The Effect of Greenways/Trails on Soil Microbial Ecosystem in Lake Johnson Park of Central North Carolina

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Background

Lake Johnson Park (LJP) is a well utilized site for leisure and recreational activities in Raleigh, North Carolina. The park consists of 300+ acres of pine and oak forests that surround a 150 acre man-made lake. LJP trailways consist of 3.5 miles of paved and 1.9 miles of unpaved trail, which is extensively used for biking, walking, jogging and running. The creation of the trail and continuous usage of it may potentially lead to a decline in soil ecosystem quality. The processes of organic matter erosion & leaching of nutrients may be exacerbated by park use.[1] Studies suggest the use of microbial community shifts as bioindicators may reflect changes in microbial mediated nutrient cycling and organic matter decomposition in shoreline and forest ecosystems.[2] We assessed the trends in soil biochemical properties and microbial community composition alongside the trail way, the lakeshore, and in the forest to compare the effect of varying intensities of anthropogenic activities on paved and unpaved soils evaluated in this park ecosystem.

Methodology

Lake Johnson Park (Fig. 1) study site consists of 5 unpaved and 4 paved locations along the trail that surrounds the lake. At each location, samples were collected from the lakeshore, in the forest, and alongside the trail. Soil enzyme assays, essential to the biogeochemical cycling of C & N, and analysis of microbial community composition via Fatty Acid Methyl Ester (FAME) profiling were performed to assess the soil biochemical and microbial compositional properties in the parks soil microbial ecosystem. Multivariate Principal Component Analysis (PCA), eigenvector and eigenvalues, was used to assess structural and functional relationships occurring in the soil microbial ecosystem.

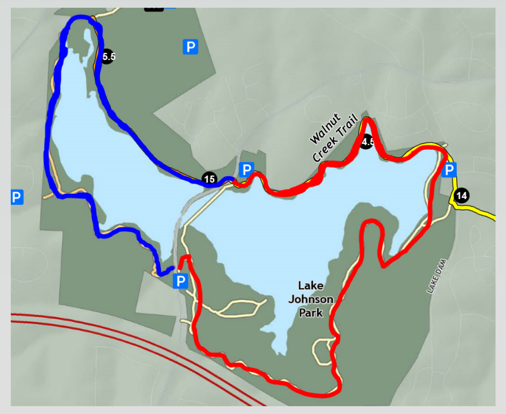

Fig. 1 Map of Lake Johnson Park trail system. The blue line shows unpaved trail; the red line shows paved trail.

Procedures

- Soil pH was read using Accument AB200 pH meter in 10mM $CaCl_2$ solution.
- Enzyme activities essential for biogeochemical cycling of carbon (β-*Glucosidase*) and nitrogen (N-*Acetyl*-β-*Glucosaminidase*) were assayed via the colorimetric method[8,9]
- Microbial community composition and structure was evaluated following soil extractions and gas chromatographic detection of Ester-linked fatty acid methyl ester (EL-FAMEs) indicators.
- Analysis of variance (ANOVA) followed by a tukey test were performed using SAS statistical package software version 9.3 (SAS Institute INC, Cary, NC). Results were considered significant at $P < 0.05$.
- Principal Component Analysis (PCA) was preformed with PC-ORD MjM software version 5.10

Results

Enzymes:

- β-*Glucosidase* activity in Lakeshore sites consistently revealed higher enzyme activities compared to forest soils analyzed, and unpaved locations revealed higher activities than soils collected from paved locations.
- N-*Acetyl*-β-*Glucosaminidase* (NAGase) activities appear to be higher in soils collected from alongside the trail in unpaved locations than in soils from paved locations alongside of the trail. For paved locations, NAGase activities were higher in Lakeshore soil compared to Forest or Alongside Trail soils.
- FAME-EL analysis reveals predominant gram positive indicator Alongside Trail-5, lowest at Lakeshore-1

