MONITORING THE DISTURBANCE OF SOIL MICRO-ECOSYSTEMS IN DORMANT TOPSOIL: USING NEMATODES AND STUDYING THE METHODS TO REHABILITATE SOIL FOR EFFECTIVE SITE RESTORATION

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ABSTRACT

In the Four Corners Area of New Mexico there are over 20,000 gas wells. Energy Companies must reclaim the abandoned well sites and access roads. Reclamation involves re-contouring, restoring topsoil, and reestablishing native vegetation. Reestablishing plant biodiversity quickly is desired to minimize soil erosion, protect surface water, and provide forage for animals. It typically takes five to seven years to re-vegetate a site with favorable soil conditions. Stable topsoil takes hundreds of years to develop and the preservation of topsoil is a critical conservation issue in maintaining a sustainable environment.

Research proves nematodes are effective indicators of soil ecosystem health (1). The goal of this year's research is to investigate whether spread or stockpiled topsoil, will more thoroughly maintain a nematode distribution consistent to native undisturbed topsoil.

The Nematodes were classified into trophics, and hand elutriation was used to extract the nematodes from the soil. The results proved the storage of topsoil directly affects the micro-ecosystem following disturbance, and topsoil spread evenly maintains a more comparable micro-ecosystem to native soil than piled topsoil. The nematode distribution in the 2010 guide for the Flora Vista native grass ecosystem (1) was modified using the trophic data.

INTRODUCTION

After well sites no longer economically produce fossil fuels, energy companies are required to restore sites to native conditions. The Bureau of Land Management and United States Forest Service provide reclamation guidelines in the Conditions of Approval (COAs) for Application Drilling (APD) permits. All companies must re-contour, restore topsoil and reestablish native vegetation to effectively abandon a site (2, 3). Reestablishing plant biodiversity quickly minimizes soil erosion, prevents surface water contamination, and provides forage for animals. Typically a site with favorable soil conditions takes five to seven years to re-vegetate.

During construction, topsoil is generally ruined due to poor management. Topsoil is the uppermost soil layer, around 5 to 15cm thick in the semi-arid climate of New Mexico. Topsoil has the highest concentration of organic matter and microorganisms enabling the uptake of nutrients and water for healthy growth in plants (4). It can take hundreds of years to develop suitable topsoil, and the preservation of topsoil is a critical conservation issue in maintaining a sustainable environment.

Soil nematodes found most prominently in topsoil are microscopic round worms that live in any soil and aquatic environment (5). Nematodes are the simplest and most numerous multi-cellular animal on Earth. Due to their simple structure, they provide effective indicators of soil micro-ecosystem health. Research has proven that nematode distribution ratios serve as a guide to environmental managers during restoration (1,6).

This is the fourth year researching nematodes as indicators of soil ecosystems health. During 2007-08, research indicated that soil chemistry was an effective, but expensive indicator; however, the use of nematodes was inconclusive and required more investigation. The 2008-09 research focused on developing a simple method for extracting nematodes and developing a key to identify native nematodes. The Baermann-Funnel was modified, and a Four Corners Nematode Key was formed (7), refer to Appendix 1. In 2009-2010, research proved nematode distributions in native topsoil were dependent on geographic area and plant community. A guide was formed for environmental managers to evaluate the current health of soil ecosystems using nematode distributions (1), refer to Appendix 2.

It was believed that the storage of topsoil directly affects the micro-ecosystem following disturbance, and topsoil spread evenly would maintain a more comparable ecosystem to native soil than stockpiled topsoil. The disturbed plot will have little to no activity at the microscopic level following disturbance. It was also believed, that the most prevalent nematode species of each trophic could be cultured to the native distribution of any specific plant community and geographic location within the San Juan Basin.

METHODS

Site Selection and Field Sampling

In the Flora Vista area of San Juan County, New Mexico, a test site was selected in undisturbed grass plant community consistent with the earlier research. The test site was a 25m by 25m square, fenced with T-posts and wire. Within the site two 3m by 5m subplots were selected, and the topsoil was removed to a depth of 12.5 cm. Topsoil from one subplot was piled as recommended in the COAs, while topsoil from the second subplot was evenly spread in a 12.5-cm layer across the surface of native soil. No vegetation was stripped in the areas where the

topsoil was placed. Every week two samples were collected from the disturbed subplots, undisturbed areas of the test site, and stored topsoils. The grab samples were placed in glass jars, labeled (sample ID; A/B; date), and refrigerated to maintain the nematodes until extraction.

Sample Elutriation Extraction

For the extraction, each sample was placed in an individual zip-lock bag, slightly moistened, and left overnight. The following morning, 100cc of soil from each sample was placed in an 8L bucket filled with three quarts of tap water. The samples were hand mixed in the water for 20 seconds and then allowed to sit for 10 seconds. The water was poured over a #35 and #400 sieve stack. The #35 sieve was rinsed and removed, trapping large debris and rocks. The #400 sieve was rinsed with tap water to remove the fine soil particles, and the remaining debris and nematodes were washed into a 200cc test tube.

The test tubes were centrifuged at 26 mph for a 5-minute cycle. Excess water was replaced with sucrose, and the test tubes were sealed. Each sample was mixed, and centrifuged for another 5-minute cycle. Then the liquid was transferred onto a 20µm sieve, leaving the soil in the test tube. The nematodes on the surface of the sieve were rinsed into a vile and sealed. Samples remained refrigerated until analyzed.

