Examination of the Relationship Between Earthworm Biodiversity and Soil Potassium Concentrations

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Abstract

Earthworms, an essential part of maintaining healthy soil, have been found to degrade different minerals including potassium, which we chose to research. Here, we examined the biodiversity of earthworms found in New Jersey and compared them to the relative potassium levels in the soil. We hypothesized that certain species of earthworms are associated with higher concentrations of potassium. We collected our 13 samples from two different locations across Tenafly NJ: rural and urban which were characterized by potassium levels of 50 PPM and 400 PPM respectively. After DNA extraction and isolation, the DNA was PCR amplified with a COI primer and underwent gel electrophoresis. However, only 6 were successfully amplified. These 6 samples were sent to GeneWiz and analyzed; all species found were *Aporrectodea limicola* so from the results collected, there was no conclusion regarding the relationship of earthworm species and soil potassium level.

Introduction

- **Earthworms**: annelid worms that burrow in soil and feed on soil nutrients and decaying organic matter.
- **Species Common to NJ (Steves et al. 2007):**
  - *Lumbricus terrestris* (common earthworm),
  - *Dendrobaena octaedra*
  - *Aporrectodea limicola*
  - *Eisenoides lonnberg*
- **Potassium**:
  - Essential for plant growth: transport sugars, synthesize proteins, produce of ATP, etc.
  - Required in large amounts (~400 PPM+) for proper growth and reproduction of plants

Table I. Potassium levels for Luster Leaf 1601 Rapitest™ Soil Test Kit:

Level	Equivalency
Surplus	900 PPM
Sufficient	600 PPM
Adequate	400 PPM
Deficient	200 PPM
Depleted	50 PPM

Figure I. Picture of *Lumbricus terrestris*
https://i.ytimg.com/vi/yhLCHX9w6dU/maxresdefault.jpg

- **Previous work done**:
  - Earthworms have the ability to convert potassium into a form that can be used by plants. (Liu, Lian, Wang, Jiang 2011)

Research Goal

•To identify and compare the species type found in varying levels of potassium in the soil using the standard DNA barcoding and NextGen sequencing.

•**Hypothesis**: We hypothesized that our results would confirm that there are some species of earthworms that are correlated with higher concentrations of potassium in the soil than others.

Methods

Sampling and Preparation:

1. **Earthworm collection**
   a. A 1-sq. ft. hole was dug in the ground, a 2-L mustard-water solution was poured to drive earthworms to the surface.
   b. Earthworms were inserted into a plastic bag with 10-mL isopropyl alcohol and stored into a cooler.
2. **Potassium testing using Luster Leaf 1601 Soil Test Kit™**
   a. Soil sample and five cups of water were added into a container and shaken for at least 1-minute.
   b. After settling, an orange potassium capsule was added.
   c. The mixture color was compared to the color chart.
3. **13 Total samples were collected from NJ**
   - 4 samples from the Tenafly Nature Center (Rural) (40.9246, -73.9450)
     - 3 samples from the parking lot (TAT-001,002,003)
     - 1 sample from the forest (TAT-004)
   - 9 samples taken from Devon Road (Urban) (40.9098, -73.9524) (TAT-005 through TAT-013)

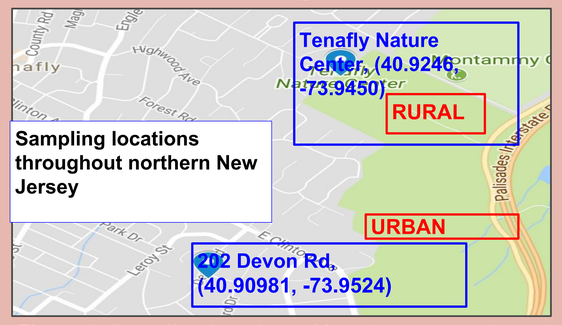


Figure II. Sampling locations. Map of samples collected across the Tenafly Nature Center and 202 Devon Rd

DNA Extraction:

1. **Lysing the cells**
   - 300 µl of guanidine HCl solution was added to each sample pellet and mechanically ground
   - Samples were heated for 5 minutes at 65 C and centrifuged for 1 minute at 13.3 rpm
2. **Binding the DNA**
   - 3-µl of silica resin was added to 50-µl of supernatant.
   - Samples were heated for 5-min at 57°C and centrifuged for 30 seconds
3. **Removing cell debris/contaminants:**
   - The supernatant was poured out, and 500-µl of wash buffer added to pellet. The sample was resuspended by pipetting and centrifuged for 30 seconds.
   - The removal of the supernatant, washing, and centrifugation was repeated, and then supernatant was completely removed.
4. **Unbinding silica from DNA**
   - 100 µl of dH2O was added to unbind the silica from the DNA.
   - 50 µl of supernatant were transferred to a new tube, and the DNA samples were stored on ice.

