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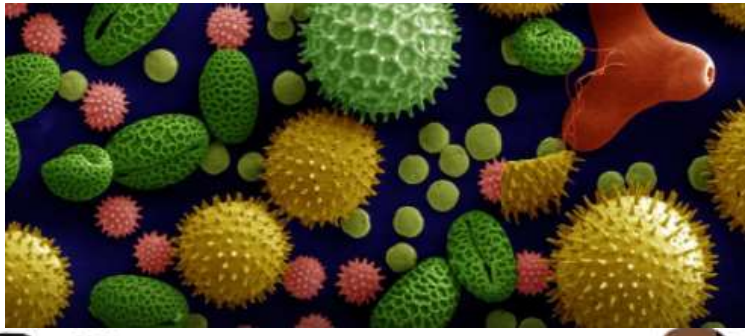
Use the Metabolic Fingerprint in Microbial Communities to Evaluate the Anthropogenic Impact on Soils

- The microbial community is defined as an “assembly” of populations of microorganisms that interact with each other, as well as with the spatial and temporal environment. Soil microbes provide ecological services to determine health in soils. Microbial communities in soils can be monitored by checking their metabolic properties.
- Microorganisms are typically the first organisms to react to chemical and physical changes in the environment.

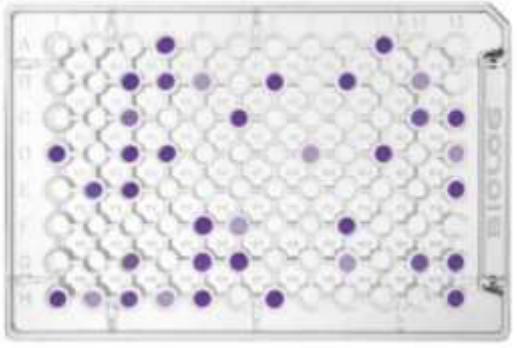


Source: Royalty-free microbiology photos, 2020

Microbial Community Analysis with EcoPlates

Source: Biolog, 2020

- Biolog EcoPlates provides a sensitive and reliable index of environmental change. Measure the metabolism of 31 carbon sources per assay, Each assay panel tests in triplicate, Simple colorimetric readout
- It is a technique to determine functional analysis diversity and structure of microbial communities, where different carbon sources are used to establish a metabolic profile of microorganisms and their behavior. It detects if bacteria can oxidize the different carbon sources where an electron transfer from the respiratory electronic transport chain to the tetrazolium salt of the medium, producing a color change that is measured in absorbance terms (Preston-Mafham, J., Boddy, L., Randerson, 2002).



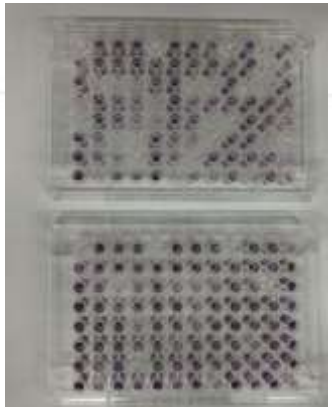
Methodology

- The aim of this study was to evaluate the anthropogenic impact on three soil samples of The Will County area at Illinois State USA: a natural reserve, an industrial park and a farmland with 15 years of herbicide (Glyphosate) use by determine the metabolic fingerprint of microbes on microplates.
- Soil samples were collected at Midewin National Tallgrass Prairie, North Island Industrial Park and Mazon Farm (15 years of Glyphosate use), utilizing a soil recovery tube and sorted during collection into Whirl-Pak bags.
- Organic Carbon (OC), pH, Nitrate (NO_3^-) and Phosphate ($PO_4^=$) were measure for each soil. OC was measured by the Walkley-Black Method and the other ones using the Soil Analysis Hach Test Kit Cat. No. 24959-00.




Methodology

- Soils were prepared for use in the EcoPlate microplate by mixing 5 g of soil from the top 4 inches and 5 g of soil from the bottom 4 inches from each collection site with 95 mL of phosphate-buffered saline (PBS) in a waring commercial blender for one minute. The soil suspension was diluted 1:100, and 1:1000.
- Each well of the EcoPlate was inoculated with 120 µL of the 1:100 and 1:1000 dilutions. The EcoPlates were incubated at 25°C. The absorbance values (optical density) of the wells were determined using a Perkin Elmer Victor x3 multilabel plate reader and measured at 24, 48, 72, 96, and 120-hour time intervals. Triplicates were done and data were compared using Analysis of Variance (ANOVA), with a $p<0.05$ as significant. IBM SPSS 25 was used to perform the analyses.