Nematode Assessment

One ml of liquid was removed with a pipette from each vile and placed on a slide. Then each slide was analyzed under an Olympus IX51 Inverted Microscope with a 100W Halogen light source and 40X Plan Fluorite Universal objectives. Images were taken with an Optixcam Summit 5MP camera processed with OC View Software 2009. Each nematode was classified by trophic, refer to Appendix3, and all data was recorded in an Access Database® for analysis.

RESULTS

The total population of nematodes present in the undisturbed Native samples decreased during the second sample, but recovered in the final sample (520, 360, 575) during the four month test period. However, the distribution stayed within a small margin of variance, refer to Figure 1. When analyzing the spread topsoil there was a substantial increase of Fungivore nematodes over the collection period, refer to Figure 2. The other nematodes decreased, but maintained a consistent ratio to native soil following the second sample on August 3, 2010, refer to Figure 3. As seen in Figure 4, the stockpiled topsoil showed a significant increase in Herbivore nematodes and a marginal decrease in Fungivore nematodes, during the test period (Herbivore 48.8%, Fungivore 14.7%). After the first month, the disturbed plot's depleted to minimal micro-organism activity, refer to Figure 5. In all samples sporadic rain storms in August and September caused slight inflation in the nematode populations, but the increase was small enough to avoid seriously skewing the sample distributions. The data for the Native grass plant community in Flora Vista was used to update the 2010 San Juan Basin Guide with the trophic distribution, refer to Appendix 2.

DISCUSSION & CONCLUSION

The nematode distributions in the native samples were expected because it is common for undisturbed topsoil to sustain a balanced micro-ecosystem throughout the seasons, unless there is a major adjustment in climate. It is believed that the ecosystem within the spread topsoil maintained due to the nutrients and healthy undisturbed soil beneath. The active plant ecosystem began to slightly adjust to the new topsoil in the last month of collection, allowing for the soil to conserve a healthy ecosystem. The Stockpiled topsoil is believed to have the major increase in the

Herbivore population because the piled soil retained more moisture than native soil. Because the soil had increased water, there was a denser population of micro-organisms and plant matter. The soil itself was sustaining a healthy micro-ecosystem, but was not fit to support a Native grass plant community in Flora Vista. The Disturbed plot was soil below the upper root zone and has a naturally less active micro-ecosystem than topsoil. The Disruption in the ecosystem was detrimental to any micro-organism living in that depth of soil because they were exposed to the elements. The rain storms did not skew the distributions; rather all the nematode trophics increased maintaining a constant distribution.

In conclusion, the nematode distributions within the spread topsoil were more consistent with the native distribution than the stock piled soil over a 5 month period. The disturbed plot had normal microscopic activity within the first sample, but depleted to nearly no activity in less than a month as predicted.

Nematodes were classified into trophics, and a few common species were identified, but due to time restraints, it is still uncertain whether each nematode trophic can be cultured. Despite time limits, literature review, and observation has revealed that both herbivore and bacterivore nematodes are commonly cultured.

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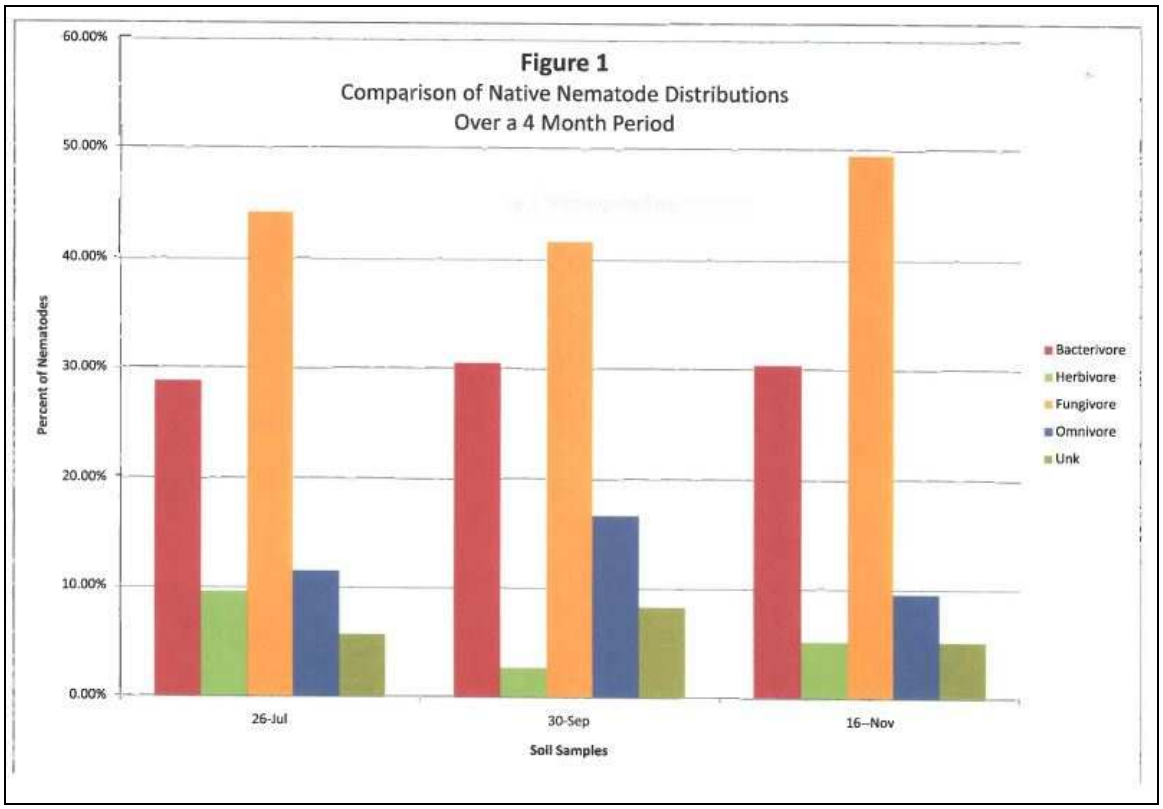


