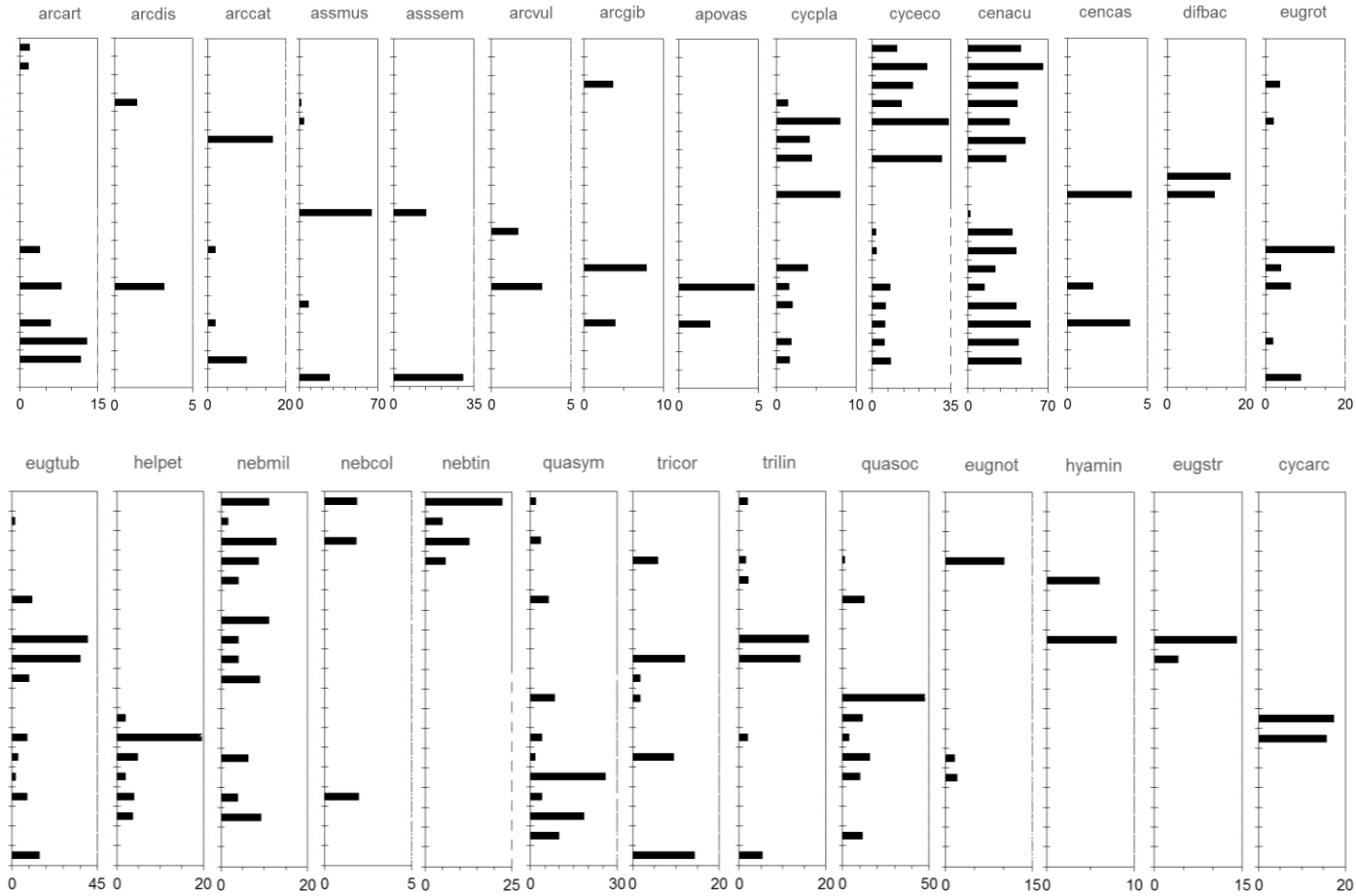

Figure 7: Testate amoebae chart. Above are the most common testate amoebae species throughout our samples. The charts show the percentage of testates in each sample.

