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Microbial Diversity of Human-Cultivated and Wild Soil on Longwood University's Campus

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Introduction

- Soils in ecosystems across the world are home to a rich tapestry of microorganisms, including bacteria, archaea, and fungi.[1]
- The highest microbial diversity is typically found in soils with a pH close to neutral.[2,3]
- Longwood University has a nutrient management program that specifies that the soil used in flower beds is composted soil from on-campus composting operations, and that all fertilizer is low in nitrogen, has no phosphorus, and is used at a bare minimum as indicated by soil tests.[4]

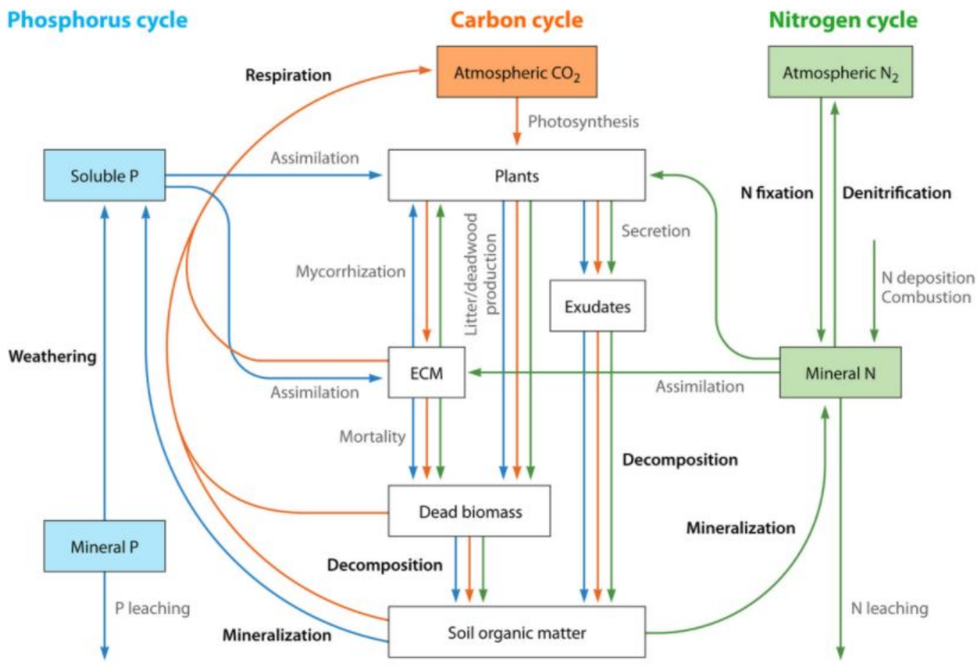


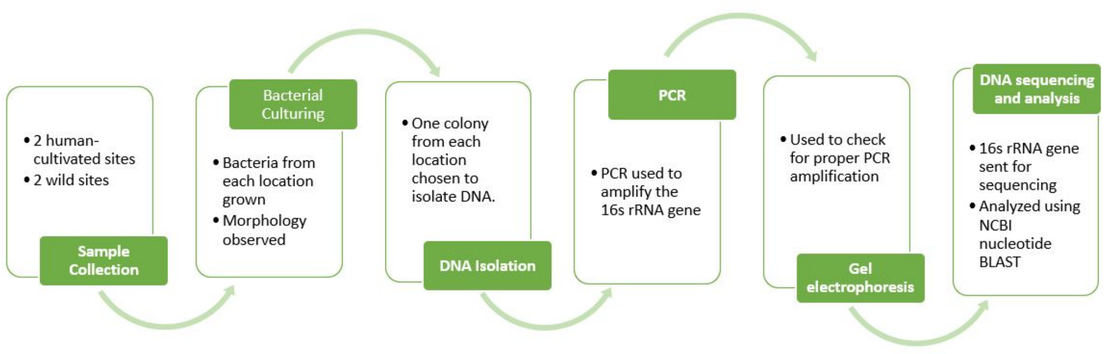
Figure 1. Role of soil microbes in biogeochemical cycles. Soil microbes play important roles in biogeochemical cycles, by decomposing organic matter, nitrogen fixation, and solubilization of inorganic phosphorus. **Bold processes** actively involve soil bacteria.[5]

Specific Aim

- Compare and contrast the microbial composition and diversity between the human-cultivated and natural sites.
- Determine if correlation exists between the soil pH of each site and the microbial composition and diversity found at that location.
- We predicted that (i) the composition of the microbial communities would vary depending on the pH of the soil, and (ii) acidic soil would show a decrease in microbial diversity in comparison to less acidic soil.