Figure 1
Comparison of Native Nematode Distributions
Over a 4 Month Period
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Percent of Nematodes
26-Jul
30-Sep
16--Nov
Soil Samples
Bacterivore
Herbivore
Fungivore
Omnivore
Unk

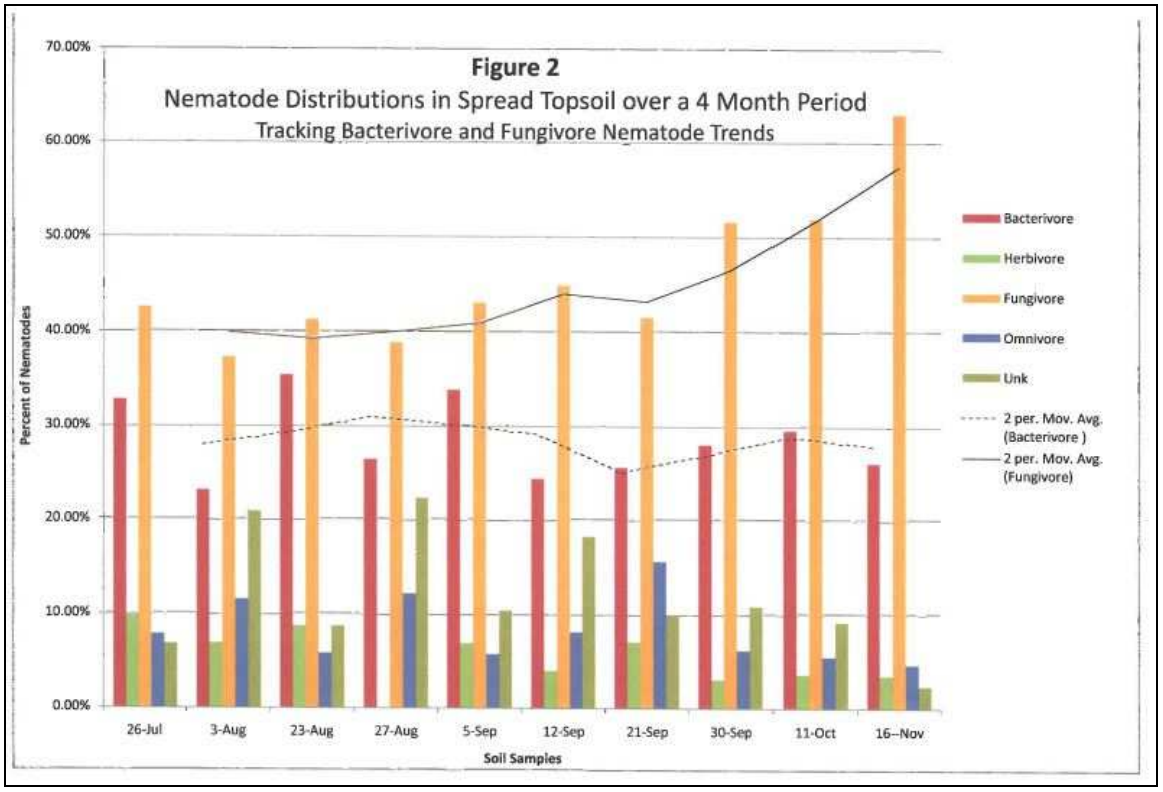


Figure 2
Nematode Distributions in Spread Topsoil over a 4 Month Period
Tracking Bacterivore and Fungivore Nematode Trends
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Percent of Nematodes
26-Jul
3-Aug
23-Aug
27-Aug
5-Sep
12-Sep
21-Sep
30-Sep
11-Oct
16--Nov
Soil Samples
Bacterivore
Herbivore
Fungivore
Omnivore
Unk
2 per. Mov. Avg. (Bacterivore)
2 per. Mov. Avg. (Fungivore)