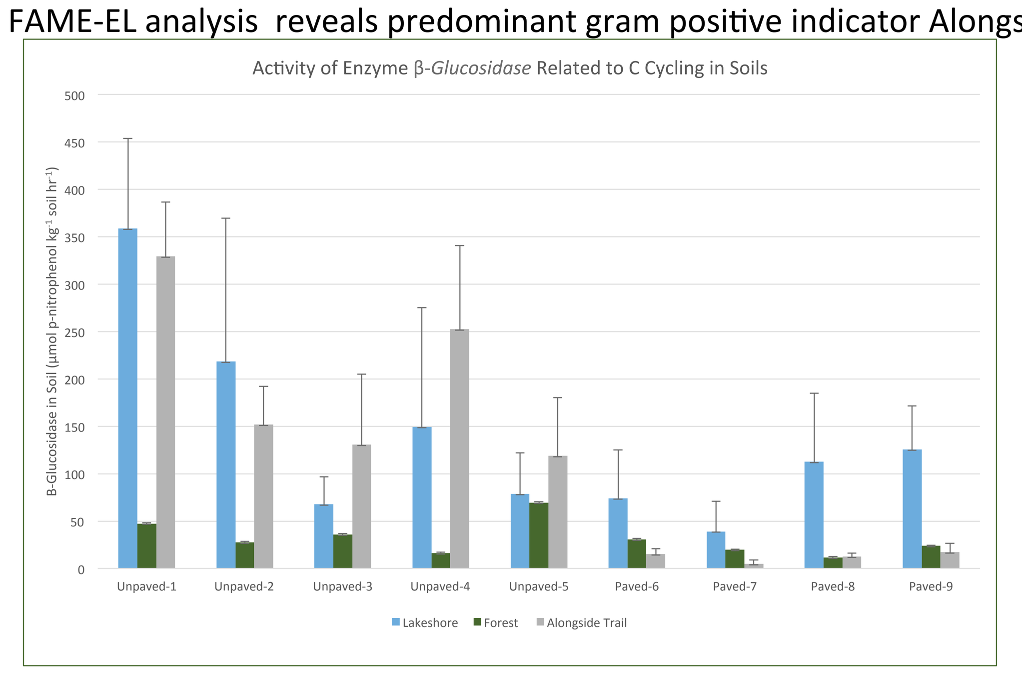

Fig. 2 Activity of β-*Glucosidase* in soils collected from the lakeshore, forest, and alongside the trail at Lake Johnson Park. Locations 1-5 are along the unpaved trail, Locations 6-9 are along the paved trail.

