1. **Enhancing the Saskatchewan Soil Health Assessment Protocol – Phase 2**
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4. Abstract

The concept of soil health recognizes soil as a living and dynamic natural system, a notion that aptly fits in the realm of biology. However, soil health tests and scoring tools are often dominated by indicators of soil fertility and chemistry. Biological indicators of soil health remain understudied and underrepresented in soil health assessments. To address this gap, here we evaluate soil attributes that reflect biological functions and vitality (including organic and total C, total N, mineralized C, extracellular enzyme activity, and phospholipid fatty acid (PLFA) analysis for microbial biomass and adaptation response ratio (ARR)). We assess if these biological indicators can be contextualized by soil classification and measure their responsiveness to agricultural management practices. Despite the dynamic nature of biological indicators of soil health, we find that soil classification by great group constrains measurements and serves as a useful contextualizing factor to adjust scoring functions. Further, we find biological indicators of soil health (namely soil organic C, total N, and P and S enzyme activity) generally improve with more regenerative crop production practices such as cover cropping or organic management. Although other indicators such as CO_2 mineralization, N and C cycling enzymes, PLFA and ARR showed fewer differences among crop production practices, all were greater under prairie grassland than cropland. These trends were also supported by soil organic matter stoichiometry (N:C, P:C, and S:C) results, suggesting that element to carbon ratios might be useful features for future soil health assessments. In contextualizing soil health scores by soil class and including biological indicators of soil health that embody soil pools, processes, and life, soil health assessments will not only better represent soil biology and appropriately contextualize soil health scores, but also move towards better targeting soil *functioning*.

5. Introduction:

Soil health assessments can improve our understanding of the relational mechanisms between and among soil, ecosystems, and society—guiding us to nurture soil attributes that promote the functions we value. Value is commonly placed on soil functions that sustain life, whether through supporting biodiversity, nutrient and carbon cycling, water cycling, social culture, and/or the provisioning food and fibre (Lehmann et al., 2020). The health metaphor has continued to proliferate in agricultural soil science, especially within soil biology (Jian et al., 2020; Liu et al., 2020) with definitions that emphasize life, i.e., the *vitality* of a soil in sustaining the socio-ecological functions of its enfolding ecosystem, by Janzen et al. (2021). As such, the biological underpinnings of health are evoked and reinforced when using soil health as a metaphor.

In agricultural settings, and most commonly in row crop production, soil health assessments have led to the development of scoring tools (Chahal et al., 2023; Gugino et al., 2009; Karlen et al., 2019; Moebius-Clune et al., 2016; Wu and Congreves, 2021). Although these scoring tools do not *quantify* soil health per se (an arguably impossible outcome), they can be illuminating *indicators* of soil health when the attributes are clearly linked to desired functions (Janzen et al. 2021). Soil health scoring frameworks typically involve obtaining measurements for soil biological, chemical, and physical attributes; quantitatively transforming the soil measurement for each attribute into a score or ranking; and integrating the numerous scores into a single soil health index (Rinot et al., 2019). Although the specific equations, thresholds, and integration strategies may differ (i.e., some have developed regionally appropriate limits, others use a broad approach; some integrate using weighted averages, others use structural equation modelling), conceptually, the goal is the same. The result is meant to serve as an easy-to-interpret index that non-specialists can use to make informed decisions when selecting and implementing soil management practices. However, interpreting soil health has its complications. Soil health is very much context dependent (Norris et al., 2020), the attributes and constraints that confer soil health are never the same in place and time (Ng and Zhang, 2019). So therein lies a conundrum. On one hand, to derive meaning, soil health assessments must be developed and applied in specific contexts (i.e., scores are only meaningful if they are regionally-representative). But, on the other hand, soil health assessments must not be overly restrictive or confined to holding meaning only for marginal or narrow scenarios, as this could result in inconsequential and inconsistent interpretations across different regions. For a test to be applicable, it involves finding the right balance where it remains meaningful at scale but can still be contextualized to any regional constraints.

There are many different soil attributes that might be used to indicate soil health. In practice, however, soil health quantification is still dominated by fertility and chemical indicators (Jian et al., 2020; Lehmann et al., 2020), despite growing appreciation of the importance of soil biology in shaping the soil vitality to sustain the socio-ecological functions of its enfolding ecosystem. Soil health is not the same as soil fertility. Whereas soil health suggests ecosystem and socioecological functions, soil fertility is primarily based in nutrient availability, linked to fertilizers, and emphasizes crop productivity. Certainly, soil fertility measures interact with and influence soil health expression (Grandy et al., 2022), but fertility is regulated by different attributes and management practices compared to those governing soil health. Fertility is informed by available nutrients such as extractable N, P, and K, and managed predominantly through fertilizer programs, in contrast to soil health management practices that are used for more holistic or ecological reasons (Bagnall et al., 2023). Like the distinction from fertility, soil inherent properties are yet other measures related to but not the same as soil health. Inherent soil properties such as texture, pH, and cation exchange capacity, etc., are important for contextualizing soil health results because they largely depend on soil forming factors (albeit there are some situations, usually with concerted effort, where these properties can be altered by soil management). Some have calibrated soil health scores according to soil texture and region (Chahal et al., 2023; Moebius-Clune et al., 2016), a combination of texture and pH (Bagnall et al., 2023), or soil type and land-use history (Maaz et al., 2023). Others have observed pH or mean annual temperature (Norris et al., 2023), or cropping system type (Wu and Congreves, 2024) as contextualizing factors.

