PRACTICAL 2: SOIL ANALYSIS LAB

Preparation

1. **Read** the Practical 2 Soil Analysis Instructions and review the associated group worksheet.
2. Watch the Soil Analysis Demonstration Video (also linked on Quercus).
3. You may also find it helpful to review your notes about biotic and abiotic factors from your Preliminary Observations in Practical 1.
4. **Print out and bring** the group worksheet to Practical 2.

Overview

Last week you toured four habitats on the UTM campus: **disturbed, wetland, grassland, and forest**. You then made some observations about what factors, both biotic and abiotic, could affect biodiversity. Some of the factors included light exposure, wind exposure, soil conditions, water availability and temperature. You also collected a soil sample from each of the habitats. In this practical, you will examine the **abiotic soil conditions** that may affect biodiversity including **salinity**, **pH**, and the macronutrients **nitrogen**, **phosphorus,** and **potassium**. Estimates of soil **organic matter content** will also be considered.

Background Information

Soil Salinity

Salts can accumulate naturally in soils due to the weathering of minerals. However, high levels of salt in soils can also build up to due to irrigation (typical in agriculture) or from the application and leeching of road salt. While natural amounts of salt may be beneficial to plants, high levels can induce salt stress that drastically inhibits plant growth.

Soil pH

Soil pH is a measure of the acidity or alkalinity of a soil. Some factors that may increase acidity (lower pH) include high levels of rainfall, acid rain, mineral weathering of soils that are high in iron, and decomposition of organic matter. Some factors that may increase alkalinity (increase pH) include accumulation of salt and the weathering of minerals containing sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^+). Soil pH is particularly important for nutrient absorption by plants. Generally, plants have an optimum pH range between 5.5 to 7.5 (although some plants prefer more acidic or more alkaline soils). Nutrients in the soil often exist as ions bound to soil particles; the pH of the soil can affect the dissociation of these nutrient ions from the soil particles, thus making them more or less available for uptake by plant roots.

Soil Nutrients

In addition to sunlight, carbon dioxide, and water, plants need a variety of macro- and micro-nutrients in order to grow and thrive. Most of these nutrients come from **decaying organic matter** in the soil; typically, the darker the soil, the higher the organic matter content. This decaying organic matter provides macronutrients (such as nitrogen, potassium, phosphorus, calcium, sulphur, and magnesium) and micronutrients (such as iron, boron, chlorine, manganese, zinc, copper, molybdenum, and nickel). We will focus on the macronutrients **nitrogen, phosphorus,** and **potassium**.

Nitrogen
Nitrogen (N) is a major component of amino acids and is required for protein synthesis (including all of the enzymes required for photosynthesis). Nitrogen is also a major component of the photosynthetic pigment chlorophyll. Although most of the Earth's atmosphere consists of nitrogen gas (N_2), plants cannot directly use this form of nitrogen. Instead, atmospheric nitrogen is converted into ammonium (NH_4^+) by nitrogen-fixing bacteria in the soil. Legumes like white clover and black medic have a mutualism with rhizobia bacteria, which fix nitrogen for the plant in return for carbon and housing in root nodules. Ammonium can also accumulate in the soil from the breakdown of decaying plant matter and animal waste. Nitrifying bacteria in the soil convert ammonium to nitrites (NO_2^-) and then nitrates (NO_3^-) that can be used by plants. Nitrogen can also be added to soils via synthetic fertilizers.

Phosphorous
Phosphorous (P) is required for DNA and lipid synthesis, as well as for the production of ATP (the primary carrier of energy in cells). Plant-available phosphorous (as $H_2PO_4^-$ or HPO_4^{2-}; dihydrogen phosphate and hydrogen phosphate, respectively) enters the soil via the weathering of rocks and minerals, the decomposition of animal waste and by the application of synthetic fertilizers.

Potassium
Potassium (K) is required by plants for carbohydrate metabolism, enzyme activation (e.g., during photosynthesis), osmotic regulation (e.g., controlling water loss via stomatal regulation), and protein synthesis. Potassium enters the soil via animal waste, decomposition of soil organic matter, and the addition of fertilizers by humans. Soil potassium exists in solution as K^+ ions that can be taken up by plant roots.

