Names of researchers

PI: Teresa Bilinski, Ph.D. Assistant Professor, Dept. Biological Sciences, St. Edward's University

Student Researchers: Maria Rodriguez (Brother Romard funding, Hook Scholar, BIOL 4157 student), Amy Ontai (McNair), Elizabeth Ademski (Hook Scholar, BIOL 4157 student), Justin Stewart (BIOL 4157 student)

- Describe the progress and status of all research conducted under your permit in 2016. Include methods, results, data analyses, and conclusions.

Objective 1

The first objective of this research is to characterize the relative abundances of fecal coliform bacteria at various locations along Bee Creek and North Hollow at Wild Basin. The goal of this research was to help us better understand how inputs from different sources – namely surface water run-off from urban areas, surface water run-off from within Wild Basin, and groundwater/spring inputs - influence water quality at Wild Basin.

Update on Objective 1

Methods

During Summer 2016 we conducted three different water sampling trips between May 18 and June 25, 2016. During the first sampling trip on May 18 (Event 1) we collected 50ml of water from 25 different sites. Of these sites, 15 water samples were collected from locations along Bee Creek, and 10 water samples were collected from "sources" of water to Bee Creek, such as ephemeral creeks and springs/seeps. While sampling on June 4 (Event 2) and June 25, 2016 (Event 3) we collected 50ml of water from 9 sites within Bee Creek and 9 potential sources to Bee Creek. For each sampling trip, the water samples were taken back to the laboratory at St. Edward's University and stored at 4 degrees C for one week or less. To quantify the concentration of total bacteria and *E. coli,* the most abundant strain of fecal coliform bacteria, in each sample, 100 microliters of water sample was plated in triplate (three replicates per sample, per media type) on two different types of bacterial growth media. We used Tryptic Soy Broth as a general growth media to quantify total bacterial concentrations in the water samples. We also used MacConkey agar, a selective and differential media, in order to quantify the relative proportions of non-pathogenic and pathogenic strains of *E. coli.* The agar plates inoculated with water samples were incubated for 24 hours at 37 degrees C. After incubation, the number of bacterial colonies on each plate were counted and recorded. Then, the concentration of colonies per milliliter of water in each sample was calculated and recorded.

Results

Overall, concentrations of total bacteria in Bee Creek increased as the creek flowed through Wild Basin from the culverts on the west side of the preserve to the waterfall

(Figure 1). Downstream of the waterfall, total bacterial concentrations decreased at some of the sampling locations, although this pattern varied between sampling events. There was high spatial and temporal variability in total bacterial concentrations in sources of water to Bee Creek (Figure 2).

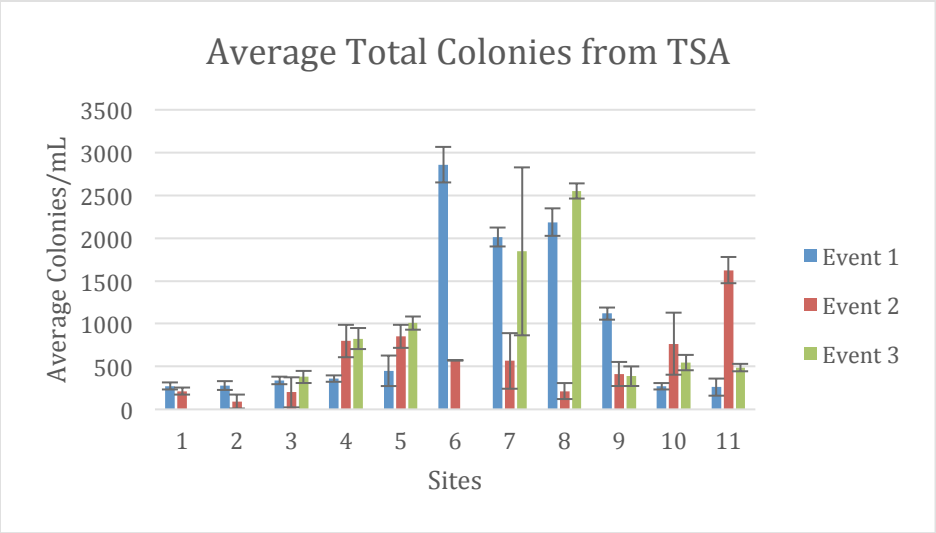


Figure 1: Total bacterial concentrations in Bee Creek.