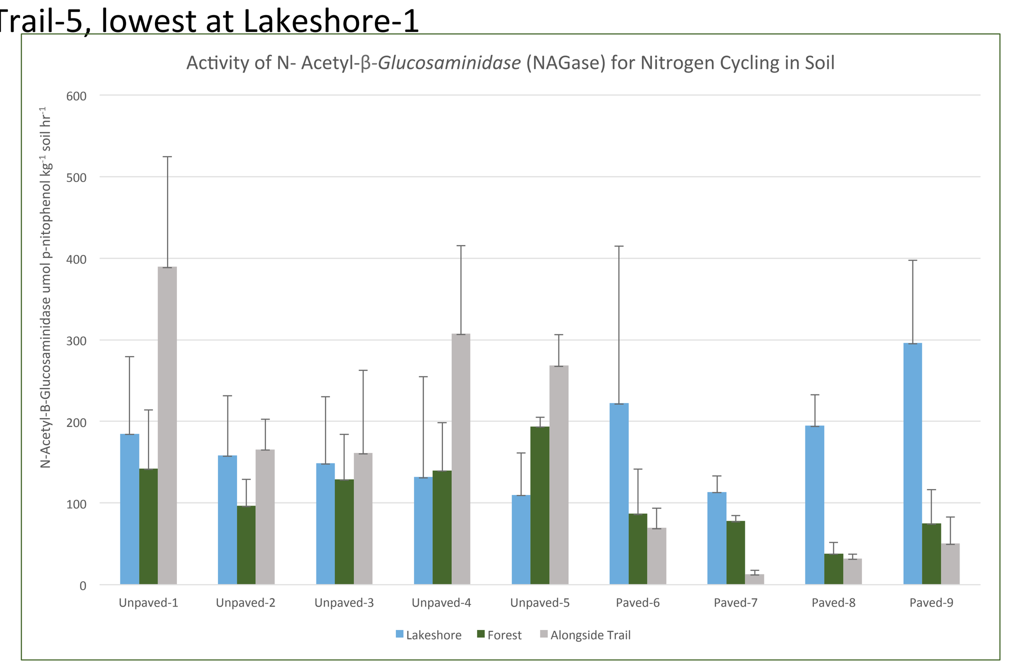

Fig. 3 Activity of N-Acetyl-β-*Glucosaminidase* in soils collected from the lakeshore, forest, and alongside the trail at Lake Johnson Park. Locations 1-5 are along the unpaved trail, Locations 6-9 are along the paved trail.

EL-FAME

- Saprophytic fungi dominated all samples analyzed
- Paved trail soils had lowest percentages of bacterial indicators compared to locations alongside the unpaved trail.