Results

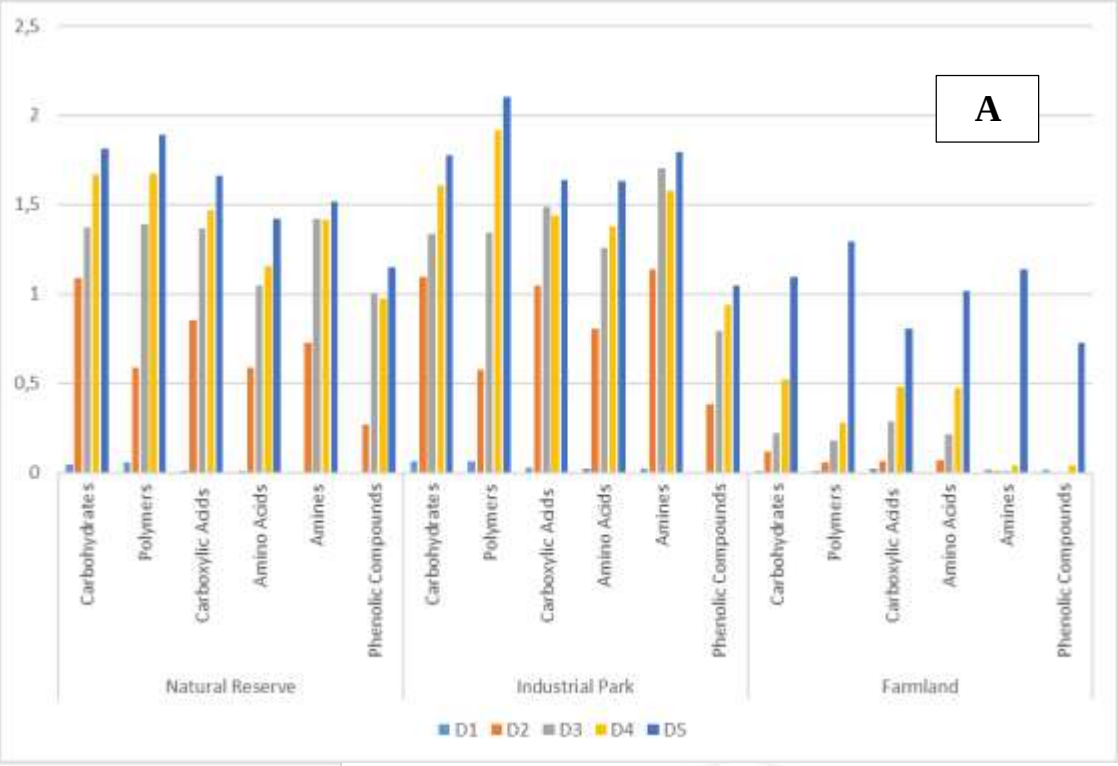
BIOLOG
EcoPlate™

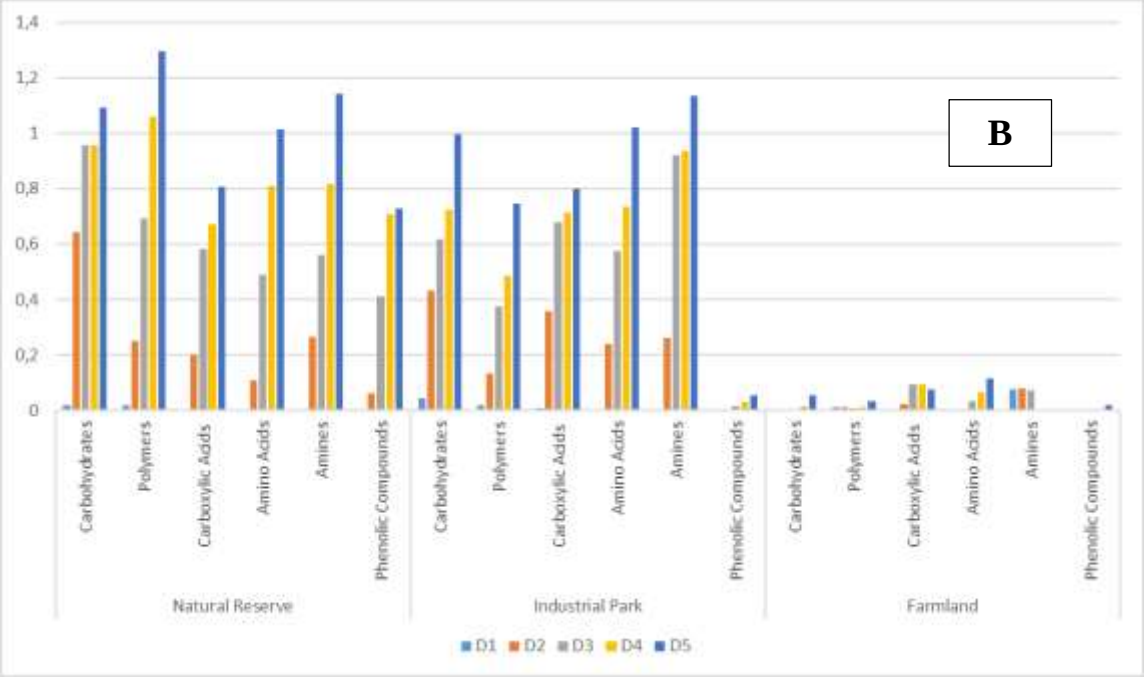
Microbial Community Analysis

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine	G1 D-Cellobiose	G2 Glucose-1-Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl-amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