PRACTICAL 2: Soil Extraction & Analysis

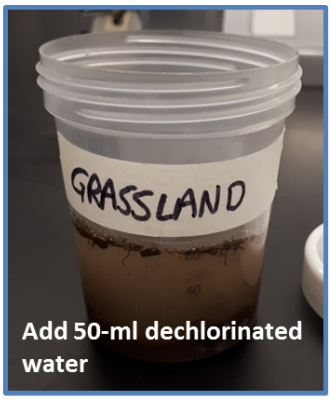
Materials

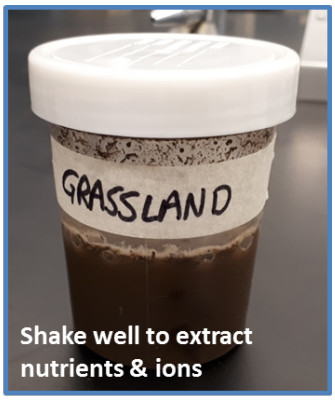
- 4x Labeled specimen cups with 10g soil samples (one from each habitat)
- 1x Specimen cup w/ RO water (negative control)
- 1x Specimen cup w/ Fertilizer (positive control)
- 4x Sharpie markers (for labelling test strips)
- 1x Electrical Conductivity (EC) meter
- 1x Container of pH testing strips
- 1x Container of Nitrate-Nitrite test strips
- 1x Container Phosphate test strips
- 1x Container Potassium test strips
- 6x micro-tubes K^+-1 test solution (in small rack)
- 1x digital timer
- 2x 1-L wash bottles w/ RO water
- 1x rinse-water container
- 4x black plastic spoons
- 4x rounds of filter paper
- 1x box Kimwipes®
- 1x stack of paper towels

Procedure

Extraction

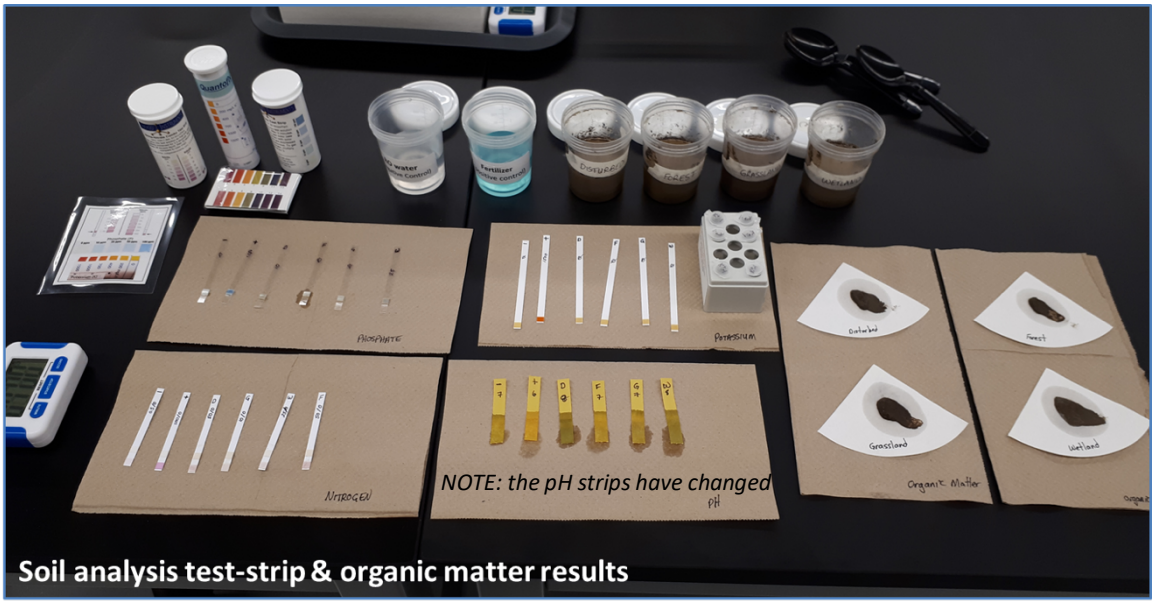
1. Obtain your labelled dry soil samples (weighed into 10 g samples for each habitat).
2. Use the RO wash bottles to add **50 ml RO water** to the soil in each specimen cup (i.e., **fill each specimen cup to the 60 ml mark**).
3. Use the lids provided to **tightly seal** your specimen cups.
4. Mix your samples (to extract salt and nutrient ions):
    a. 1 minute – shake vigorously
    b. 1 minute – rest
    c. Repeat the shake-rest cycle 5 times in total
    d. Allow soil to settle for ~5 minutes after the last shake
    e. While waiting for the final rest, move on to Step 5 to set up your testing area.





Setup for Soil Analysis

5. Layout and label the following test strips from the containers on your bench (*see the picture of the set up below*):
    a. **6** Nitrate-Nitrite test strips (white strip with 2 pads)
    b. **6** Phosphate test strips (clear strip with 1 pad)
    c. **6** Potassium test strips (white strip with orange pad)
    d. **6** pH test strips (white strip with orange pad – don't mix it up with the Potassium)
    e. Tightly seal the test strip containers after taking what you need. **Do not get the strips wet before using!**
    f. Be careful not to touch the test pad end.
    g. **You should label the strips** for each habitat/control so that you can keep track of them.