Table3. Percent FAME indicators of bacterial and fungal populations

	Bacteria (%)							Fungi (%)				Protozoa (%)	Actinomycetes (%)		
	G+				G-			AM		Saprophytic					
System	i15:0	a15:0	a17:0	i17:0	i17:0 3OH	cy17:0	cy19:0	16:1w5c	18:3w6c	18:1w9c	18:2w6c	20:4w6c	10Me 16:0	10Me 17:0	10Me 18:0
Lakeshore-1	2.68	0.92	0.72	0.85	0.39	0.92	4.05	0.97	2.93	6.93	10.82	1.64	3.87	1.68	2.52
Forest-1	3.75	1.56	0.87	1.28	0.01	0.73	5.69	2.17	2.70	11.45	4.59	0.86	3.14	1.04	1.69
Alongside Trail-1	5.64	2.52	1.65	1.79	0.09	1.73	5.04	5.94	0.29	7.13	10.53	0.88	4.20	1.02	1.82
Lakeshore-2	3.47	1.50	0.73	0.93	0.00	0.88	3.44	2.23	2.53	9.51	6.08	0.63	3.49	1.29	1.17
Forest-2	3.89	1.51	0.91	1.20	0.02	0.92	5.67	3.15	2.37	6.98	6.18	0.86	3.56	0.86	2.01
Alongside Trail-2	5.06	2.38	1.28	1.58	0.04	1.62	4.14	6.49	0.00	7.84	9.45	0.95	4.47	0.68	1.38
Lakeshore-3	3.65	2.12	0.93	1.08	0.00	1.11	2.84	2.95	0.84	11.55	3.52	0.69	2.61	0.52	1.14
Forest-3	3.34	1.73	1.12	1.06	0.01	0.75	4.49	2.50	3.50	14.20	7.93	0.55	2.35	0.72	1.40
Alongside Trail-3	4.01	2.44	1.14	1.26	0.05	1.35	3.14	4.93	1.34	10.29	9.72	0.48	2.30	0.90	1.67
Lakeshore-4	3.93	2.01	1.29	1.33	0.01	1.33	3.34	2.42	4.06	4.96	3.68	1.02	4.11	1.11	0.65
Forest-4	3.73	2.12	1.22	1.23	0.01	1.07	4.69	3.62	1.23	10.67	6.58	0.59	2.60	0.76	1.49
Alongside Trail-4	5.59	2.25	1.49	1.63	0.07	1.40	5.50	4.81	2.22	4.22	6.32	1.14	4.60	1.33	1.70
Lakeshore-5	3.60	2.42	0.86	1.09	0.02	1.22	2.79	2.84	1.13	9.03	4.26	0.35	2.66	0.64	1.04
Forest-5	3.44	1.73	1.10	1.02	0.03	0.89	5.47	3.10	2.74	3.71	7.65	0.60	2.90	0.73	1.07
Alongside Trail-5	6.19	3.08	1.87	1.91	0.10	1.61	5.22	6.56	0.54	0.00	5.46	0.82	4.44	1.63	2.41
Lakeshore-6	3.87	1.33	1.01	1.14	0.04	0.99	4.39	1.61	4.02	7.54	7.84	0.65	5.10	1.32	1.44
Forest-6	3.12	1.18	0.99	0.85	0.01	0.79	4.33	2.30	2.27	14.81	6.45	0.61	3.41	0.85	0.87
Alongside Trail-6	3.87	2.09	1.44	1.43	0.11	1.21	3.31	2.30	3.34	11.30	11.24	0.53	3.51	1.23	1.69
Lakeshore-7	3.64	1.75	0.80	1.25	0.00	0.91	3.69	2.75	2.66	15.48	2.68	1.08	3.03	0.91	1.38
Forest-7	3.28	1.16	0.73	0.83	0.01	0.62	4.27	1.74	2.76	12.63	7.15	0.67	3.41	0.70	1.06
Alongside Trail-7	4.73	2.61	1.22	1.71	0.00	1.74	2.47	2.37	1.68	8.06	3.81	0.65	3.77	0.85	2.33
Lakeshore-8	3.80	0.96	0.87	0.97	0.05	0.99	5.71	2.34	2.80	4.54	7.99	0.84	4.96	0.97	1.13
Forest-8	3.80	1.51	0.75	1.17	0.00	0.89	5.95	2.76	3.20	12.16	6.29	2.40	3.14	0.82	1.69
Alongside Trail-8	3.73	1.56	0.99	1.17	0.00	1.22	2.22	3.64	1.90	13.94	9.18	0.54	2.62	0.61	1.29
Lakeshore-9	3.59	1.08	0.90	1.18	0.01	0.95	4.01	2.81	4.10	3.30	12.74	0.84	3.65	1.07	1.29
Forest-9	3.70	1.72	0.87	1.16	0.00	0.76	5.17	3.11	2.91	13.12	5.92	0.68	2.68	0.73	1.74
Alongside Trail-9	3.11	1.56	0.97	1.21	0.00	1.19	3.04	6.55	2.21	15.74	6.28	0.53	2.67	0.77	1.14

Table 1. Percentage of fatty acid indicators in soils collected from the lakeshore, forest, and alongside the trail at Lake Johnson Park. Blue color indicates locations with the lowest percentage of specific fatty acid indicator. Orange color indicates locations with the highest percentage of specific fatty acid indicator. Locations 1-5 are along the unpaved trail, and Locations 6-9 are along the paved trail.

PCA

- PCA shows that Forest soils were strongly influenced by Fungi and F:B ratio, compared to the other soils. However, soils collected from lakeshore, and alongside trail (grouped in the upper left quadrant) were influenced by all of the bacteria.