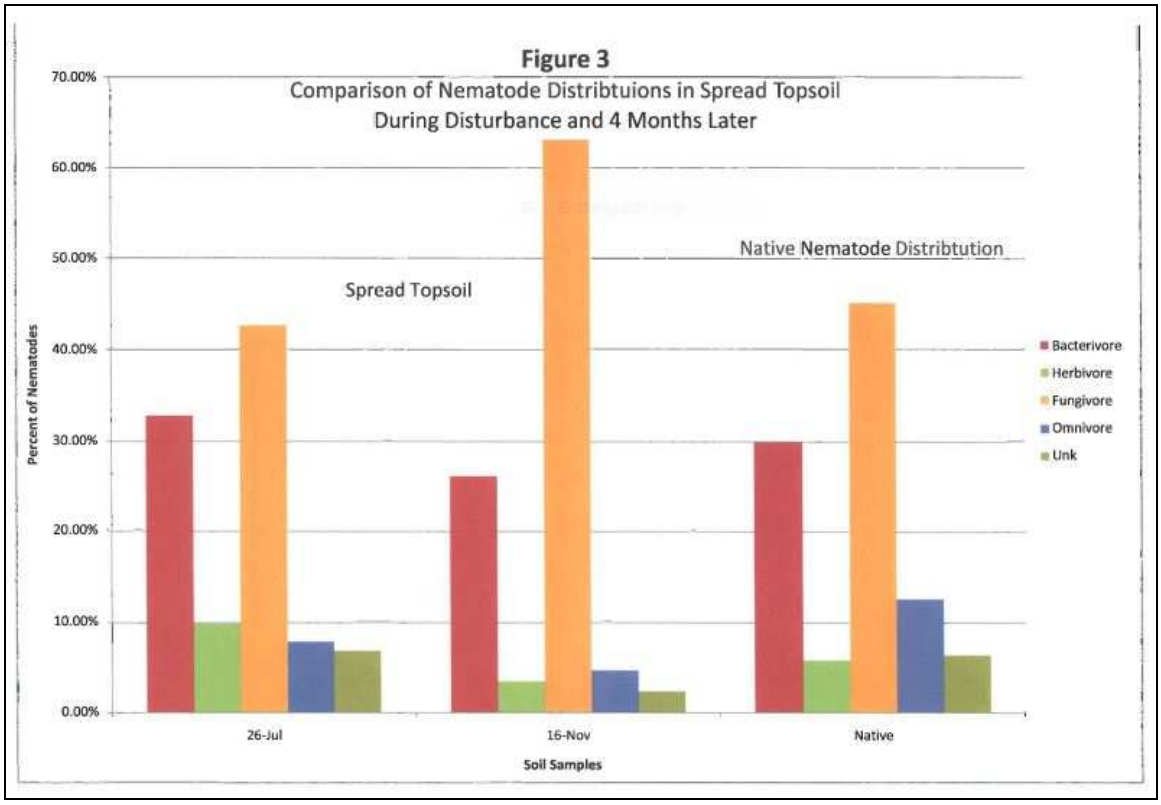


Figure 3
Comparison of Nematode Distribtuions in Spread Topsoil
During Disturbance and 4 Months Later
Native Nematode Distribtution
Spread Topsoil
Percent of Nematodes
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
26-Jul
16-Nov
Native
Soil Samples
Bacterivore
Herbivore
Fungivore
Omnivore
Unk

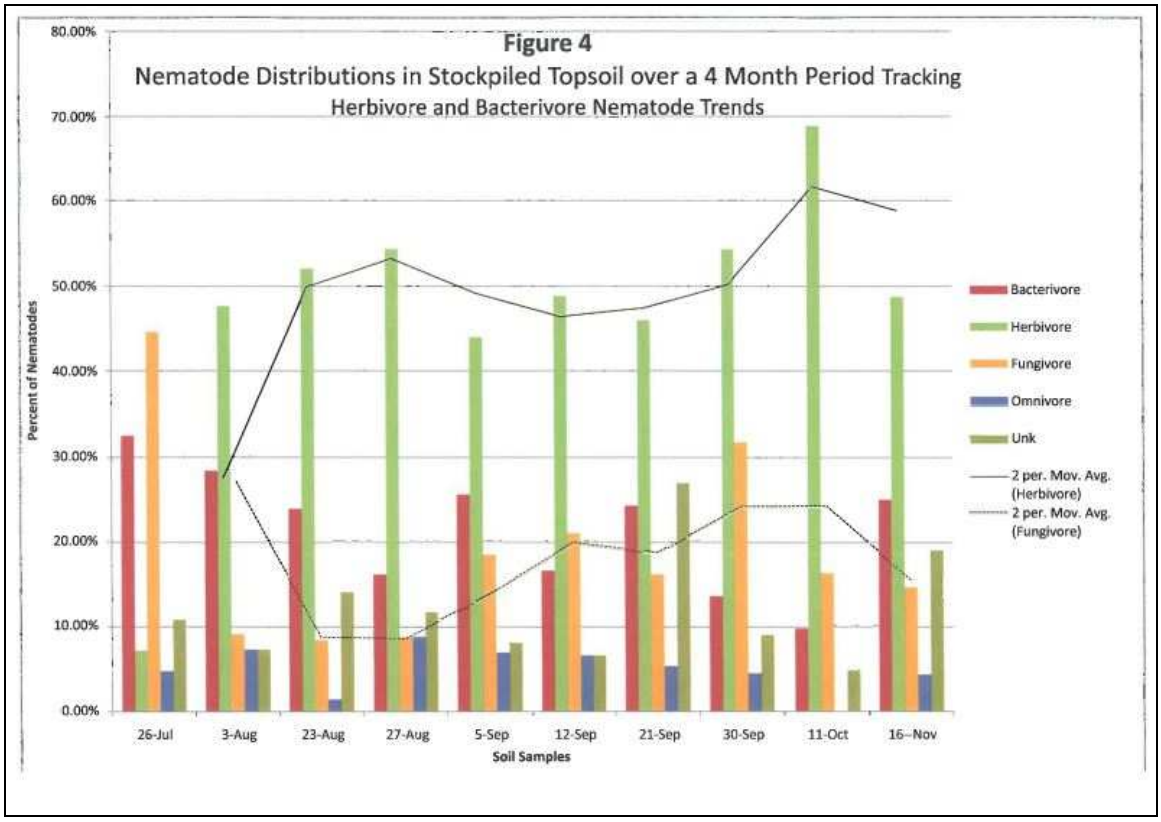


Figure 4
Nematode Distributions in Stockpiled Topsoil over a 4 Month Period Tracking
Herbivore and Bacterivore Nematode Trends
Percent of Nematodes
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
26-Jul
3-Aug
23-Aug
27-Aug
5-Sep
12-Sep
21-Sep
30-Sep
11-Oct
16--Nov
Soil Samples
Bacterivore
Herbivore
Fungivore
Omnivore
Unk
2 per. Mov. Avg. (Herbivore)
2 per. Mov. Avg. (Fungivore)

