Mini but Mighty: Assessing Liming Effects on Soil Biota Communities

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Introduction

- The need to feed the world's increasing population has led to agricultural intensification and heavy reliance on fertilizers, which increase soil acidification. Acidic soils limit crop growth and productivity and negatively impact soil microorganisms (Junior et al., 2020).
- In Alberta, there are 1 mil. acres of strongly acidic soils (pH < 5.5) and 4.5 mil. acres of moderately acidic soils (pH = 5.5-6.0) (Alberta Agriculture, Food, and Rural Development, 1996).
- Acidic soils are deficient in beneficial nutrients such as phosphorus, calcium, magnesium and molybdenum (Liang et al., 2021). As pH decreases, aluminum (Al^{3+}) and manganese (Mn^{2+}) solubility increases causing phytotoxic effects on plant roots and shoots (Yang et al., 2009).
- **Liming** is used to counteract soil acidity. Liming materials include carbonates, oxides, and hydroxide mixtures of calcium and magnesium which raise soil pH and neutralize metal toxins (Junior et al., 2020).
  - **Hydrated Lime** ($Ca(OH)_2$) is a product from quicklime (CaO) hydration (Bessaim et al., 2018).
  - **Sugar Beet Lime** is a byproduct of the purification process of raw sugar and is comprised of $CaCO_3$, organic matter, and water (Engel et al., 2020).
  - **Agricultural Lime** is naturally mined limestone ($CaCO_3$) which can be used or modified into subsequent liming sources. (Dowling et al., 2015).
  - **Cement Kiln Dust** is a byproduct produced in millions of tons by the cement production industry and has found to have alkaline properties (Zawrah et al., 2021).
- Soil biota (i.e., bacterial, fungi, nematodes) play a crucial role in the cycling of nitrogen, phosphorus, carbon and sulfur (Singh, 2017). Low pH decreases diversity, abundance and activity of soil biota (Rousk et al., 2010).
- **Objective:** To assess the impact of liming on soil pH and microbial and nematode communities.

Methodology

- Prior to liming, soil samples were collected from two different sites in contrasting zones: Breton (grey soils) and CDC-North (black soils) to establish a baseline for the experiment.
- Lime sources: Agricultural Lime, Sugar Beet, Hydrated Lime, Cement Kiln Dust. Control (no lime) plots are also included.
- Samples were assessed across different growing seasons (wheat-oats-canola) rotation at two sampling depths of 0 cm-7.5 cm and 7.5 cm-15 cm.
- Soil pH was measured in calcium chloride at the Natural Resources Analytical Laboratory (NRAL).
- Bacterial DNA was extracted from the soil using a DNeasy PowerSoil Pro Kit (Qiagen, USA). Nanodrop spectroscopy was used to assess the quality and purity of the extracted DNA.
- Genomic DNA was amplified using Polymerase Chain Reaction (PCR) techniques, which is where a targeted sequence of DNA can be amplified into numerous copies (Garibyan & Avashia, 2013).
  - The primers 341-F (Forward) and 805-R (Reverse) were used to amplify the V3-V4 region in bacteria.
- Afterwards, the PCR products were visualized using agarose gel electrophoresis.
- Samples were sent to the University of Laval for sequencing.
- Nematodes were also analyzed using microscopic techniques.

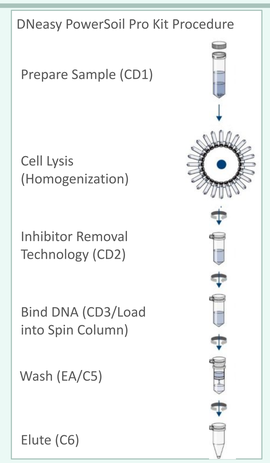

Fig. 1 Illustrated process of DNA extraction using a DNeasy Powersoil Pro Kit (Qiagen, USA).

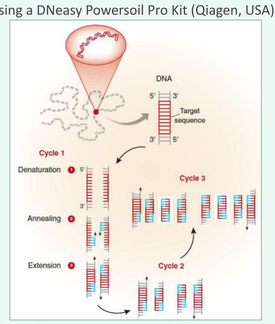

Fig. 2 Illustrated process of PCR amplification. (Garibyan & Avashia, 2013).

Results

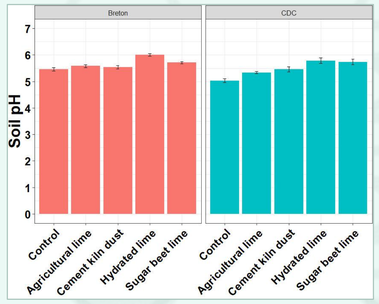

Fig. 3 Soil pH measured at the two sites (Breton and CDC-North) in plots treated with the different lime sources.