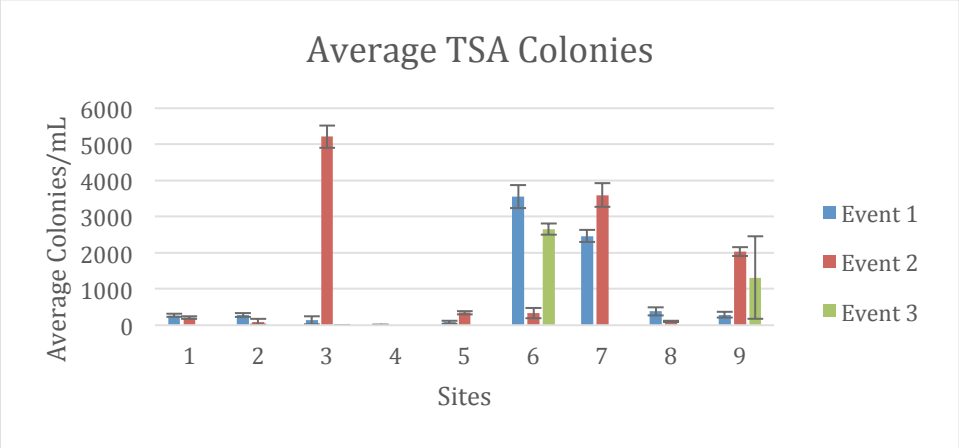


Figure 2: Total bacterial concentrations in water sources that flow into Bee Creek.

There were fecal coliform bacteria in all of the water samples collected during this research study. The concentration of non-pathogenic E. coli remained low throughout each sampling event along Bee Creek and for sources sites. However, there was a high degree of variability in the ratio of non-pathogenic to pathogenic *E. coli* in both water samples collected from Bee Creek (Figure 3) and water samples collected from sources to Bee Creek (Figure 4). As shown in Figure 3, there were very different patterns in the spatial distribution of pathogenic strains of *E. coli* in Bee Creek at the different sampling events. During the May 18 and June 25 sampling event pathogenic *E. coli* concentrations in Bee Creek were highest downstream of the waterfall, and remained relatively high until downstream of the confluence with North Hollow. By contrast, on June 4 pathogenic *E. coli* concentrations were highest upstream of the waterfall, closest

to where Bee Creek enters the preserve. The sources to Bee Creek had relatively low concentrations of pathogenic *E. coli* compared with non-pathogenic strains during all sampling events except on May 18 when a spring source that flows into Bee Creek directly downstream of the waterfall had very high concentrations of pathogenic *E. coli* (Figure 4). It is possible that some spring and ephemeral creek sources to Bee Creek contribute to elevated levels of bacterial concentrations in Bee Creek at Wild Bain.

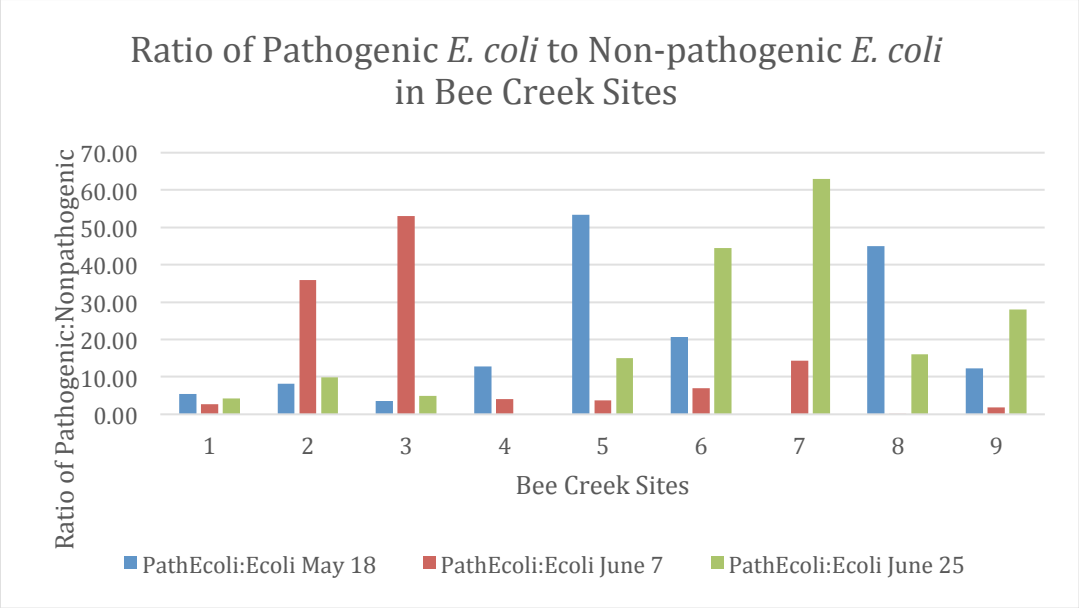


Figure 3: The ratio of the concentrations (colonies/ml) of pathogenic strains of *E. coli* to non-pathogenic strains of *E. coli* within Bee Creek samples.