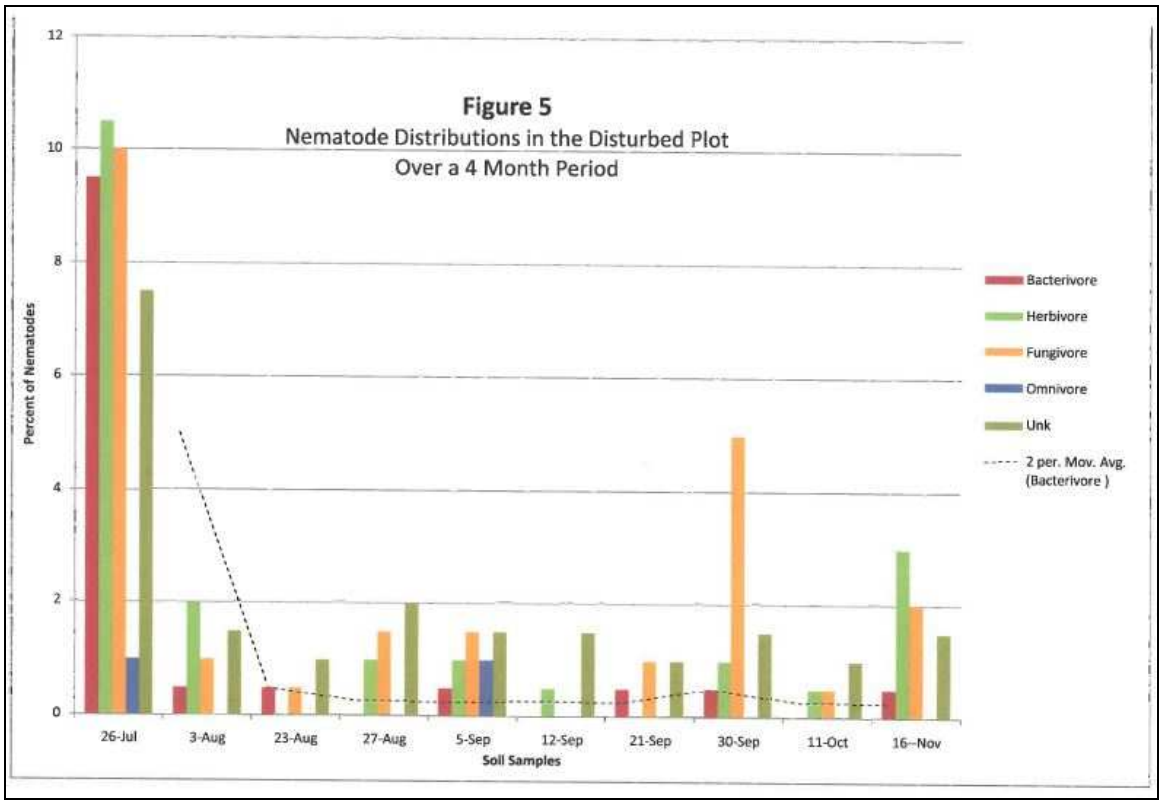


Figure 5
Nematode Distributions in the Disturbed Plot
Over a 4 Month Period
Percent of Nematodes
12
10
8
6
4
2
0
26-Jul
3-Aug
23-Aug
27-Aug
5-Sep
12-Sep
21-Sep
30-Sep
11-Oct
16--Nov
Soil Samples
Bacterivore
Herbivore
Fungivore
Omnivore
Unk
2 per. Mov. Avg. (Bacterivore)