The scientific community may not agree on which soil attributes (and how many) should be selected to indicate soil health, but there is general consensus that carbon-based indicators are crucial and must be included, especially soil organic C and/or mineralizable C. Soil carbon indicators are emblematic in that they blend spheres of soil biology, chemistry, and physics—but in the era of 'net-zero carbon farming', it demands that the role of soil biology in conferring soil health is more closely evaluated. The pressing question may not be "which soil attributes should be selected as soil health indicators", but rather "are soil functions and vitality being adequately indicated?" For soil biology, the functions and vitality might be best represented if we consider carbon-based indicators like organic C and active C alongside microbial respiration, biomass, enzyme activity, and stress responses. In theory, these measures would help interpret soil functions like carbon and nutrient cycling. Other soil physicochemical functions would necessitate a different collection of attributes or processes, such as soil aggregate resiliency, soil water infiltration, conductivity, holding capacity. Without minimizing the importance of soil physicochemical indicators, Lehmann et al. (2020) emphasized the need for a greater biological perspective and representation in soil health indices. However, this raises important questions, such as: are biological indicators of soil health too dynamic to illustrate soil health? What are the sufficiently reliable biological patterns that provide meaningful information about a soil's health status, which can be acted upon via soil management? How might biological indicators be contextualized to derive meaning?

Better understanding the biological indicators of soil health must begin by analysing attributes that reflect biological functions and vitality, understanding their responsiveness to agricultural management practices, and identifying an appropriate contextualizing factor to apply results at scale. Fierer et al. (2021) recommended efforts to improve the interpretability of soil biological indicators of health, and multi-site studies to determine soil biological attributes consistently provide relevant indicators of soil health. In this paper, we evaluate several soil biological indicators (including organic and total C, total N, mineralized C, microbial phospholipid fatty acid biomass, enzyme activity, and adaptation response ratio) across a wide latitudinal gradient in a region of agricultural importance. Our objective is to test soil classification as one approach for contextualizing soil health attributes, exploring on how soil great groups—an already agreed upon method of characterizing soils—might control soil biological indicators. In doing so, we also evaluate different degrees of soil health management in agricultural crop production, and their role in influencing these soil biological indicators.

6. Objectives and the *progress towards meeting each objective*

Objectives	**Progress (e.g. completed/in progress)**
a) Build on the SK Soil Health Testing Protocol so that it outputs soil zone-specific scores	**Completed.** Our study demonstrates that biological indicators of soil health can be adequately contextualized by soil classification, and managed in agricultural systems to indicate improved soil functioning. We recommend, for this region, soil classification *by great group* (Brown, Dark Brown, Black) is an important contextualizing factor to adjust soil health scores, thereby improving the meaningfulness of soil health assessments.
b) Incorporate novel microbial measurements of soil health into the testing protocol	**Completed.** We evaluated several different biological indicators of soil health (including organic and total C, total N, mineralized C, extracellular enzyme activity, and phospholipid fatty acid (PLFA) analysis for microbial biomass and adaptation response ratio (ARR)). To target soil *functioning*, we incorporated the measurement of soil carbon and nitrogen pools alongside *processes* like CO_2 mineralization and enzyme activity, and soil *life* such as microbial biomass and biomarkers.
c) Explore early-indicators of soil health change for when producers incorporate regenerative agricultural practices on farm	**Completed.** Farmers following conventional, organic, and regenerative farming practices were included in our network of Saskatchewan farmers. Samples from native prairie grassland were also included as a reference and optimal scenario of soil health management. Carbon dioxide flux indices is proving to be effective in showing early changes in soil health due to management.

7. Methodology:

Study region

This study focusses on soils in the province of Saskatchewan in Canada within the prairie ecozone formed under grasslands and aspen parklands. This is an important agricultural cropping region that produces cereal, oilseed, and legume crops—experiencing a humid continental climate in the central and eastern parts of the province, and a semi-arid and steppe climate in the southwestern parts. The dominant soil order in the agricultural production region is Chernozemic soils, encompassing soils in the Brown, Dark Brown, and Black Great Groups according to the Canadian System of Soil Classification (SCWG 1998).

Soil sample collection and analysis

This study encompasses 153 soil samples collected from across latitudes of 50° to 53°N and longitudes of 103° to 106°W. This project focuses on soil surface samples from the 0-15 cm depth, each collected as a spatially representative composite of six cores from a 22-hectare area of a field. Overall, 57 samples were from Brown soils, 57 samples were Dark Brown soils, and 39 samples were Black soils. Of the 153 samples, 87 represent conventional cropping systems (annual crops such as cereals, oilseeds, and/or pulses as per typical production), 24 represent organic cropping systems (annual crops such as cereals, oilseeds, and/or pulses grown under certified organic production practices), 24 represent cropping systems that included cover crops in rotation (i.e., post-harvest or in-season cover crops were grown the annual crop rotation), and 18 represent prairie grasslands.