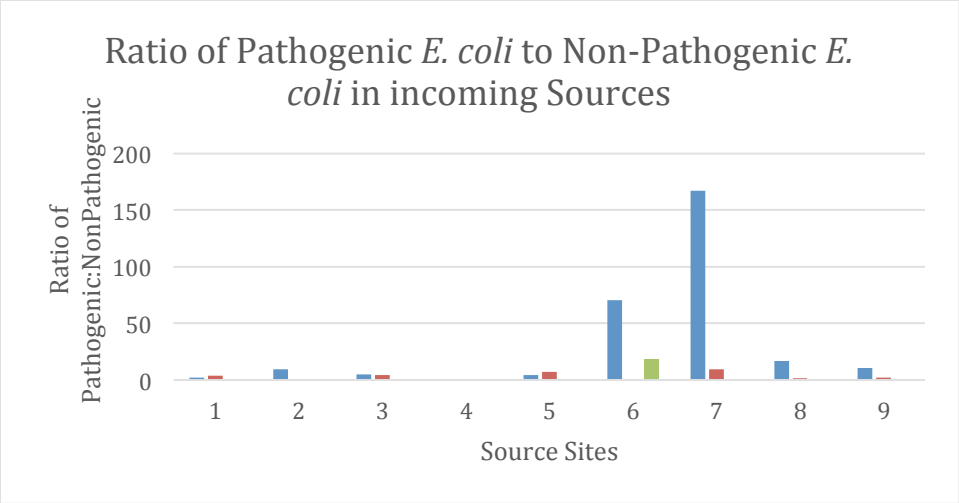


Figure 4: The ratio of the concentrations (colonies/ml) of pathogenic strains of *E. coli* to non-pathogenic strains of *E. coli* within water samples collected from sources to Bee Creek.

Current & Future Work

Currently we are preparing water samples collected from Bee Creek for a molecular characterization of the relative abundances of fecal coliform bacteria compared with other bacterial strains. To this end, we have extracted DNA from 15 samples collected in June 2016 and 5 samples collected in November 2016. We will send these samples to a DNA sequencing facility for 16S rRNA gene sequencing. These data will be used to calculate the relative proportions of different bacterial taxa, and will provide another way of investigating the proportion of harmful fecal coliform bacteria in Bee Creek.

Objective 2

The second objective of this research is to characterize soil ecology at 13 grid points at Wild Basin. Our primary focus was to link soil characteristics to the above-ground vegetation. For example, we aimed to understand how the percent coverage of trees, as well and the relative abundance of Ashe Juniper, affects soil carbon, soil water-holding capacity, soil pH, microbial respiration and leaf litter decomposition.

Update on Objective 2

In summer of 2016, six plots were established at five of the long-term sampling sites across Wild Basin. Of these six plots, three were representative of Ashe juniper-dominated forests while three were established as oak-dominated areas. The criteria for oak-dominated or Ashe juniper-dominated was determined by the amount of oak or Ashe Juniper species within the area, along with the leaf litter observed upon the ground. Of the 13 grid points established for long-term sampling, grid points 4, 5, 8, 6, and 7 were visited, and this study's sampling plots were within 40 meters of the grid points' center. Sampling plots each had a 5.6 m radius, an area of 98.2 m^2. Tree species inside of plots were identified and measured at diameter breast height. Three soil samples were collected from random points inside the 98.2 m^2 plot, along with a composite soil sample which included soil from all of the three areas around the plot. Soil samples were collected using a soil corer, which was drilled into the ground until bedrock in order to sample the entire root zone for accurate soil organic matter measurements (Olson and Al-Kaisi 2015). The soil depth varied from 5-20 cm deep across the six sample sites. Soil temperature at each soil collection site was recorded in the field using a HANNA® Digital Soil Thermometer with a 5 inch probe. Soil samples were stored at 4 degrees Celsius until analysis.

Sample Analysis

Characterizing Plant Biomass

All woody species were counted in each plot, and their identity was recorded. The diameter at breast height (DBH) was also measured for each tree. DBH measurements of either Ashe juniper or oak-deciduous species were totalled at each site and used, in addition to number of Ashe juniper and oak-deciduous seedlings counted, to characterize woody species dominance at each site.

Table 1. Total DBH and seedlings at each site.