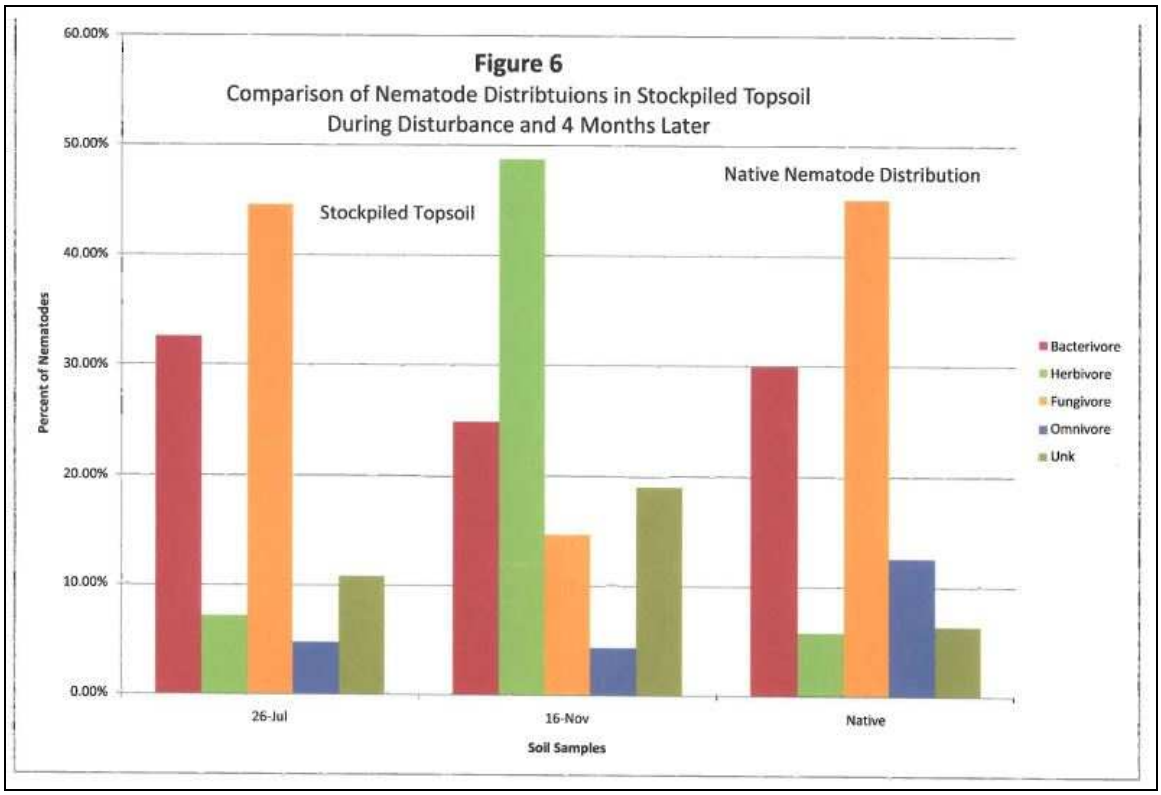
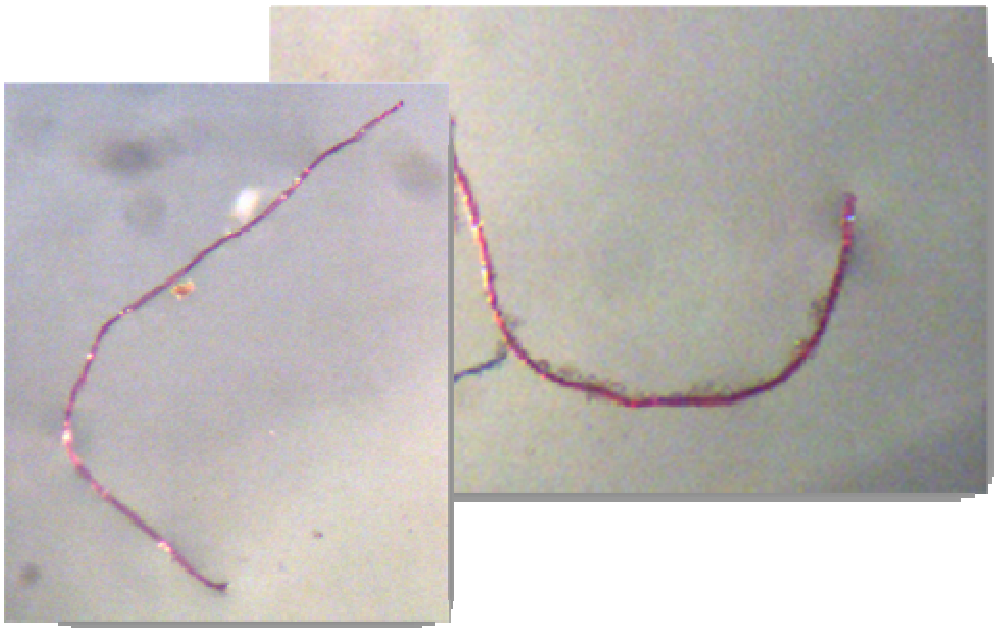


Figure 6
Comparison of Nematode Distribtuions in Stockpiled Topsoil
During Disturbance and 4 Months Later
Stockpiled Topsoil
Native Nematode Distribution
Percent of Nematodes
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
26-Jul
16-Nov
Native
Soil Samples
Bacterivore
Herbivore
Fungivore
Omnivore
Unk

APPENDIX 1

FOUR CORNERS NEMATODE KEY

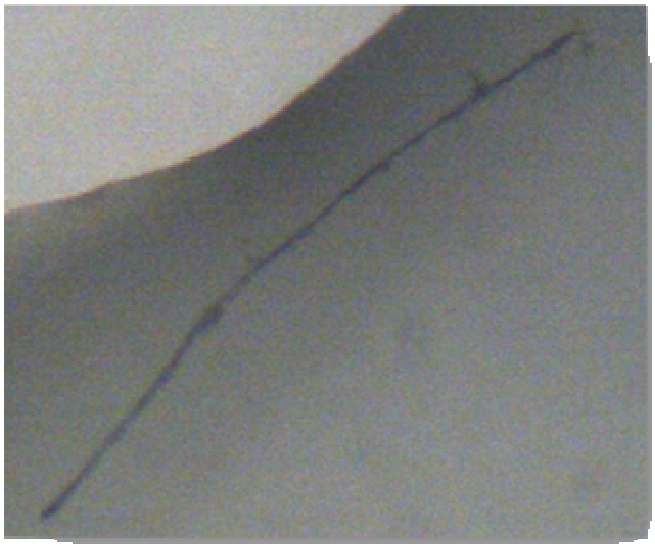


Red Nematode

A nematode that has a coating color of red as seen under the 40X microscope light. Ranges about middle sized in comparison to other nematodes found in Four Corners Samples.

Clear-Short Nematodes

A nematode with a clear or white coloring and short in appearance compared to other nematodes in Four Corners samples. Commonly found alive more frequently than any other nematode found.