Photos showing soil sample collection (left), a field under regenerative cover crop production after cash crop harvest (top right), and conventional management (bottom right).

The soil samples were collected during Sept and Oct 2021 using a Dutch auger (5 cm diam). Augers were cleaned with ethanol between sample collection to mitigate microbial contamination between samples. All samples were placed in a cooler on ice during field work and transportation to the lab prior to analysis. Sub-samples were frozen at -40°C prior to microbial and enzyme assay analyses, while another sub-sample was air dried and sieved to 2mm prior to other analyses.

Soil samples were analyzed for 10 key biological indicators of soil health, including soil organic C, total C, total N, CO_2 mineralization, extracellular enzyme activity for cycling N, C, P, and S compounds, and phospholipid fatty acid

(PLFA) analysis for microbial biomass and adaptation response ratio (a phenotypic expression indicator). Briefly, soil organic C, total C, and total N were determined via the dry combustion method, and after carbonates were removed for organic C determination (Rutherford et al., 2007; Skjemstad and Baldock, 2007). Soil total concentrations for P and S were measured via inductively coupled plasma-optical emission spectroscopy, after soil was digested with HNO_3. For soil CO_2-C mineralization, a modified 24-hr 'burst' test was conducted where Petri dishes were filled with dry soil, deionized water was added to reach 50% water-filled pore space, and samples were incubated in a sealed 1 L mason jar at 23 °C for 24 hrs whereupon a gas sample was collected and analyzed for CO_2-C via gas chromatography (Rochette and Bertrand, 2007). Extraction, separation, and detection of soil microbial PLFA followed the method described by (Helgason et al., 2010). Briefly, the soil sub-samples were freeze-dried, ground to a powdery texture, and soil PLFA were extracted following methylation of isolated phospholipids, identified by comparing fatty acid methyl ester peaks to the library of known standard biomarkers. We also estimated a key phenotypic ratio (indicative of changes in cell wall fatty acid structure due to environmental changes), based on biomarker chemical synthesis pathways and as identified by Norris et al. (2023) as the adaptation response ratio (ARR), (α15:0 + α17.0)/(*i*15:0 + *i*17:0). Further, we fluorometrically measured extracellular enzyme activity for β-1,4-N-acetylglucosaminidase (NAG, a proxy indicator for N cycling), β-glucosidase (BG, an indicator for organic matter quality), alkaline phosphatase (AP, an indicator for P cycling), and arylsulfatase (AS, an indicator for S cycling) following the protocol developed by Bell et al. (2013).

Although the focus of this study was on biological indicators of soil health, we also collected data on soil organic matter stoichiometry. We evaluated N:C, P:C and S:C ratios (rather than the more commonly used C:N, C;P, and C:S ratios) because the former are considered more direct measures of the element enrichment of organic matter (Tipping et al., 2016). For each ratio, we use SOC as the C denominator, and total N, total P, and total S as the numerators. We acknowledge that the total N, P, and S concentrations include both inorganic and organic fractions, however, note that organic compounds make up the vast majority of these pools. Thus N:C, P:C and S:C are essentially concentrations of N, P and S in the organic matter.

Soil health scoring functions

Scoring functions were developed using a similar approach as described by Wu and Congreves (2021). Briefly, the measured values for each soil attributes were transformed into a soil health score (0-100, low to high) computed from the z-value of the data distribution of the measured values within the dataset. For this study, all soil attributes followed the "more is better" scoring type assumption. Scores were first computed by amalgamating the data from all soil great groups and subsequently by separating the data by great group. The scores were ranked into categories of "very poor" (<20%), "poor" (20-40%), "medium" (40-60), "good" (60-80%), or "very good" (>80%).

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 10.0.0, GraphPad Software, Boston, Massachusetts USA. The alpha threshold and confidence level was set at 0.05. To visualize the data, patterns, and relationships among variables, a principal component analysis (PCA) was performed by soil great group. For all the cropland sites, soil attribute measurements collected from each great group (Brown, Dark Brown, and Black) were compared via a one-way analysis of variance Kruskal-Wallis test followed by a Dunn's multiple means comparison test. For Dark Brown soils, four different management scenarios (conventional cropland, cover cropped cropland, organic cropland, and prairie grassland) were compared via a one-way analysis of variance Kruskal-Wallis test followed by a Dunn's multiple means comparison test.

8. Results

Biological soil health indicators

The cumulative proportion of variance explained by the first two principal components (PCs) was 71%. Soil TC, TN, and SOC were strongly correlated (values close to 1) on PC1 (Figure 1). The microbial biomass and extracellular enzyme variables were reasonably correlated (values between 0.5 and 1) on PC1, but formed two different groupings on PC2 where AP, PLFA, and AS were somewhat corrected to each other, separately from BG and NAG (Figure 1). The PC score plot showed distinct groupings by soil class, demonstrating that soil class significantly influenced the dimension reduction achieved by the PCA (Figure 1).