Site	AJ Total DBH	AJ Total Seedling	O-De Total DBH	O-De Total Seedling	Dominance
1	119.37	14	0	0	AJ
2	195.76	13	113	134	O-De

3	128.60	1	87.22	50	O-De
4	320.86	1	.64	0	AJ
5	214.86	1	314.80	10	O-De
6	436.78	0	19.57	7	AJ

pH, Soil Organic Carbon (SOC) and Water Loss

The pH of each soil sample was measured within 12 days of soil sample collection. Five mg of soil were added to 10 mL of water (Robertson et al. 1999) in 50 mL falcon tubes and then shaken at 153 rpm for three hours at 21 degrees celsius. Then, the mixture was left to settle overnight. In the morning, pH measurements were taken using a calibrated Accumet Basic AB15 model. Each of the three soil samples taken from a site were measured three times, totalling nine pH measurements per site.

Measurement of soil organic carbon (SOC) was done within three weeks of soil collection. Before measuring the SOC, soils were sieved through a 2000 micron sieve. Then, eight grams of soil were measured into DYN A MED disposable aluminum dishes and were placed in a Thermo Scientific Heratherm Oven for 24 hours at 105 degrees celsius. Afterwards, each soil sample was weighed. Each dried sample was then combusted at 524 degrees Celsius for 3 hours in a Ney R Vulcan R 3-550 furnace and weighed again. SOC was calculated by subtracting the weight of the sample after the furnace from the weight of the sample after the 24-hour drying period (Hoogsteen et al. 2015). Additionally, the water loss (WL) of the soil was calculated by subtracting the weight of soil after drying from the original weight of soil. Three measurements were taken per site.

Microbial Carbon Utilization

Within five weeks of soil collection, microorganisms were extracted from soil samples by adding 10 g of soil to 100 mL of a sterilized 1% solution of NaCl in deionized water (Gomez, et al. 2006; Bissett, et al. 2011). At 25 degrees Celsius, soil solutions were shaken for one hour and then pre-incubated in the dark for 18 hours at 21 degrees Celsius to allow for the microorganisms to metabolize the standing stocks of organic carbon in the soil samples (Gomez, et al 2006). Ten-fold serial dilutions of the the supernatant from the pre-incubated soil solutions was prepared with sterilized 1% NaCl in 15 mL falcon tubes to a 10^{-3} concentration. Then, 100µL of the 10^{-3} dilution was added to each well in the Biolog EcoPlate™, which contained in each of their 96 microtiter wells a single carbon compound (31 C sources with 3 replicates and a control well with no carbon) and a redox dye (tetrazolium violet) which changed color according to the degree of oxidative catabolism in each well (Garau et al. 2007; Frąc et al. 2012). Each Biolog EcoPlate™ was incubated at 21 degrees Celsius in the dark (Bissett et al. 2011). The color development in each well was read visually, and on a Fischer Scientific Multiscan FC spectrophotometer at 590nm, every 6 and 18 hours for 78 hours (Gomez, et al. 2006; Bissett, et al. 2011). Each soil site was assigned a Biolog EcoPlate™, totalling six Biolog EcoPlates™.

The absorbance readings at specific time points were blanked against the control well at that same reading time. The blanked absorbance values were then used to describe the functional diversity of microbial communities at each reading time by calculating the number of substrates used (absorbance>.25) by the community to determine community richness,which in this study

simply means a numerical value to quantify the diversity of each site. All measurements reported are at the 78 hour reading time, as this reading time provided the best discrimination amongst samples (Gomez et al. 2006). Richness of functional groups of carbon sources between sites were also noted (Garau et al. 2007; Gryta et al. 2014).

Statistical Analyses

Using Minitab 17, two-way t-tests were performed on the SOC, WL, and pH data to determine if there was a significant difference between oak-deciduous-dominated and Ashe juniper-dominated areas. Additionally, a one-way ANOVA was run on the data to determine any significant difference between the pH, SOC, and WL of sites 1-6, and a Pearson correlation was run to determine the relationship between pH and SOC. A post-hoc Tukey HSD test was also run on pH using Minitab 17.

For the Ecoplate™ data, two-way t-tests in Minitab 17 were run on the average richness of Ashe juniper-dominated sites and oak-deciduous-dominated sites to determine a significant difference. Two-way t-tests were also run on the average richness between AJ and O-De sites according to carbon substrate guild.