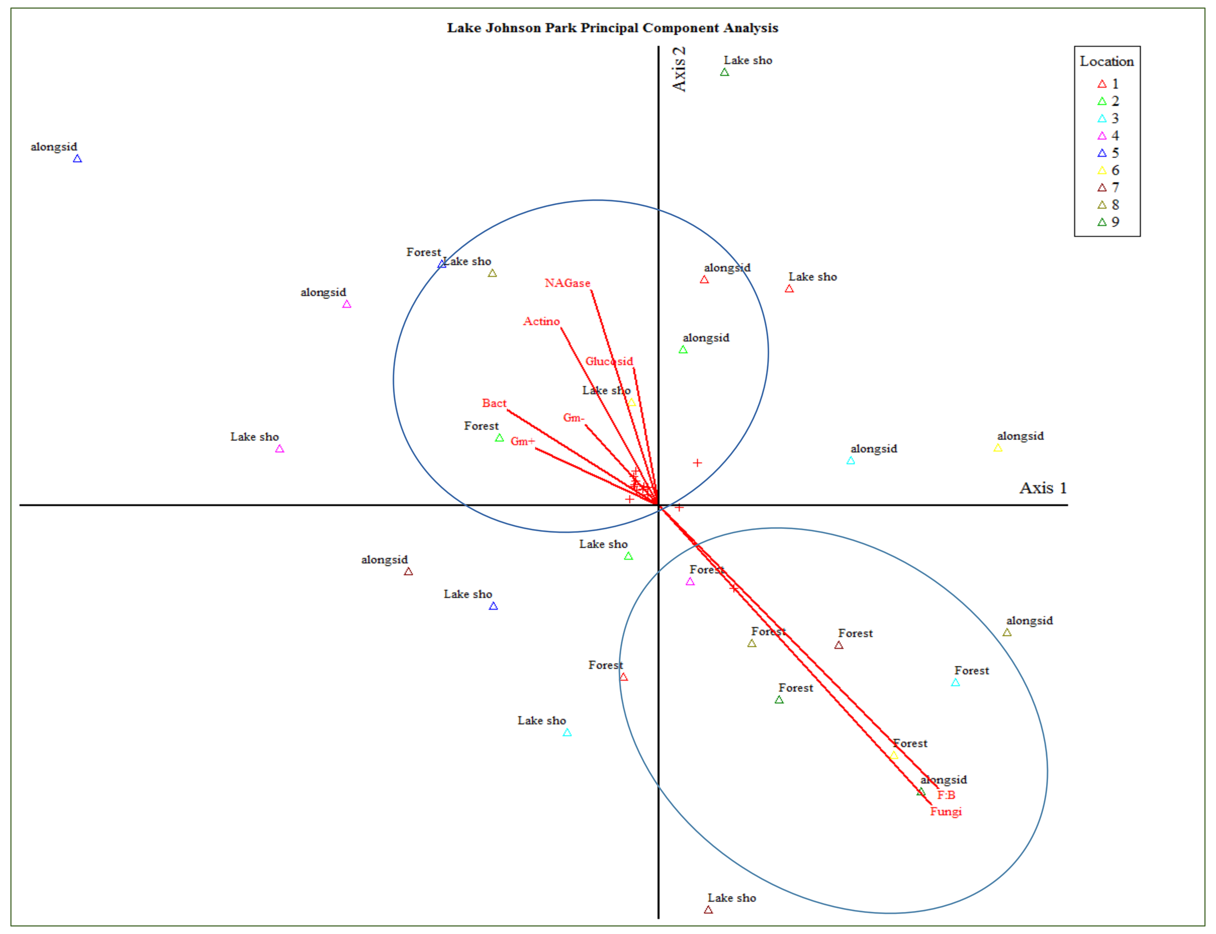

Fig. 4 Principal component analysis (PCA) graph identifying relationships among soil microbial communities and enzyme activities from soil samples collected from 9 locations at Lake Johnson Park. Colored triangles denote locations, and captions denote where soils were collected: Lakeshore, Forest, or Alongside Trail.