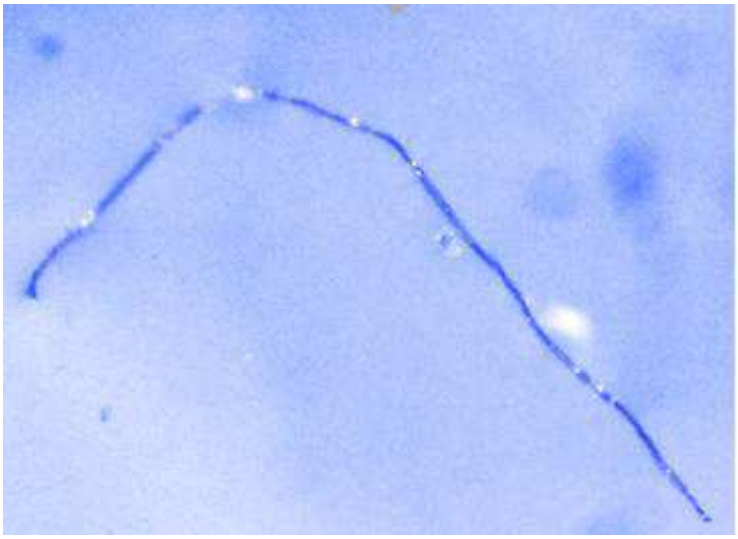


Black Nematodes

Longer nematodes with a dark brown or black tint are not roots (don't have the frayed ends).

Long-Clear Nematode

A medium to long sized nematode that tends to be larger in width with a clear complexion and alive more often than most with the exception of clear-short found frequently throughout samples.