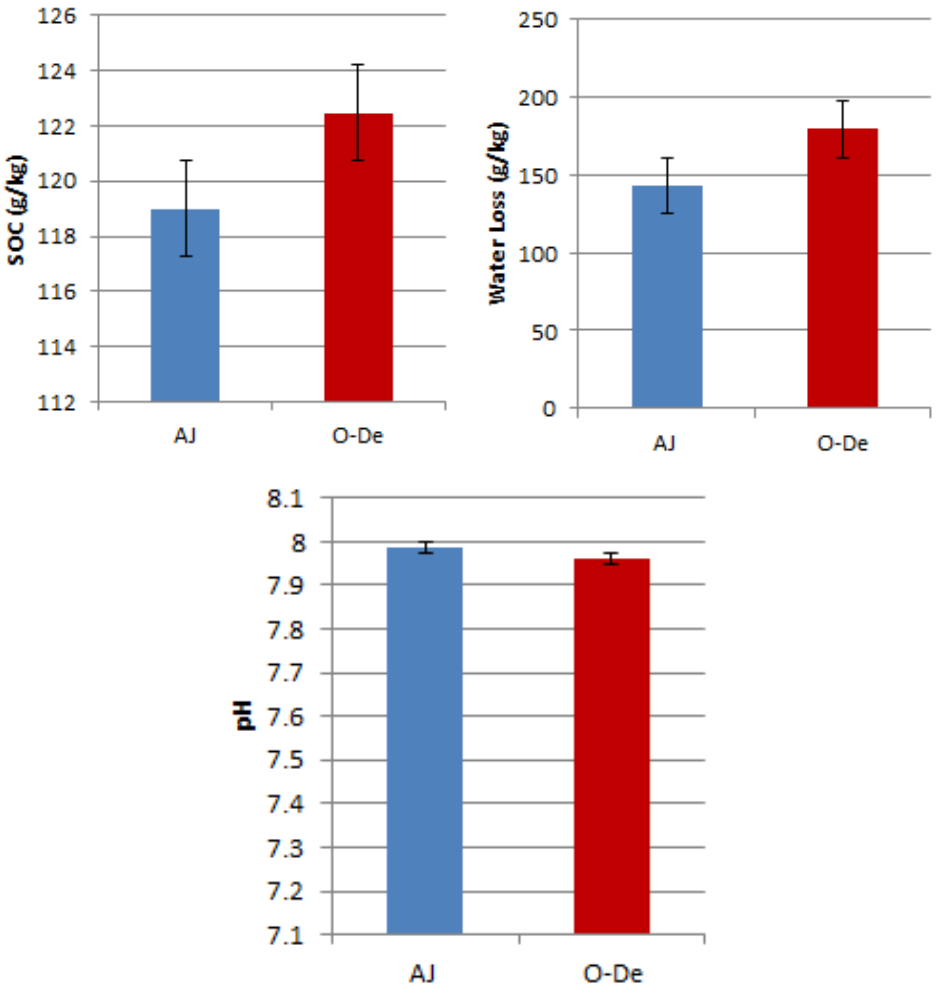
Results

pH, Soil Organic Carbon, and Water Loss

The mean ($\bar{x}$), standard deviation (σ), and range (Ra) of pH, SOC, and WL for AJ and O-De is reported in Table 2. The measurements of pH, SOC, and WL between AJ and O-De sites showed no significant difference (p=0.602, p=0.857, and p=0.112, respectively). There was a moderate-to-weak positive correlation between pH and SOC (r=-0.4282 p-value=0.3967), as shown in Fig. 5.

Table 2. Mean, standard deviation, and Range data sets.

Dominance	Variable	$\bar{x}$	σ	Ra
AJ	pH	7.99	0.21	0.71
	SOC	11.90	5.37	15.91
	WL	14.31	5.63	18.57
O-De	pH	7.96	0.14	0.48
	SOC	12.25	1.63	5.25
	WL	17.94	2.94	10.69



Figures 2-4 (From top left to right to bottom). Soil Organic Carbon of AJ v. O-De areas (1). pH in AJ v. O-De (2). Percent Water Loss in AJ v. O-De (3).