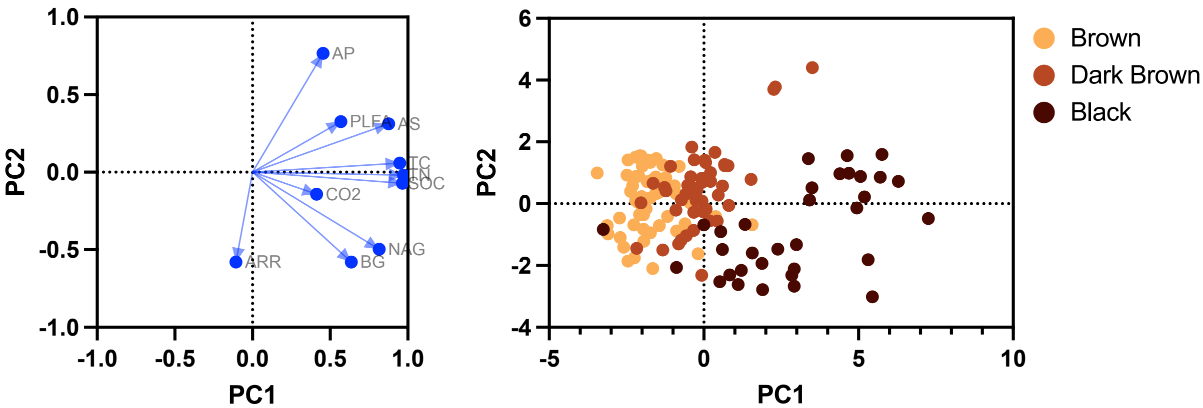


Figure 1. Principal component analysis (PCA) loadings plot (left) and scores plot (right). The loadings plot shows the correlation between variables on the first two principal components (PC 1 and 2). Variables clustered together are correlated, and the strength of the correlation is indicated by the values closer to 1 or -1. The scores plot shows the dimension reduction achieved by the PCA, and that clear separation is achieved by soil great group.

Soil attributes by soil class

Soil great group significantly influenced most soil attributes measured, whether those indicated soil carbon levels and mineralization, extracellular enzyme activity, or phospholipid fatty acid microbial biomass. Generally, all measures followed a prevailing pattern where Black > Dark Brown > Brown soils, with only a few exceptions (Figure 2). Median soil total C, SOC, and total N levels in Black soils were 1.6 to 1.7 times greater than Dark Brown soils, which were 1.4 times greater than Brown soils ($P < 0.01$). Soil CO_2 mineralization also differed by great group ($P < 0.01$) with Dark Brown and Black soils producing 1.5 times more than Brown soils. In a similar pattern to the carbon and nitrogen indicators, median soil NAG activity in Black soils were 2.1 times greater than Dark Brown soils, which were 1.3 times that of Brown soils ($P < 0.01$). Median soil BG in the Black soils was 1.2 to 1.5 times greater than the Dark Brown and Brown soils ($P < 0.01$), which did not differ from each other. Median AP activity also differed by soil ($P = 0.03$), but in a different way as the above enzymes—in this case, AP levels in Dark Brown soils were 1.4 times that of the Brown soils but not different from Black soils. Median AS activity in the Black and Dark Brown soils were, together, ~1.7 times greater than Brown soils ($P < 0.01$). A similar pattern was also observed for PLFA microbial biomass, where Black and Dark Brown soils had 1.3 times greater biomass than Brown soils ($P < 0.01$). Microbial ARR was not influenced by soil class ($P = 0.39$).