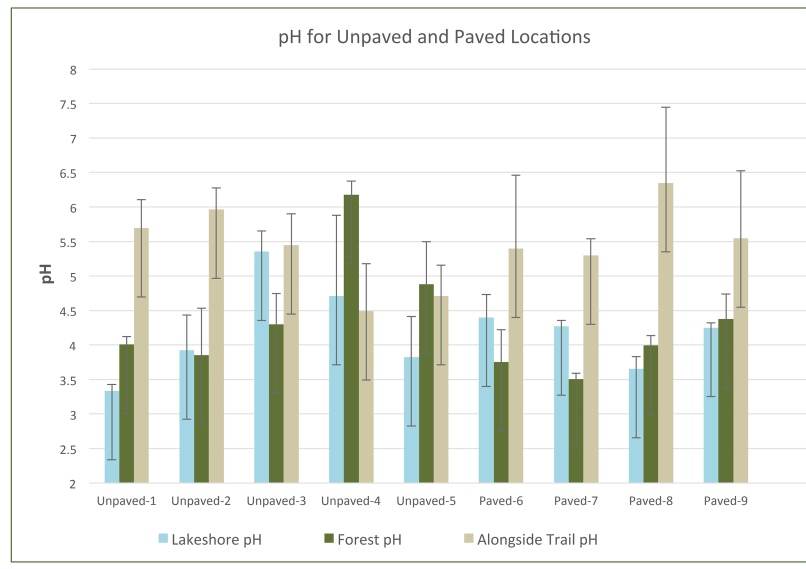

Fig. 5 pH values obtained from samples collected from the lakeshore, forest, and alongside the trail at Lake Johnson Park. Locations 1-5 are along the unpaved trail, Locations 6-9 are along the paved trail.

Discussion

- Hydrolytic enzymes produced by plants, animals, and microbes make nutrients available by breaking down large macromolecules from a variety of substrates to easily absorbable forms.[3] Enzymes are sensitive indicators of soil quality, and are influenced by pH, substrate availability, soil texture, temperature, and anthropogenic impacts.[3]
- When comparing Alongside Trail data, β-*Glucosidase* activities were significantly lower ($p<0.05$) for locations along paved trails than for locations along unpaved trails (Fig. 2). β-*Glucosidase* is involved in the hydrolysis of soil glycosides. The end product is glucose, an important energy source for microorganisms.[4] Similarly, N-Acetyl-β-*Glucosaminidase* levels were significantly lower ($p<0.05$) alongside the trail at paved locations 7, 8, and 9 than at unpaved locations 1, 4, and 5 (Fig. 3). Lack of vegetation along the paved trails may have contributed to low substrate availability, consequently limiting enzyme activity. Extracellular enzyme synthesis is often induced by presence of appropriate substrate.[5]
- Differences/Changes in microbial community composition (Gram+, Gram-, Actinomycetes, AM fungi, saprophytic fungi, protozoa) may be associated with nutritional stress or limited resource availability.[6] Diversity of microorganisms also influences plant growth and development.[7] Data from Alongside Trail samples showed higher Gram+ and Gram- FAME indicators in the unpaved trail relative to the paved trail (Table 1). This may indicate soils are disturbed alongside unpaved trails. The decreased microbial diversity may also be due to the increased popularity, and therefore increased usage, of the paved trail.
- Forest soils had high percentages of saprophytic fungi fatty acid indicators (Table 1). PCA also revealed greater fungal biomass and fungal:bacterial ratios in soils collected from the forest than in soils from the lakeshore or alongside the trail. Saprophytic fungi dominate forest soils because they are responsible for initiating the degradation process of woody material, promoting fungal growth[6, 10]. Locations that fall in Quadrant 2 are likely to have lower fungal biomass and fungal:bacterial ratios (Fig. 4).
- All soil samples were in the acidic range (3.3-6.4) (Fig. 5). Location 8, Alongside Trail sample, had a significantly higher pH than Forest and Lakeshore.

Future Work

- Assaying the soil enzyme activities that are essential to the biogeochemical cycling of S & P will give a more complete idea of the effect of trail disturbances.
- Characterizing additional physicochemical properties of the soil such as total carbon, nitrogen, and phosphorous content will give a better indication of soil health.
- DNA extraction and sequencing will enhance identification of key microbes within studied soils.
- Continued monitoring will help detect any future shifts in microbial communities.

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