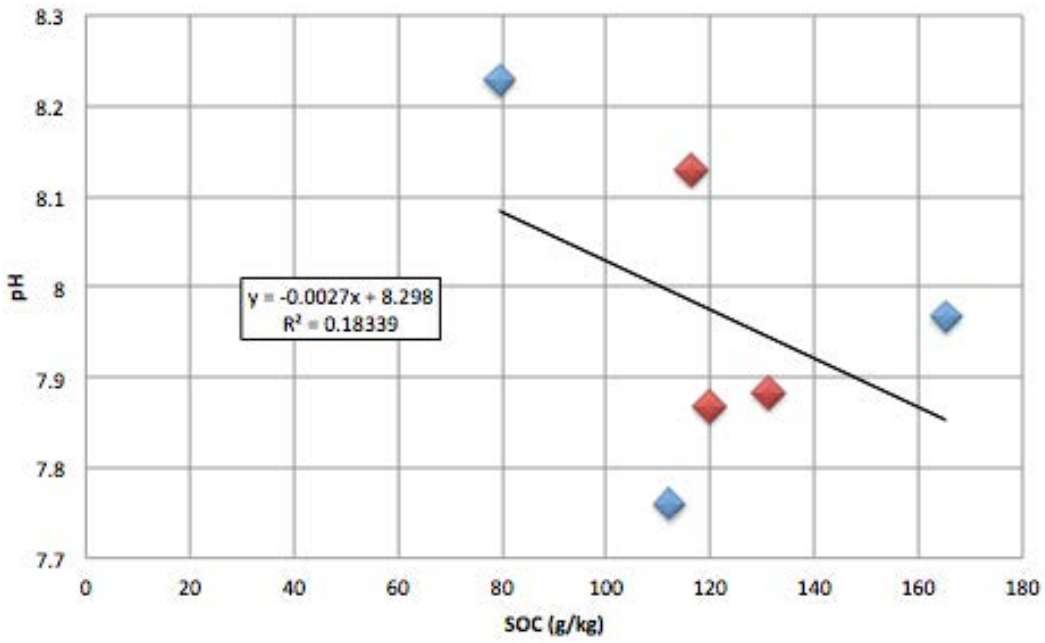


Figure 5. SOC vs. pH, blue points are AJ, red points are O-De. r=-0.4282 p-value=0.3967.

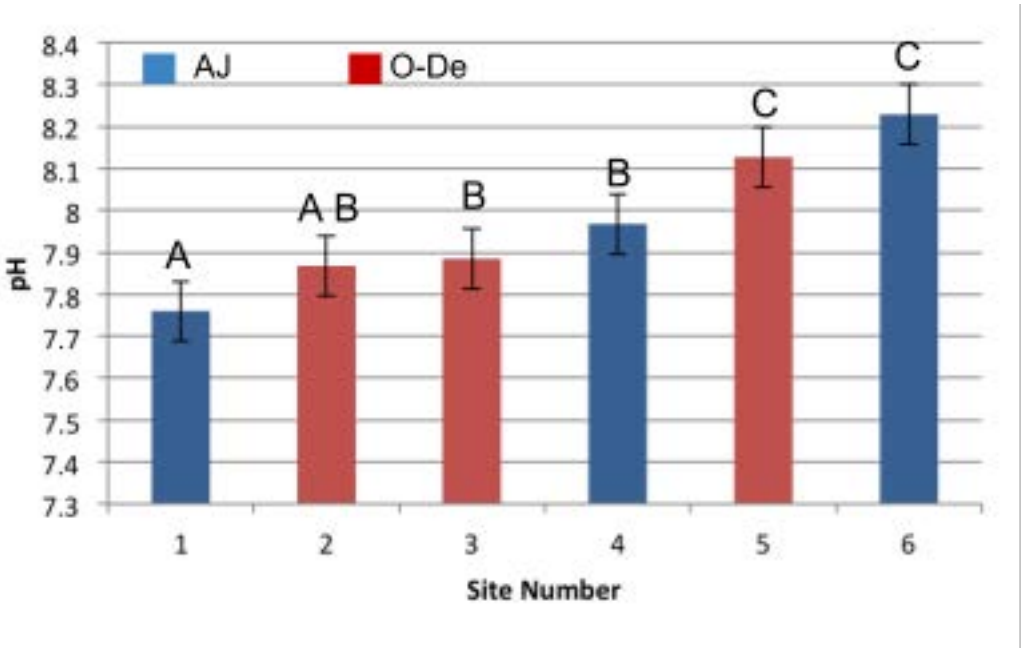


Figure 6. pH across different sites. Means that do not share a letter are significantly different.

A one-way ANOVA revealed that there was no significant difference between SOC and WL across different sites (p=0.142, p=0.134, respectively). However, it was shown that there was a significant difference in pH across sites (p=0.000). A post-hoc Tukey HSD test was then run on the data, with the results shown in Fig. 6.

Carbon substrate

The mean ($\bar{x}$), standard deviation (σ), and range (Ra) of AJ and O-De sites are shown in Table 3. Oak-dominated sites were found to have a significantly higher richness than Ashe juniper-dominated sites (p=0.016). Additionally, the substrates L-arginine, Pyruvic acid methyl ester, L-asparagine, Tween 40, Tween 80, D-mannitol, 4-Hydroxy benzoic acid, and L-serine were consistently oxidized (abs>.25) across all six sites. The substrates D-xylose, i-erythritol, 2-Hydroxy benzoic acid, L-phenylalanine, L-threonine, and D-cellobiose were not significantly oxidized (abs>.15) in any of the six sites. All other carbon sources were utilized by at least one site. Of the sites, Site 3 had the highest species richness, and site 6 had the lowest species richness.