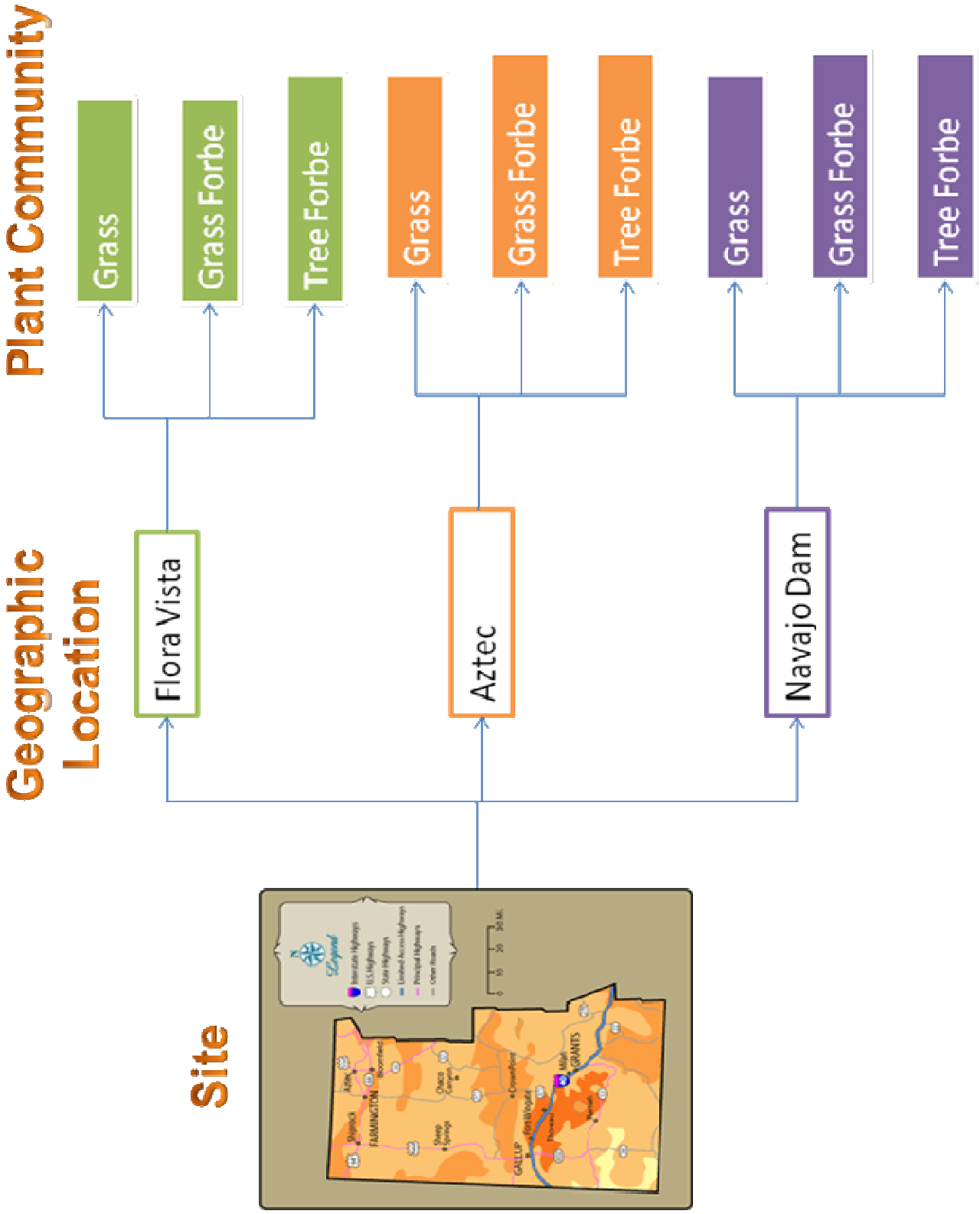


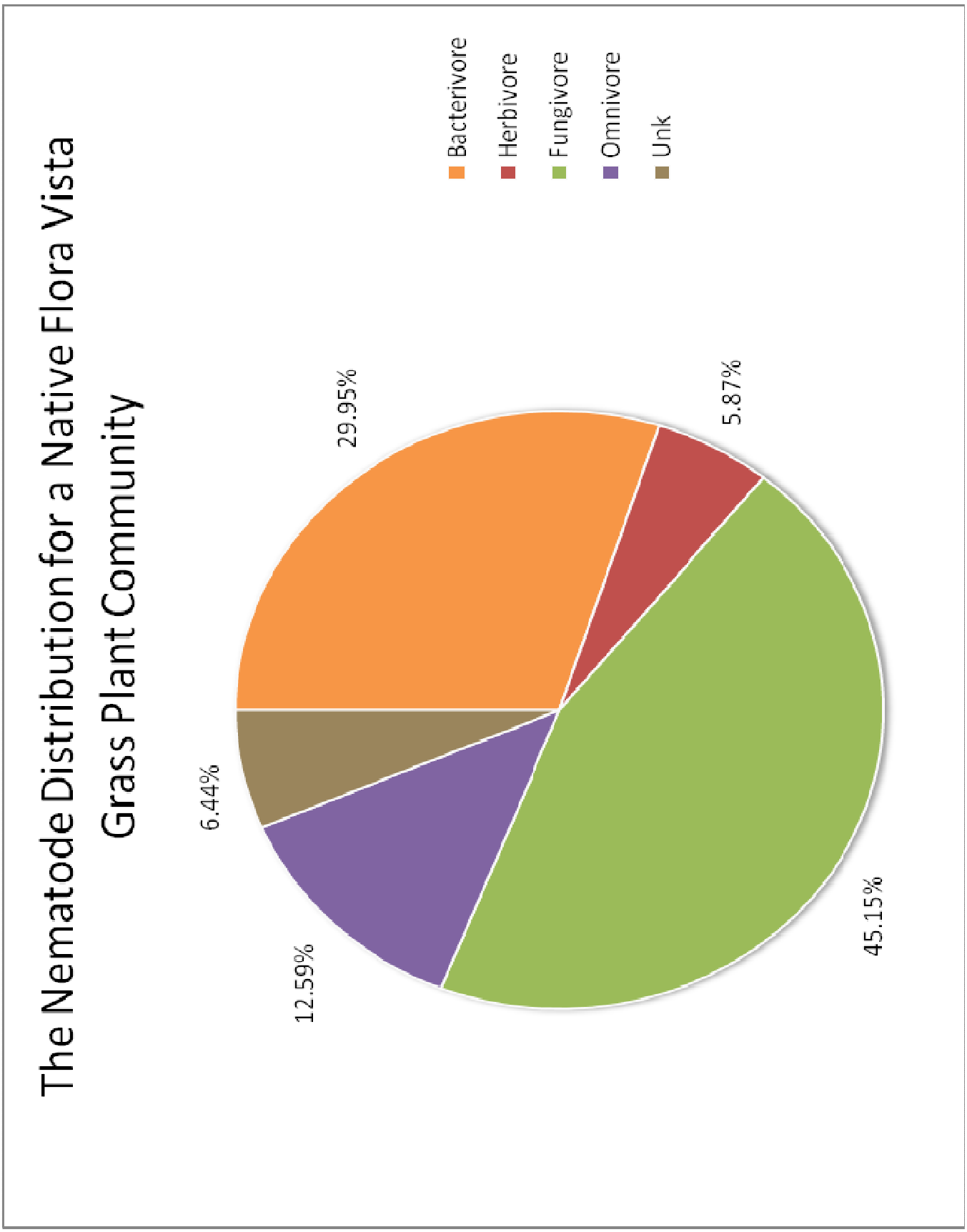
Blue Nematodes

A medium to long nematode with a blue tint that is not all too common in 25g samples of the Four Corners samples. More often died than not.

APPENDIX 2

ENHANCED 2010 GUIDE


Plant Community
Grass
Grass Forbe
Tree Forbe
Grass
Grass Forbe
Tree Forbe
Grass
Grass Forbe
Tree Forbe
Geographic Location
Flora Vista
Aztec
Navajo Dam
Site


The Nematode Distribution for a Native Flora Vista Grass Plant Community
Bacterivore
Herbivore
Fungivore
Omnivore
Unk
29.95%
5.87%
45.15%
12.59%
6.44%

APPENDIX 3

NEMATODE TROPHIC KEY

Photos by Researcher and references (8, 9, 10)

Bacteriavore

(Eats primarily Bacteria in the soil)

Has protruding lips, a clean stomodeum tract to the Basal Bulb and intestines

Has prtruding lips with the clean stomodeum tract and enlarged Basal Bulb, as well as a thicker mesenteron

Fungivore

(Eats primarily Fungus that lives in soil)

(Aphylenchus) Has an enlarged Median, visible stemedeum tract from mouth to intestine, and a small stylet.

Herbivore

(Eats from protiens made and nutrients collected by plants)

Has obvious long stylet with a longer body of presister, the Stomodeum leads to a diagonal intestinal wall

Has Obvious longer stylet and shorter stomodeum with more of a mesenteron

Juvenile, has tapered intestinal wall

Omnivore

(Eats most any micro-organism prominent in Healthy Native soil)

Has elongated stylet with no knob, neither bulb is immediately visible, the body is long and thick

The long stylet intercepts to meseteron with a flat line an distinct cuticles line the body.

Carnivore

(Eats primarily other nematodes and Micro-organisms in the soil, very rare)

N/A

Has enlarged tooth in protruding lips.

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