Figure 2. Human-cultivated and wild sample sites. Soil samples were collected from four locations on Longwood University campus. Two human-cultivated sites were sampled: a campus flower bed and the soccer field at Griffin Boulevard (top row). Two wild sites were also sampled: the forest floor and the creek behind the Environmental Education Center (bottom row).

Methods



Results

	pH	Nitrate (ppm)	Nitrite (ppm)
Human-cultivated sites:			
Flower bed (FB)	6.4	5	0
Soccer field (SF)	6.8	5	0
Wild sites:			
Forest (F)	6.2	5	0
Creek (C)	6.4	5	0

Table 1. pH and other chemical characteristics of soil from each of the four test sites.

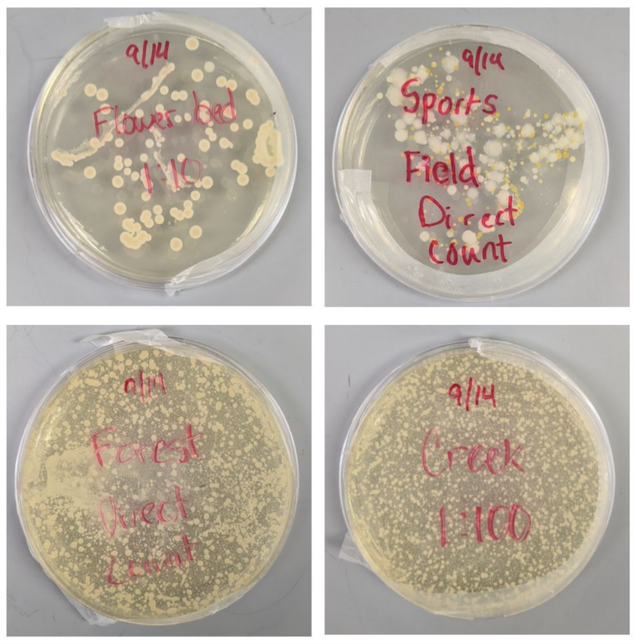


Figure 3. Bacterial growth of human-cultivated and wild sites. *Top row* - human-cultivated sites. *Bottom row* - wild sites

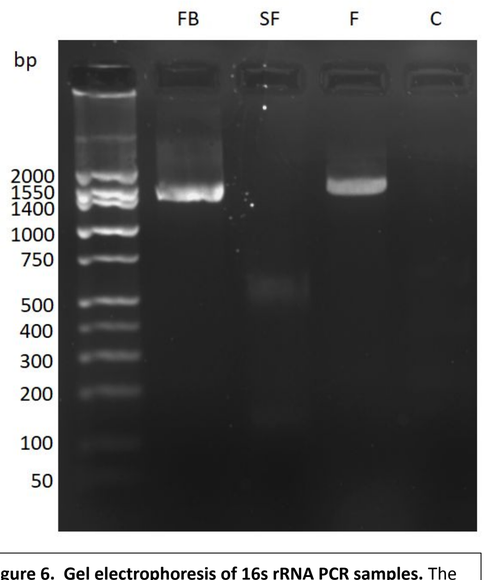


Figure 6. Gel electrophoresis of 16s rRNA PCR samples. The bacterial 16s rRNA gene was amplified using PCR, and run through gel electrophoresis to test for successful PCR amplification. The flower bed, soccer field, and forest samples were successfully amplified. The creek sample was not successfully amplified.