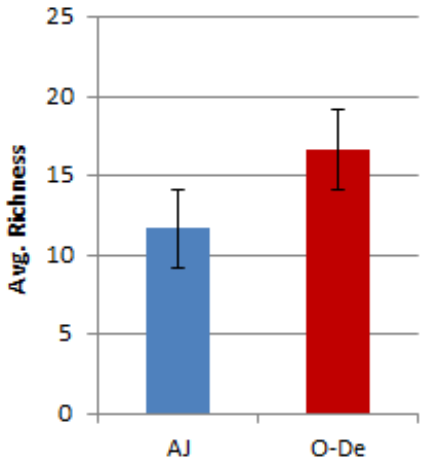
Substrate data were further analyzed by breaking the substrates into guilds (carbohydrates, carboxylic and acetic acids, polymers, amino acids, and amines/amides). Oak-deciduous-dominated sites were found to utilize carboxylic and acetic acids significantly more than Ashe juniper-dominated sites (p=0.005), while no significant differences were observed between utilization of the other four guilds. Additionally, a correlation was run on organic matter and species richness, showing a weak, insignificant correlation (r=-0.264, p-value=0.613).

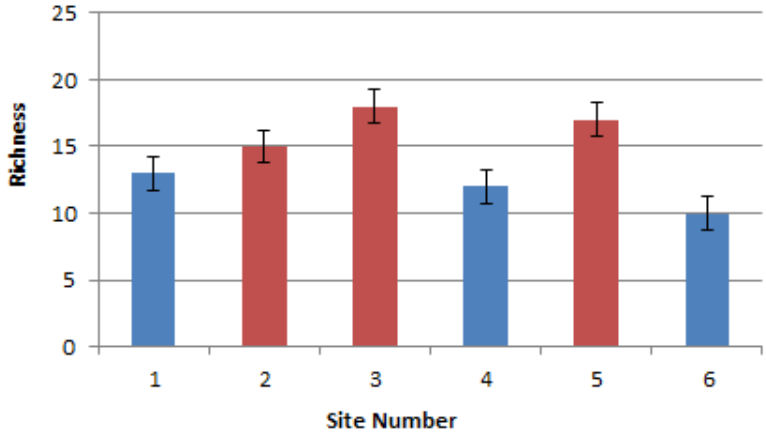
Table 3. Mean, standard deviation, and range of Richness at each site.

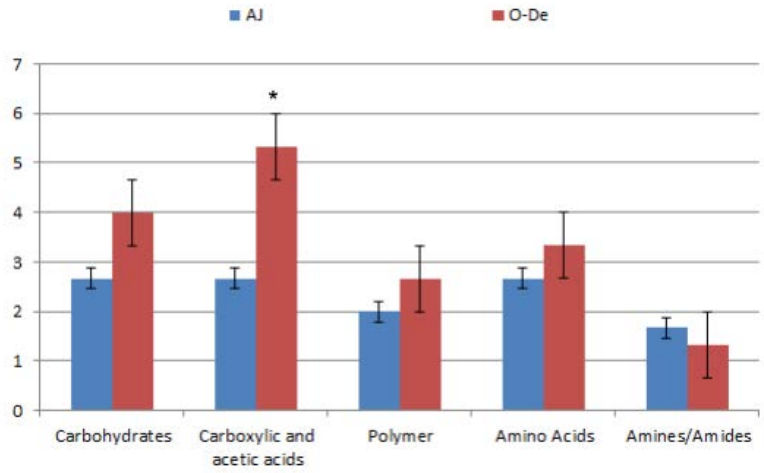
Dominance	$\bar{x}$	σ	Ra
AJ	11.67	1.53	3.00
O-De	16.67	1.53	3.00

Table 4. Sources of carbon utilized at abs590 at each site at 78 hours.