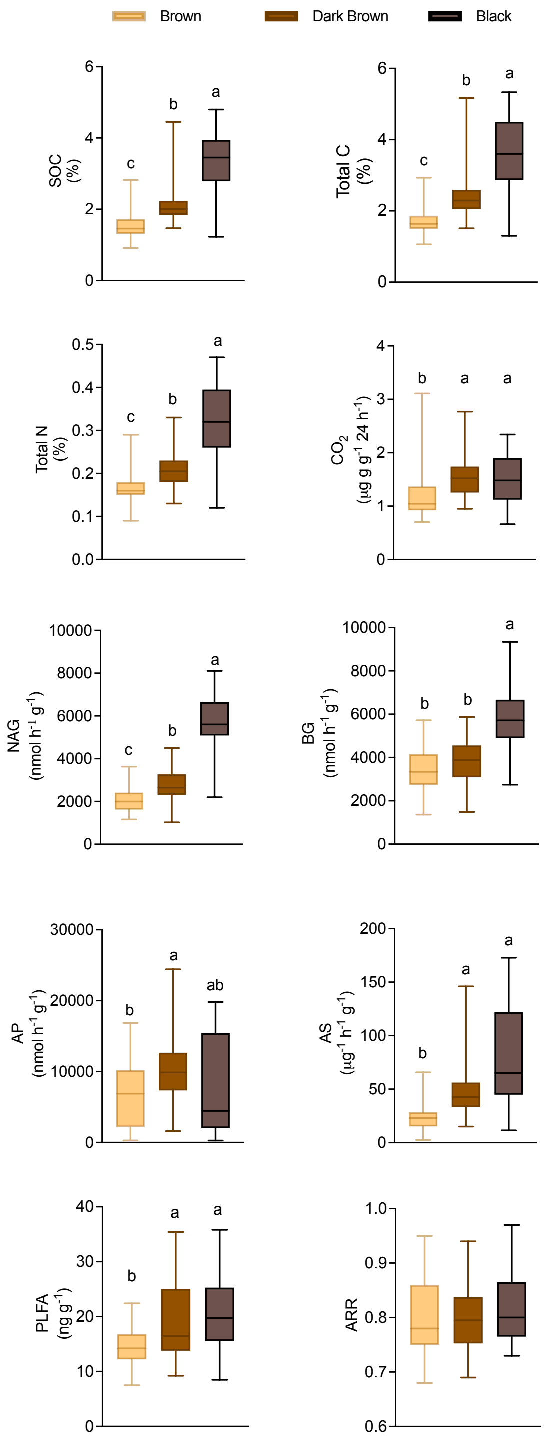


Figure 2. Soil attribute measurements as influenced by soil great group (Brown, Dark Brown, Black). Boxplot area indicates the first and third quartiles, the central line represents the median, and the whiskers represent the range. Bars with different letters are significantly different ($P < 0.05$). SOC (soil organic C), NAG (β-1,4-N-acetylglucosaminidase), BG (β-glucosidase), AP (alkaline phosphatase), AS (arylsulfatase), PLFA (microbial phospholipid fatty acid biomass), and ARR (adaptation response ratio).

Soil scoring functions and scores by soil class

The soil health scoring functions and resulting scores significantly differed depending on whether they were adjusted for classification (by great group) or not (Figure 3). The most consequential differences were observed for SOC, total C and N, enzyme activities, and PLFA microbial biomass, whereas a more marginal difference was observed for soil CO_2 mineralization and microbial phenotype (Figure 3). Overall, the adjustment for soil classification had a marked impact on the interpretation of biological soil health. For example, a SOC level of 3% would be ranked as "good" (the second highest category, with a score of 60-80%) if soil classification was not considered. However, when adjusted for soil class, 3% SOC would be considered "very good" (the highest category, a score > 80%) for Dark Brown and Brown soils, but "poor" (the second lowest category, a score between 20-40%) for Black soils (Figure 3). The same magnitude of consequence applies to all other attributes except CO_2 mineralization where scores and ranking was similar regardless of the soil class (Figure 3).

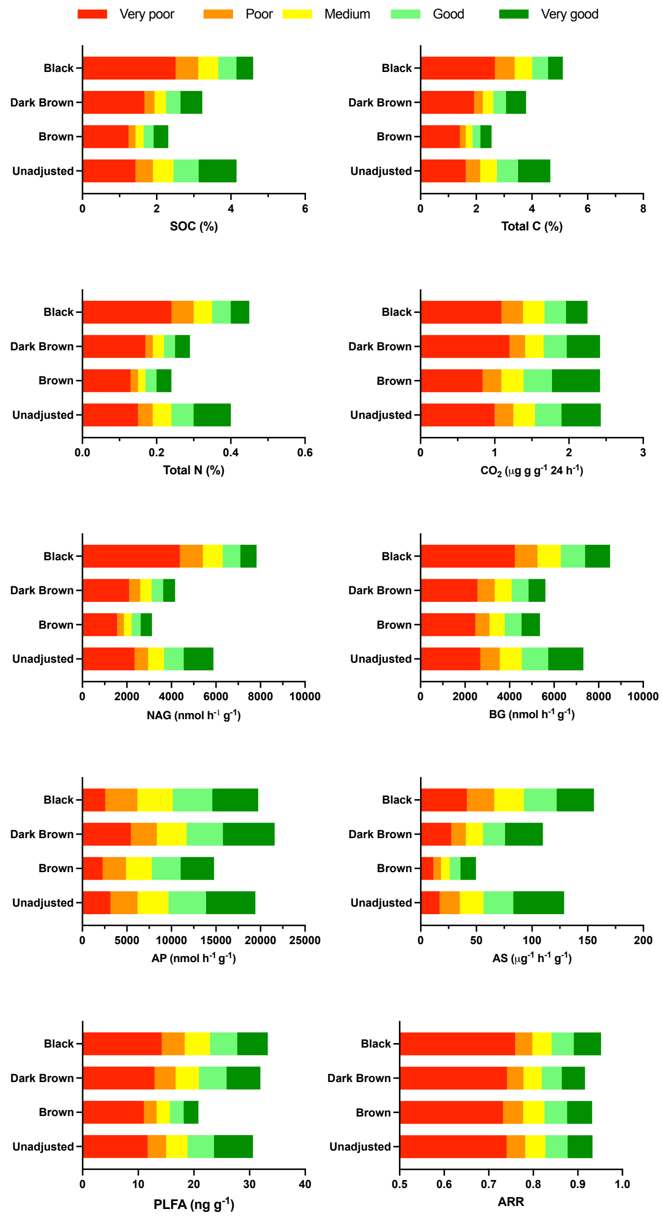


Figure 3. Soil attribute measurements and corresponding soil health scores and ranking, when adjusted by soil great group or left unadjusted. SOC (soil organic C, NAG (β-1,4-N-acetylglucosaminidase), BG (β-glucosidase), AP (alkaline phosphatase), AS (arylsulfatase), PLFA (microbial phospholipid fatty acid biomass), and ARR (adaptation response ratio).