Statistical Analysis

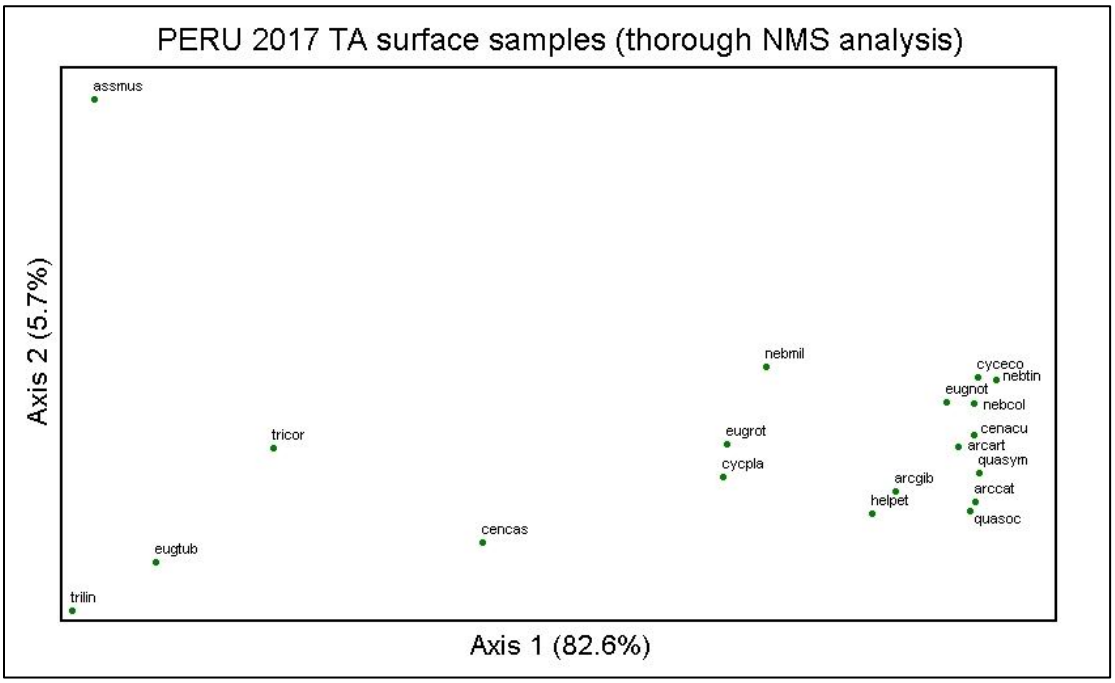

Figure 8: NMS plot. This figure quantifies how similar the testate taxa identified are to each other by displaying their proximity.