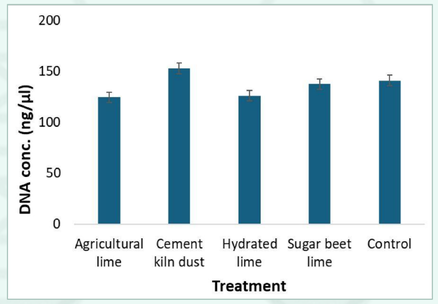

Fig. 4 Genomic DNA concentration measured in soil samples collected from limed and control (no lime) plots.

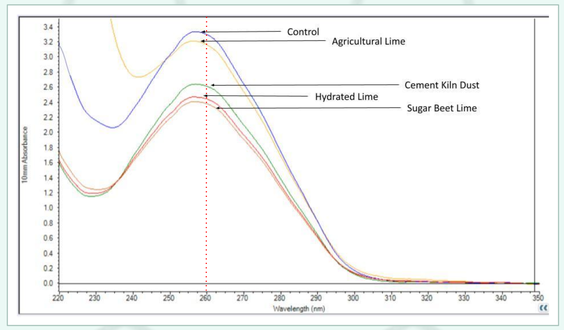

Fig. 5 Nanodrop spectroscopy graph displaying selected samples treated with different lime sources. The red dashed line shows the wavelength for maximum absorbance of nucleic acids.

Sample ID	Nucleic acid conc. (ng/µl)	A260 (Abs)	A280 (Abs)	260/280 ratio	260/230 ratio
Control	164.9	3.298	1.751	1.88	1.53
Cement Kiln Dust	130.4	2.609	1.384	1.89	2.27
Sugar Beet Lime	118.9	2.378	1.363	1.88	1.92
Hydrated Lime	122.1	2.442	1.300	1.88	2.06
Agricultural Lime	158.0	3.161	1.693	1.87	0.74

Table 1 Nanodrop results for selected samples treated with different lime types. Desire 260/280 ratio is ~1.8. (Thermo Scientific, 2003).

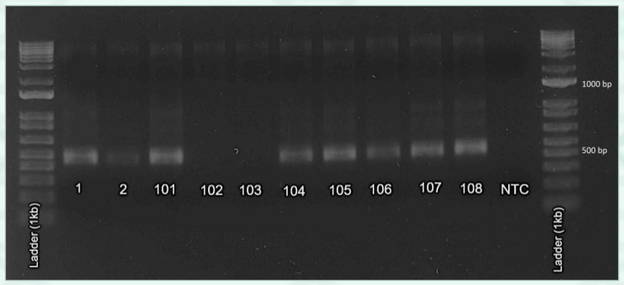

Fig. 6 Agarose gel electrophoresis pattern of PCR products of bacteria amplified with universal primers (341F and 805R) using samples collected from Breton. 500-600 base pairs expected. Samples: 1 (Baseline Breton), 2 (Baseline CDC-N), 101 (Hydrated Lime), 102 (Cement Kiln Dust), 103 (Control), 104 (Sugar Beet Lime), 105 (Agricultural Lime), 106 (Agricultural Lime), 107 (Hydrated Lime) and 108 (Sugar Beet Lime).

Fig. 7.1 Picture of full nematode body under Low Microscope Power (10x).

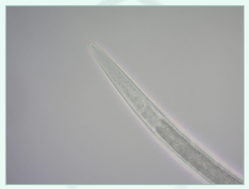

Fig. 7.2 Picture of nematode head under High Microscope Power (40x). Used to identify species and niche (Free-Living Nematode)

Fig. 7.3 Picture of nematode tail and reproductive organ under High Microscope Power (40x).

Discussion

- All liming sources used in the study at the two sites increased soil pH.
  - There was no significant increase of soil pH in the control (no lime) plots.
  - There was a significant increase in soil pH in both sites when Hydrated Lime was used.
- Nanodrop spectroscopy results demonstrated that genomic DNA is present in soil samples.
  - Fig. 4 indicates that Cement Kiln Dust had the highest concentration of DNA.
  - Fig. 5 indicates that the control (no lime) had the highest absorbency, consistent with the data shown in Table 1.
- Single bands observed on the agarose gel showed that the V3/V4 region was successfully amplified and thus bacterial DNA was present in the soil samples.
- Some free-living nematodes were present in limited quantities in selected soil samples.
- Overall, the observations show that the limed soils comprise of bacterial and nematode communities both of which can be beneficial in nutrient cycling, decomposition, and can be used as indicators of soil biological health.

Conclusion

- Liming is effective in increasing soil pH and thus can be used to manage soil acidity.
- Liming has an effect on soil biota present in the soil, which could be crucial in maintaining healthy soils. Cement Kiln Dust has the potential to also be used as an effective liming source, limiting the amount ending up in landfills.
- Further studies are needed to observe the long term effects of liming on soil microbial and nematode communities.
  - Next steps include the analysis of nematodes from the soil and DNA extraction of fungal microbes using appropriate primers targeting the Internal Transcribed Spacer (ITS) region.
  - Microbial and nematode DNA will be sequenced to assess liming effects on these communities and how they influence soil health.
  - The study is ongoing to determine the long term effects of lime application on soil chemical and biological properties.

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