Soil health by management

In the Dark Brown class, soil management had a significant impact on soil attribute values and their corresponding soil health scores. In general, prairie grassland soils had the greatest values and ranking (nearly all categorized as “very good” with scores > 80%), whereas lower values and rankings were associated with soils under crop production (Figure 4). However, the differences among crop production type were less clear depending on the soil attribute. Soil organic C, total C and N, AP, and AS had a similar trend, in that greater levels and “good” scores were associated with organic cropping systems and incrementally lower values and “medium and poor” scores were associated with cover cropped and conventional cropping systems (Figure 4). Soil CO_2 mineralization, BG activity, PLFA, and AAR however, had little to no significant differences between the three different crop production types; and NAG activity was greater in conventional cropping systems than cover cropped systems but not different than organic cropping systems (Figure 4). For CO_2, NAG activity, and ARR the scoring categories accentuated the numerical trends, i.e., the relatively higher attribute values associated with conventional cropping systems ranked as “good”, whereas the lower attribute values in the cover cropped or organic cropping systems ranked lower (Figure 4). For PFLA biomass, conventional and cover cropped cropland both ranked as “medium”, whereas organic cropland had “poor” levels of biomass (Figure 4).

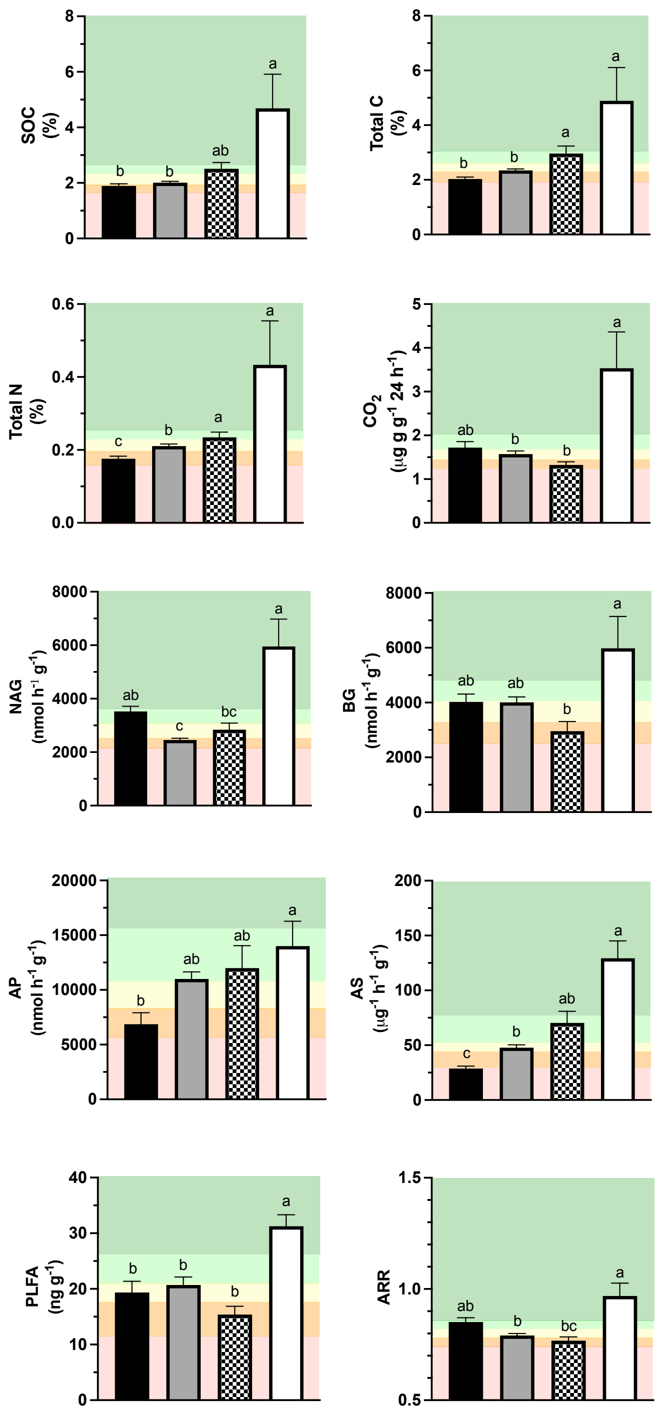


Figure 4. Soil attribute measurements as influenced by agricultural production practices in the Dark Brown class (black bar = conventional cropland; gray bar = cover cropped cropland; checkered bar = organic cropland; white bar = prairie grassland). Bars with different letters are significantly different ($P < 0.05$). The background shading color indicates the corresponding soil health score and ranking for each measure (red = very poor; orange = poor; yellow = medium; light green = good; dark green = very good). SOC (soil organic C), NAG (β-1,4-N-acetylglucosaminidase), BG (β-glucosidase), AP (alkaline phosphatase), AS (arylsulfatase), PLFA (microbial phospholipid fatty acid biomass), and ARR (adaptation response ratio).