Soil Analysis

6. **Electrical Conductivity (EC) Test (for testing salinity)**
   a. Remove the protective cap from the EC meter.
   b. Press the **'ON/OFF'** button to turn the meter on.
   c. Press the **'MODE'** button to get conductivity readings in **µS/cm**.
   d. Immerse the meter into the **watery layer of the soil sample** (no more than 1 inch).
   e. Wait for the display to stabilize (~10-30 seconds).
   f. Press the 'HOLD' button and record the conductivity of the solution.
   g. Rinse the probe thoroughly using the RO wash bottle – Do this over the **RINSE-WATER container** (NOT over your sample container).
   h. Carefully dab the probe with Kimwipes® to remove excess water.
   i. Press the **'CLEAR'** button to reset the probe before taking the reading for the next habitat or control.
   j. **Repeat these steps with all test samples** (including positive and negative controls).
   k. **Record the EC values** in the group worksheet. **The higher the conductivity value, the higher the salinity** of the sample.
   l. When finished with all samples, rinse the EC probe with fresh RO water and dab the probe dry with Kimwipes®.You can leave the probe open to air-dry while you do the other soil tests.

EC Test

Remember to replace the cap at the end of the practical (only once the probe is dry).

7. **Phosphorus Test Strips**
   a. *Coordinate with your group to do **all soil solution samples** and **controls** at the same time for easier comparison*.
   b. Dip a phosphorus test strip into the controls and the watery layer of the soil sample for **1-2 seconds**.
   c. Remove the test strip and gently shake off excess liquid.
   d. Wait **7 minutes** and compare the darkest part of the pad (bump side) with the colour chart – do not wait too long to do this as any colour change may fade as the strip dries out.
   e. **Record your results** in the group worksheet.

NOTE: Make sure the phosphorous test strip has the paper square at the top of the bump (it can fall out if the strips are shaken).

8. **Nitrite-Nitrate Test Strips (for indicating nitrogen content)**
   a. *Coordinate with your group to do **all soil solution samples** and **controls** at the same time for easier comparison*.
   b. Dip the nitrite-nitrate test strip into the controls and watery layer of the soil sample for **2-3 seconds** – make sure both pads are immersed.
   c. Remove the test strip and gently shake off excess liquid.
   d. Wait **1-3 minutes** and compare test pads to the colour chart – do not wait too long to do this as any colour change will fade as the strip dries out.
      - Nitrite (upper pad)
      - Nitrate (lower pad)
   e. **Record the values** in the group worksheet.

9. **pH Test Strips**
   a. *Coordinate with your group to do **all soil solution samples** and **controls** at the same time for easier comparison*.
   b. Dip a test strip into the controls and watery layer of the soil sample for 1-2 sections and remove and gently shake off excess liquid.
   c. Compare to the colour scale (on the side of the container) **within 30 seconds**. Do not allow the test strip to dry before reading against the colour scale.
   d. **Record the pH values** in the group worksheet.

10. **Soil Colour/Organic Matter Content**
    a. Use the spoons provided to scoop some of the wet soil from the bottom each habitat sample (avoid scooping too much excess water) onto a separate piece of filter paper and let it sit for a few minutes.
        - You can put the filter paper on top of a few sheets of paper towel to help the water absorb a bit faster (and make clean-up easier).
        - Make sure to keep track of which sample is on each piece of filter paper.
    b. After the filter paper has removed some of the moisture (your samples should still be moist but not shiny) compare the colour of each sample.
    c. Rank your samples from **lightest/lowest organic content (1)** to **darkest/highest organic content (4)** and record this ranking in your group worksheet.

11. **Potassium Test Strips**
    NOTE: DO THIS TEST LAST, AS THE TEST STRIP WILL BLEED COLOUR INTO YOUR TEST SAMPLES
    a. *Coordinate with your group to do **all soil solution samples** and **controls** at the same time for easier comparison*.
    b. Dip a test strip into controls and the clear, watery layer of the soil sample for **60 seconds**.
    c. Shake off excess liquid and place the test strip into the **K^+-1** testing solution (one test strip into each of the 6 clear micro-tube) for **60 seconds**.
    d. Gently shake off excess liquid and compare the test pad with the colour chart – do not wait too long to do this as any colour change may fade as the strip dries out.
    e. **Record your results** in the group worksheet.

12. **Enter your group data in the class spreadsheet** and **complete the group worksheet**.

13. **CLEAN-UP**
    d. **Soil Samples** – **do not pour soil directly down the sink!**
        - **Remove any masking tape/labels** from the cups.
        - Shake specimen containers to re-suspend the soil and empty the contents (water + soil) into the large **soil settling bins**.
        - Put the empty specimen containers (with lids removed) into the soapy water bins.
    e. **Rinse-Water Containers** – pour into the sink and return the empty container to your bench.
    f. **Positive and Negative controls** – leave at your bench.
    g. **Spoons** – wash, dry and return them to your bench.
    h. **Used test strips** – discard in regular garbage.
    i. **Soiled filter paper/paper towel** – discard in regular garbage.
    j. **EC meter** – If the probe end is still slightly wet, leave the cap off to allow it to dry. If it is dry, replace the cap.
    k. **Tidy** and **wipe down your benches**.
    l. **Wash your hands**.