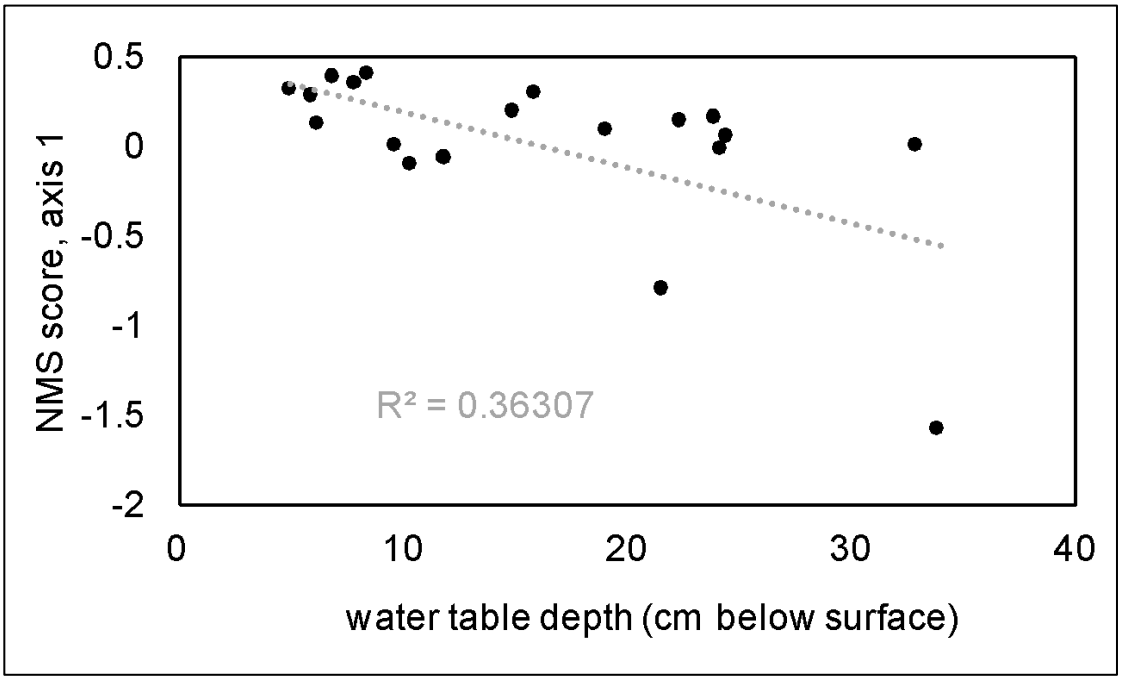

Figure 9: Axis 1 plotted against water table depth (WTD).

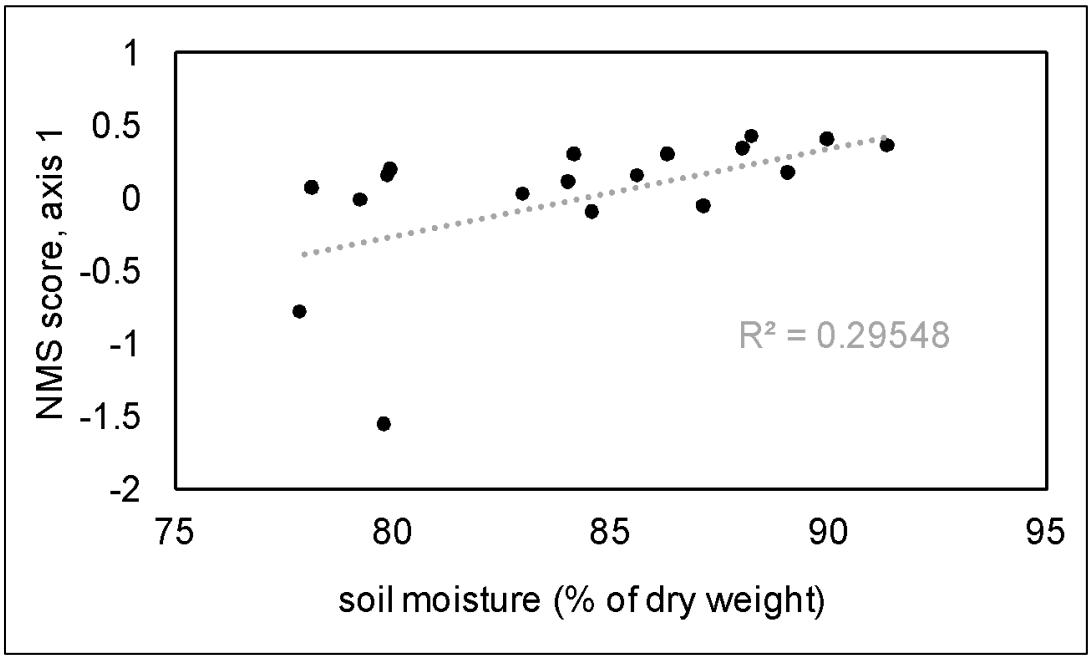

Figure 10: Axis 1 plotted against soil moisture.