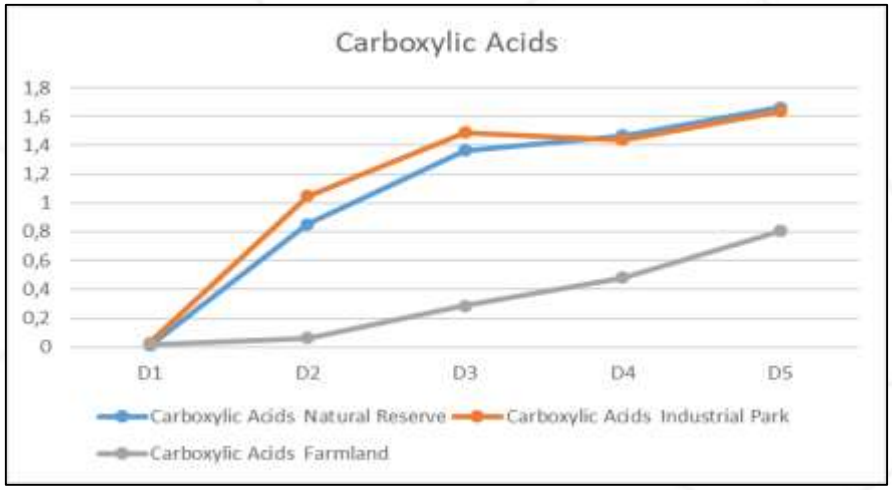
FIGURE 1. Carbon Sources in EcoPlate

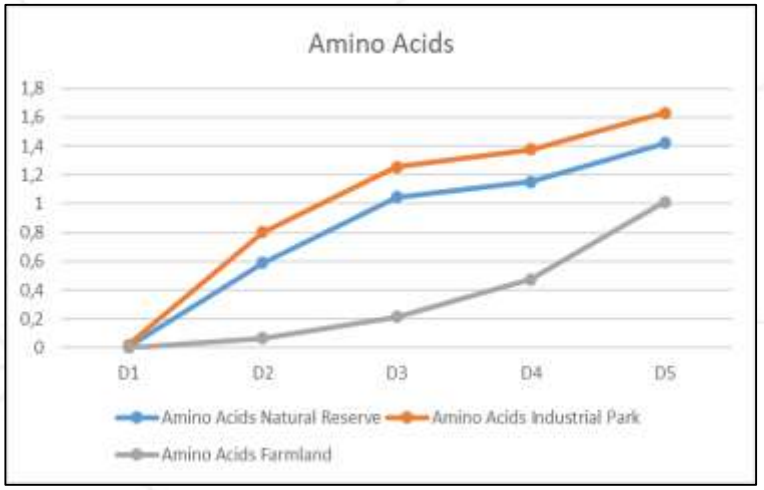
Results

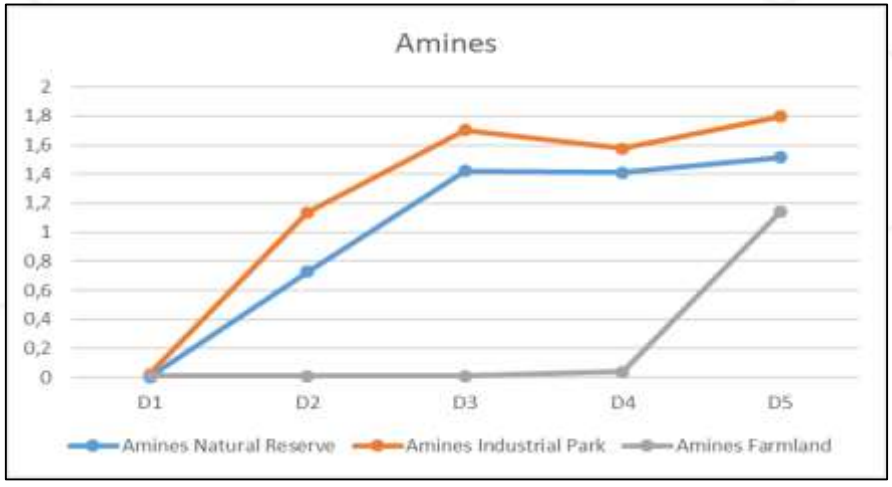


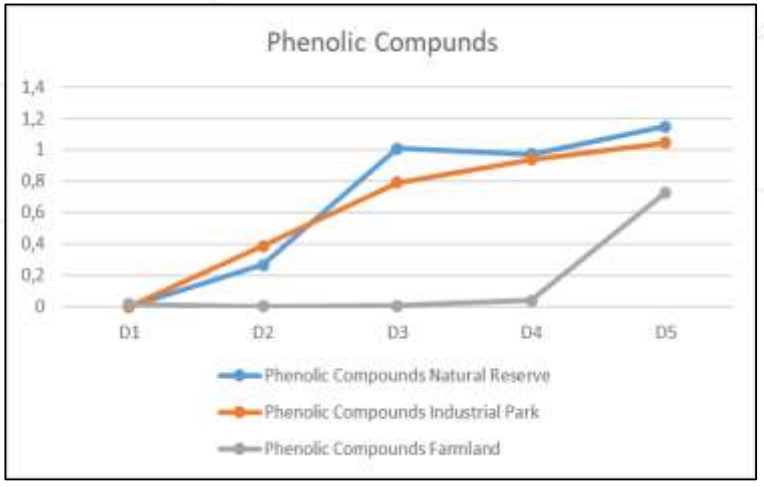


Optical Densities to Natural Reserve, Industrial Park and Farmland Microbial Soil Communities from D1 to D5 of 1:100 (A) and 1:1000 (B) Dilutions









Optical Densities by Carbon Sources: Carbohydrates, Polymers, Carboxylic Acids, Amino Acids, Amines, And Phenolic Compounds of Microbial Soil Communities by Natural Reserve, Industrial Park and Farmland from D1 to D5 of 1:100 and 1:1000 Dilutions

Discussion

- According to The European Soil Data Centre (ESDAC, 2020), soil biodiversity is the variation in soil life, from genes to communities, and the ecological complexes of which they are part, that is from soil micro-habitats to landscapes. In this context, microbial metabolic responses obtained in this study, provides important information about impact that anthropogenic sources made on soils.
- Through the collection of environmental samples, it was found that soil samples obtained from areas with high glyphosate exposure (Farmland) had lower optical densities than areas with no glyphosate exposure (Natural Reserve and Industrial Park). This aligns with the findings that suggests that long-term applications of glyphosate influence microbial diversity and community composition (Newman *et al*, 2016; Kuklinsky-Sobral *et al*, 2005; Lancaster, Hollister, Senseman, & Gentry, 2010).
- In addition to that, data showed how the communities exhibit differences in adaptation through time when they must use to specific carbon sources. This agrees Arteaga-Garibay et al (2016) where they determined the physiological profile of the microbial community using a set of substrates and carbon sources to establishing a characteristic response pattern without isolation of axenic crops.

Conclusion

- Results indicate that EcoPlate (Biolog, Inc.) can be used to monitor changes in soil microbial communities over time, as well as, the metabolic use of carbon sources of these communities in soils. On the other hand, the study gave evidence of changes in soils where anthropogenic activities are on it. The extensive herbicide use is an example of that. This example indicated that the adaptation period of microbes is higher than the ones in natural environments to be capable to use different carbon sources. Future work is needed to determine how additional carbon of anthropogenic sources can be use as food by microbes and their effect over time.

Acknowledgements

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Thank you for your attention