Soil carbon, nitrogen, phosphorous, and sulphur stoichiometry
To generally evaluate the degree of soil organic matter degradation, soil N:C, P:C, and S:C ratios were plotted against SOC% (Figure 5). In theory, higher ratios *and* lower SOC concentrations would indicate nutrient rich organic matter, whereas lower ratios *and* higher SOC concentrations would indicate nutrient poor organic matter. Our results showed that soils from the Brown class or from conventional croplands tended to be more nutrient-enriched as these datapoints clustered on the left side of the scatterplot, whereas soils from the Black class or from prairie grassland tended to be more nutrient-restrained by clustering on the right side. The P:C and C:S results tended to have relatively greater spread than N:C results, which generally clustered around a more consistent range except for Brown soils.

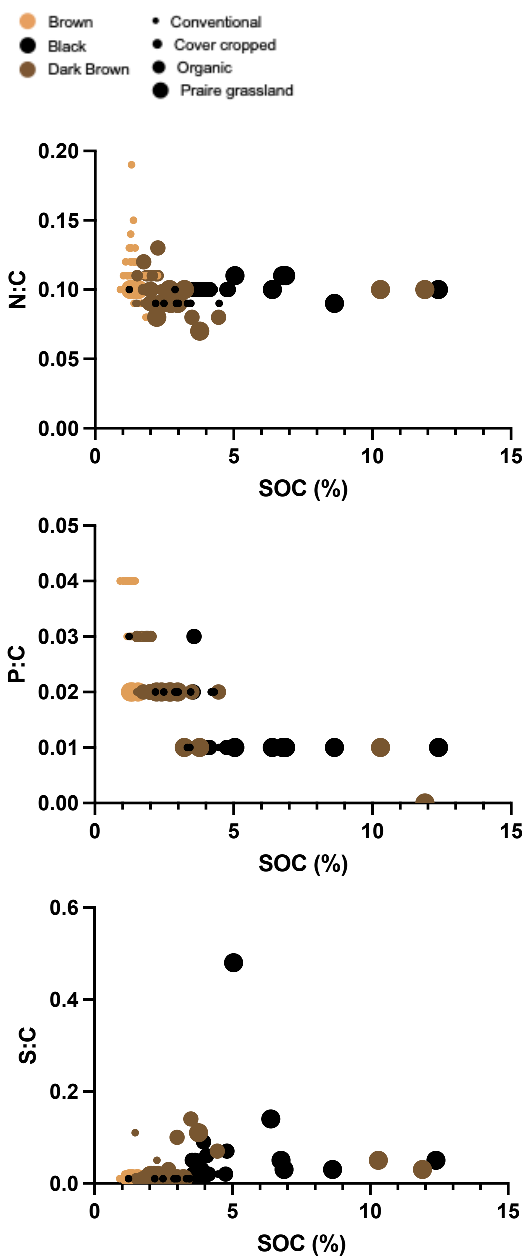


Figure 5. Element ratios to soil organic C (SOC) versus SOC for all soils. The left end of each graph indicates nutrient-enriched organic matter, whereas the right end of each graph indicates more nutrient-restrained organic matter.

9. Discussion

In evaluating biological soil health indicators, we found that soil class constrained measurements and served as a useful contextualizing factor to adjust scoring functions. Others have also pointed out the need to adjust scores for appropriate categorical variables that help explain the diversity and heterogeneity of soils (Nunes et al., 2021), such as by soil texture (Moebius-Clune et al., 2016; Chahal et al., 2023), by soil mineralogy and land use history (Crow et al., 2023; Maaz et al., 2023) and by the type of cropping system (Wu and Congreves, 2024). What is most effective as a contextualizing variable in one region or setting, may not be for others. For example, clay mineralogy can vary greatly in weathered soils, and was identified as the most influential inherent soil property for volcanic and tropical soils (Maaz et al., 2023). For prairie regions (like in Saskatchewan Canada), soils have developed from native grassland where the vegetation and climate strongly regulate the accumulation of soil organic matter. Typically, in this region, there has been abundant below-ground additions of organic matter to the soil through the root systems of grasses, and the environment has regulated net carbon exchange to favour soil organic carbon accumulation. In Saskatchewan, soils predominantly belong to the Chernozemic order. This order is subdivided into great groups, a classification that reflects the differences in the strength or intensity of the dominant soil forming process on horizination (SCWG 1998). The great groups are categorized based on the color of the surface horizon, which is indicative of the amount of soil organic matter. In our study, soil great group was an appropriate contextualizing variable for scoring biological indicators of soil health. Our results showed a gradient where soil biological measurements are limited to lower thresholds for the Brown soils than the Dark Brown and the Black soils, respectively. As policy begins to mandate soil health stewardship and carbon sequestration, is important to account for the soil class differences in this region—to refrain from penalizing soils with lower inherent thresholds of biological soil health, or from inadvertently rewarding poor soil health management decisions for the simple reason that the soils may be naturally high in organic matter and biological activity (i.e., not appropriate to justify decisions that would eventually degrade biological soil health indicators in regions that have inherently higher scores). This framework of soil health scoring by classification might be applicable to other grassland regions around the world.