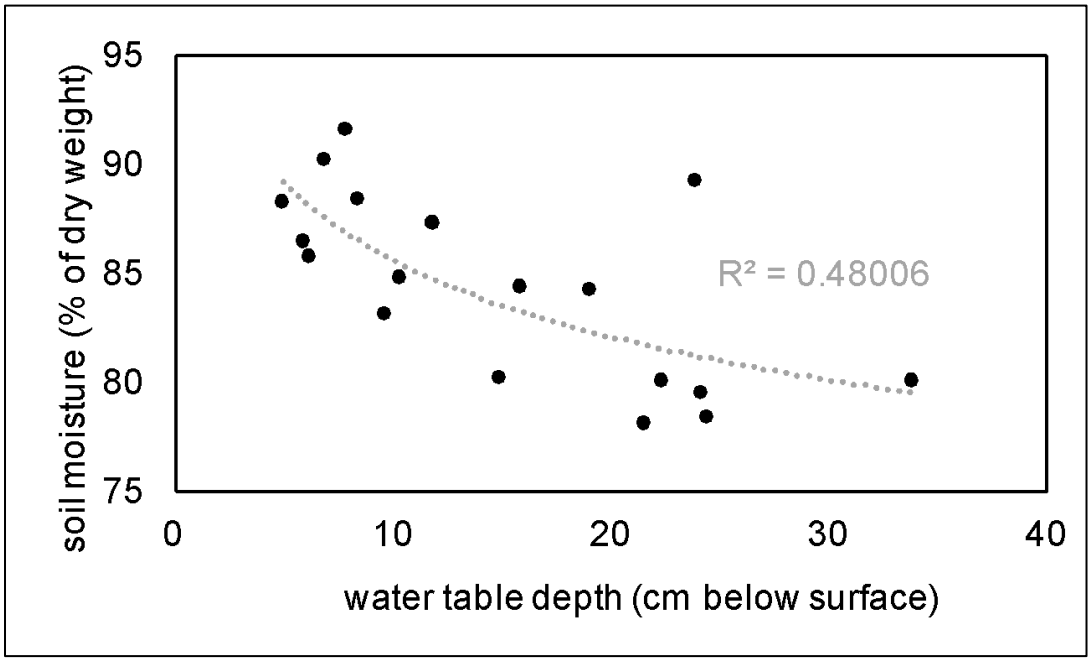

Figure 11: Soil moisture plotted against water table depth.

A training set was developed using non-metric multidimensional scaling analysis (NMS), which was performed in PC-ORD; the NMS analysis shows a good spread of the testate amoebae along Axis 1. Axis 1 represents the environmental preferences of the testate amoebae based on drier or wetter environmental conditions, and explains 82.6% of the distance between the taxa (Figure 8). In the NMS plot, *Cyclopyxis ecornis*, *Nebela tincta*, *Euglypha noteeth*, *Nebela collaris-bohemica, Centropyxis aculeata, Arcella artocrea, Quadrulella symmetrica, Arcella catinus, and Quadrulella soccerball* all fall within the same or similar columns along Axis 1, preferring wetter environmental conditions. Plotting the Axis 1 scores against water table depth measurements from each of the twenty samples reveals a statistically strong correlation, indicating that Axis 1 can largely be explained by water table depth ($R^2 = 0.36$) (Figure 9). The correlation was not as strong between soil moisture and Axis 1 ($R^2 = 0.30$) (Figure 10). Plotting water table depth (cm below surface) against soil moisture (% of dry weight) displays a statistically weaker correlation than the above two correlations ($R^2 = 0.23$) (Figure 11).

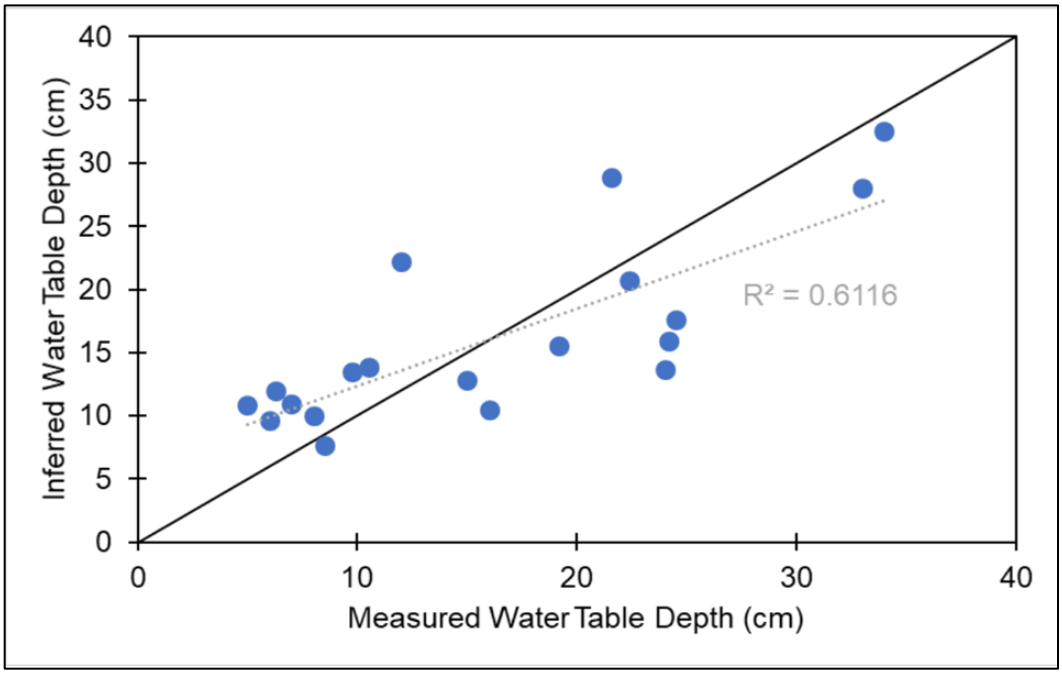

Figure 12: Transfer function. The transfer function successfully predicts WTD measurements.