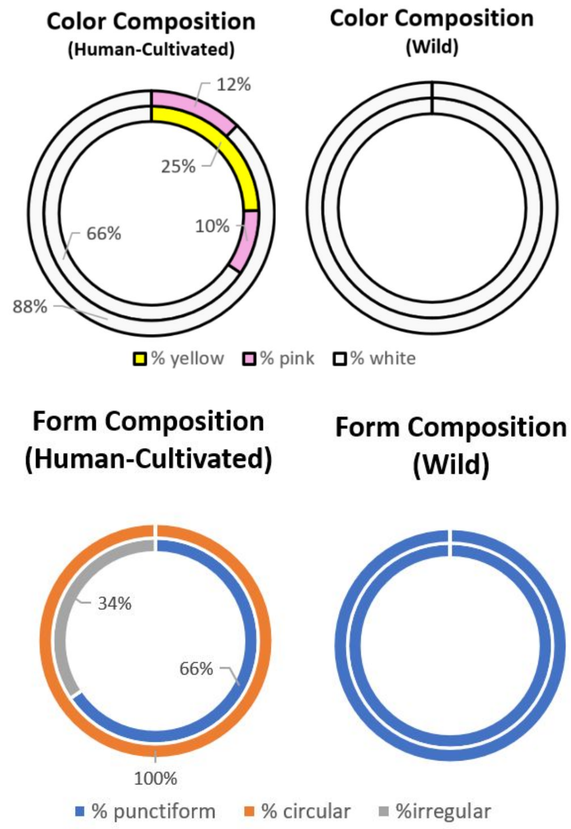


Figure 4. Color and form composition of bacterial colonies from human-cultivated and wild sites. *Inside rings - soccer field, forest. Outside rings - flower bed, creek.*

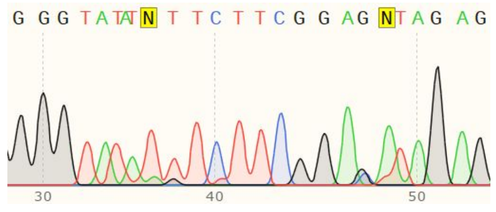


Figure 5. 16s rRNA DNA sequences isolated from the flower bed bacterial colony. After PCR amplification, the bacterial 16s rRNA DNA sequences from each of the chosen colonies was sent for DNA sequencing.

Isolate (soil)	Closest BLAST alignment	Phylum	% identity	# of gaps	Previously found in
Flower bed	***Flavobacterium hercynium***	Bacteroidetes	97%	0/879	Stream water, compost
	Flavobacterium hydatis	Bacteroidetes	97%	2/879	Gills of diseased fish
Soccer field	***Pseudomonas fragi***	Proteobacteria	99%	1/873	Soil, freshwater, spoiled meat/milk
	Pseudomonas psychrophila	Proteobacteria	99%	1/873	Arctic water, cold room storage
Forest	***Bacillus simplex***	Firmicutes	99%	1/1052	Rhizospheres of plants
	[Brevibacterium] frigoritolerans*	Actinobacteria	99%	1/1052	Arid soil of Morocco, roots of plants in Nebraska

Table 2. Top BLAST alignments for the sequenced bacterial colonies. The 16s rRNA DNA sequences from each of the bacterial colonies were run through BLAST, and the top two closest matches by % identify and number of gaps are displayed below.

Conclusions

- Human-cultivated sites have pH values closer to neutral than wild sites → may be the result of Longwood's nutrient management system.
- More bacterial diversity was found in the human-cultivated samples, especially the soccer field, which is consistent with our hypothesis that most diversity would be found at the location with a pH closest to neutral.
- *Flavobacterium hercynium* was first isolated from a freshwater creek in Germany in 2007[6], later found in compost in Japan.[7]
- *Pseudomonas fragi* has been shown to play a role as a promoter of plant growth.[8]
- *Bacillus simplex* has previously been found in the microbial communities associated with plant roots, and shown to promote the growth of lateral root systems.[9]

Future Directions

- Recollect and sequence a soil sample from the creek shore, in order to allow a comparison with the forest floor sample.
- Collect samples from more locations around campus, and sequence multiple colonies from each location to develop a more solid understanding of the microbial compositions.
- Collect samples from locations around campus with a wider range of pH values.

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