Polymerase Chain Reaction and Gel Electrophoresis:

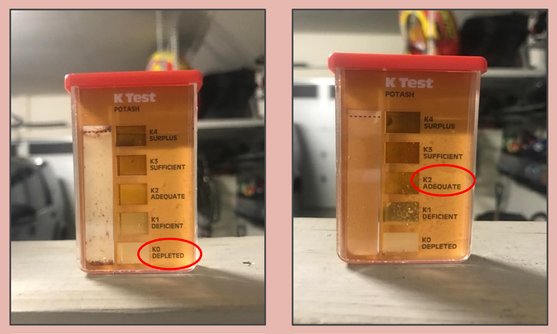
1. **DNA Amplification (PCR)**
   - 5 µl of each (defrosted) sample was added to 10.5 µl COI primer and 12.5 µl Taq mix, and placed in the thermal cycler for 2.5 hours
2. **Gel Electrophoresis:**
   - 2 µl of each amplified DNA sample was mixed with loading dye and loaded into a 2% agarose gel with a positive control and DNA ladder
   - The gel was electrophoresed 30 minutes at 130 V
   - Samples that produced the target bands (~300 bp) were sent to GeneWiz for sequencing in both the forward and reverse directions

DNA Sequencing, Editing, and Analysis

- The sequenced DNA was uploaded into the DNA subway platform; then were trimmed, paired, and edited for consensus
- Sequences were matched to those in BLAST and GenBank to identify species

Results

Figure III. Gel electrophoresis of samples. Indicates that wells #5, 6, 9-12 were successfully amplified the Cytochrome Oxidase I (COI) gene (650-700 bp section) that was targeted in the PCR.



A. Rural | B. Urban

Figure IV. Potassium soil level results. A. indicates depleted potassium levels (~50PPM) from soil taken from rural areas. B. indicates adequate potassium levels (~400PPM) in soil taken from urban areas.

Results (continued)

Table II. Species found and correlated potassium levels.

Location	Samples	Species	Potassium level
Tenafly Nature Center	TAT-001 thru TAT-004	N/A	Depleted (~50PPM)
202 Devon Road	TAT-005 thru TAT-013	*Aporrectodea limicola*	Adequate (~400PPM)

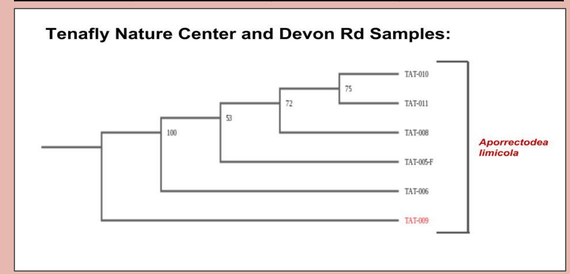


Figure V. Phylogenetic trees of selected samples. A split in the branches indicates a common ancestor. All samples are closely related as they are all the same species.

Figure IV. Picture of *Aporrectodea limicola*, the species of earthworms found in this study.
http://www.discoverlife.org/mp/20q?search=Aporrectodea&guide=Earthwormsz

Discussion

From the 13 earthworms we sampled, only 6 of them were successfully DNA amplified. Although we did find a significant difference in potassium soil levels between the two locations, all of our samples were of the same species which was *Aporrectodea limicola*. Therefore, we were unable to come to a conclusion of whether certain earthworm species were associated with lower or higher levels of potassium in the soil. Surprisingly, the species found was not the common earthworm, *Lumbricus terrestris*. If this study was to be repeated we would put more emphasis on the DNA extraction process since only 6 of the 13 sampled species were successfully extracted and analyzed. In addition, if there were more species sampled there would have been a higher chance of earthworm diversity; it also would have made our study much more accurate.

References

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