The transfer function, which was developed using the R package rioja, successfully predicts water table depth measurements (0.61) (Figure 12). That said, it seems to slightly overestimate wet assemblages (as seen by the blue dots above the 1:1 line towards the left end of the relationship) and slightly underestimate a few of the mid- to high-range assemblages (as seen by the blue dots below the 1:1 line towards the center of the graph). Lastly, the transfer function provides a WTD optimum for each testate amoeba taxon (Figure 13). This can be used in paleohydrological reconstructions.

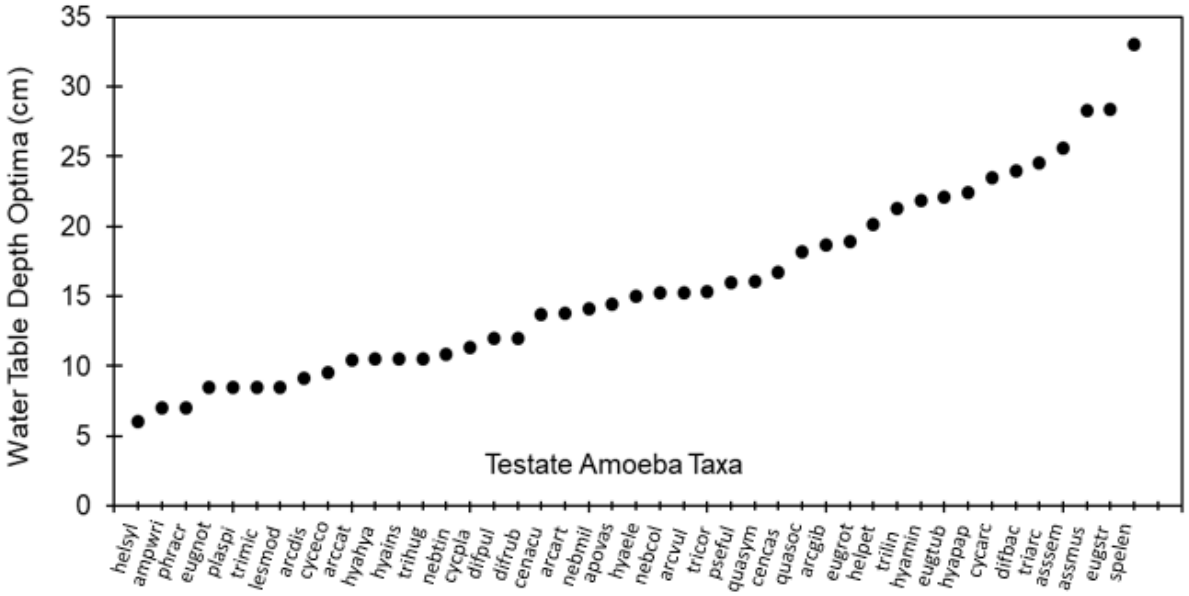

Figure 13: Testate amoebae taxa plotted against water table depth optima. This graphs displays what water table depth each taxon primarily thrives in.

CHAPTER IV

CONCLUSION

Bofedales are often studied for their importance as carbon sinks and underlying peat composition that provide insight into past changes in hydrology and temperature. Based on the above analyses, water table depth appears to be the main control of testate amoebae concentrations in the bofedales of Quilleta and Murmurani.

The testates found at the lower water table depths between 5 and 10 cm include *Heleopera sylvatica, Amphitrema wrightianum, Phryganella acropodia, Euglypha noteeth,* and *Placocista spinosa*. On the opposite spectrum in the higher water table depths between 30 and 35cm, testates found include *Trigonopyxis arcula, Assulina muscorum, Assulina seminulum, Euglypha strigosa,* and *Spenoderia lenta*. Our future work includes finishing the analysis of the 50 soil samples taken in Quilleta and Murmurani to create a more accurate transfer function. The transfer function generated successfully predicted water table measurements, and can be applied to reconstructing paleohydrological changes in the bofedales of Peru.

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