Site	1	2	3	4	5	6
Dominance	AJ	O-De	O-De	AJ	O-De	AJ
Water	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Beta-methyl-D-glucoside	0.0648	0.1690	0.3688	0.0293	0.5484	0.0001
D-galactonic acid-gamma-lactone	0.2016	0.3422	0.3720	0.4071	0.2546	0.2867
L-arginine	0.2948	0.4547	0.4240	0.5143	0.2520	0.2402
Pyruvic acid methyl ester	0.2528	0.3530	0.3405	0.3385	0.3481	0.1912
D-xylose	0.0029	0.0059	0.0066	0.0000	0.0000	0.0017
D-galacturonic acid	0.1795	0.3467	0.5647	0.2118	0.5554	0.1243
L-asparagine	0.6275	0.6923	0.5871	0.7404	0.9665	0.6496
Tween 40	0.6262	0.5975	0.9029	0.7624	0.8990	1.0850
i-erythritol	0.0937	0.0158	0.0083	0.0077	0.0087	0.0005
2-Hydroxy benzoic acid	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
L-phenylalanine	0.0179	0.0269	0.0126	0.1504	0.0141	0.0035
Tween 80	0.6238	0.6106	0.6602	0.4856	0.7889	0.7929
D-mannitol	0.3761	0.4076	0.5258	0.5445	0.3106	0.3951
4-Hydroxy benzoic acid	0.3202	0.3854	0.3574	0.3500	0.4271	0.3174
L-serine	0.3938	0.2747	0.3569	0.5175	0.3325	0.4080
Alpha-cyclodextrin	0.0198	0.0125	0.9029	0.0030	0.0174	0.0068
N-acetyl-D-glucosamine	0.4416	0.2634	0.0083	0.3117	0.2740	0.3058
Gamma-hydroxybutyric acid	0.3584	0.3996	0.0000	0.1785	0.2753	0.1104
L-threonine	0.0216	0.0036	0.0126	0.0003	0.0000	0.0000
Glycogen	0.1215	0.0525	0.6602	0.0243	0.0714	0.0190
D-glucosaminic acid	0.2109	0.0168	0.5258	0.0081	0.2102	0.1981
Itaconic acid	0.1066	0.1086	0.3574	0.0123	0.2814	0.0995
Glycyl-L-glutamic acid	0.0281	0.0980	0.3569	0.0283	0.0397	0.0270
D-cellobiose	0.0381	0.0178	0.0148	0.0012	0.0176	0.0000
Glucose-1-phosphate	0.0000	0.0282	0.5366	0.0099	0.0216	0.0000
Alpha-ketobutyric acid	0.0468	0.0053	0.1463	0.0000	0.0000	0.0000
Phenylethylamine	0.4227	0.2926	0.0204	0.1730	0.3045	0.2921
Alpha-D-lactose	0.0000	0.0025	0.7362	0.0266	0.0000	0.0000
D,L-alpha-glycerol phosphate	0.1311	0.2155	0.0541	0.1579	0.1555	0.1564
D-malic acid	0.8602	2.0271	0.0316	0.2665	1.4001	0.0102
Putrescine	0.2630	0.3612	0.1448	0.3555	0.3236	0.2561
Richness	13.0000	15.0000	18.0000	12.0000	17.0000	10.0000







Figures 7, 8, 9 (Top left to bottom). Average richness of the sites at abs>.25 (7), richness of either AJ or O-De sites by carbon substrate guild (8), and comparison between carbon guilds (9)

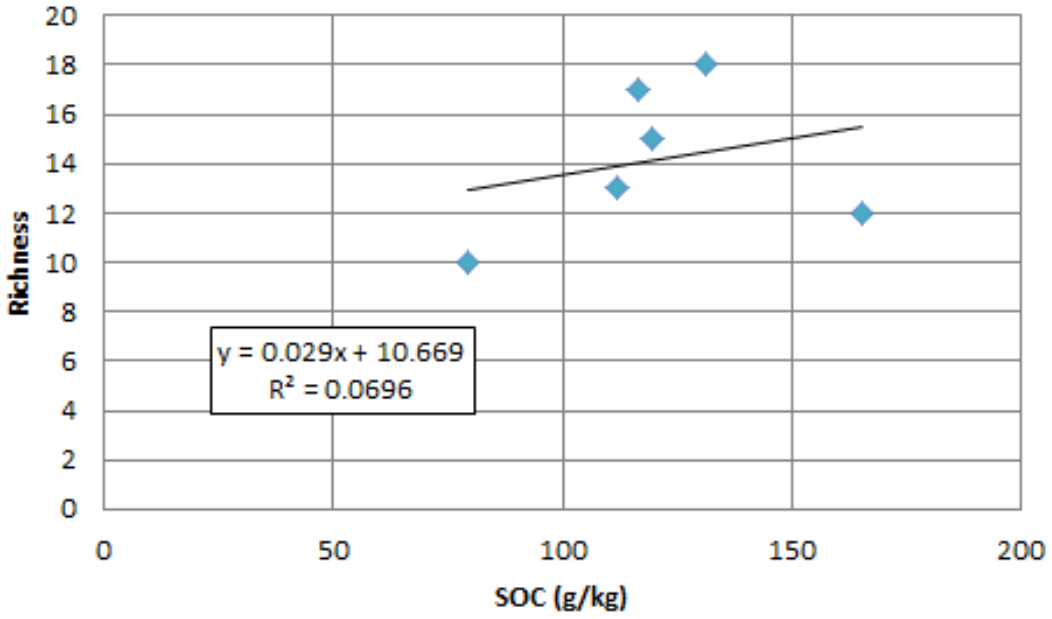


Figure 10. Soil Organic Carbon vs. Richness. Pearson correlation of SOC and R = -0.264, P-Value= 0.613