Soil health is traditionally envisaged as the confluence of several soil biological, chemical, and physical attributes (Moebius-Clune et al., 2016). But recently, only a few soil attributes are considered illustrative of soil health, such as soil organic C, aggregate stability, and 24 h C mineralization (Bagnall et al., 2023). These are arguably more indicative of soil biological functioning than chemistry or physics alone. Others also recommend indicators that are more so in biological realm, for example, soil organic C, active C, soil protein (Wu and Congreves 2021). Some argue that current frameworks for soil health modelling do not align with the three categories (biology, chemistry, and physical) but rather crosscut them or else fall squarely in the domain of soil biology (Janzen et al., 2021; Crow et al., 2023; Maaz et al., 2023). Likewise, Lehmann et al. (2020) emphasized the need for a greater biological perspective and representation in soil health indices. However, there is a concern that soil biological measures are too changeable, ephemeral, and irregular to adequately indicate soil health and functioning, thereby limiting their effectiveness to draw inferences about soil health (Fierer et al., 2021). In our study, we demonstrate that clear patterns based on soil class can be drawn from biological indicators of soil health—sufficient to contextualize the measurements for soil health scoring. As soil health research continues to progress, we recommend that soil biological indicators are not only included in minimum datasets but that they are properly contextualized so that meaningful interpretations can be drawn.

It was interesting that ARR values (the adaptation response ratio, where an increase in the anteiso to iso branching chemistry indicates an increase in fluidity of the cell membranes) was not influenced by soil class in our study, because others found ARR strongly associated with environmental conditions (Norris et al., 2023). It is possible that ARR indicates microbial differences across very broad geographic regions (i.e., from North to South America as in Norris' work) but is less responsive to environmental differences within a relatively more uniform region like the Saskatchewan prairies. By not showing differences across soil great groups, perhaps ARR is a measure that can help delineate regions with common soil formation processes, prior to subdividing the region into groups to further contextualize scoring functions.

When interpreting the measures of potential activities of the microbial extracellular enzymes via a soil health scoring lens, the common assumption is that greater activity indicates a healthier soil (Fierer et al., 2021). The rationale for this belief is that greater potential enzyme activity means that the inorganic nutrient end-product (which the enzyme is targeting) is more limiting, and there is an ample organic supply pool for mineralization. This assumption and rationale are best suited for row crop production systems where avoiding excess inorganic nutrient levels is crucial for minimizing nutrient losses from the system and risking environmental degradation due to such losses. However, there are some important caveats and scenarios where this assumption may not hold up, as Fierer et al. (2021) outlines. For example, activities of enzymes associated with C, N, P metabolism do not always accurately predict the limiting nutrient, higher enzyme activities can be interpreted as either more nutrient availability or reduced nutrient availability, and enzymes typically measured represent a small subset of potentially important enzymes (Fierer et al., 2021). Despite these caveats, there is potential utility of microbial extracellular enzyme activity data, and research such as ours is needed to assess whether consistent trends can be drawn in response to soil classification and agricultural management. As our PCA results demonstrated, the enzyme activity measures were not only important factors in explaining the variability of the soil biology dataset, but also showed clear differences by soil class (especially NAG and BG) and by agricultural management (more so AP and AS activity).

To be a useful tool in practice, soil scoring must be able to identify differences due to management (Congreves et al., 2015; Lehmann et al., 2020). As expected, we found significantly higher soil health scores in prairie grassland than in agriculturally cropped systems, and this agrees with other studies (Maaz et al., 2023). For some biological soil health indicators like SOC, TC, and TN, cover cropped and organic crop management were associated with higher soil health scores and rankings than conventional crop production. Where differences were not indicated by multiple means comparisons, then they were accentuated by different soil health scores and rankings. Carbon and nutrient management are key distinctions among the different cropping systems, where greater carbon inputs are generally favoured with more regenerative systems (cover cropped or organic management) via carbon-based nutrient additions, longer rotations, or cover cropping, than with conventional approaches. In other work focused on the same semi-arid region as the present study, where moisture typically limits building soil health and over relatively short period of study (3 years) soil health indicator differences were associated with more perennial systems than annual crop rotations (Wu et al., 2024). Although numerical differences between cropland management were rather subtle for some indicators (i.e., mainly the carbon cycling indicators of CO_2 mineralization rates, NAG and BG activity, and PLFA biomass), others were more discernible (i.e., total N concentration, AP and AS activity). The β-glucosidase enzyme plays an important role in the degradation of soil organic C and plant residues by influencing and catalyzing the hydrolysis of cellulose, the most abundant polysaccharide, to provide simple sugars for the soil microbial population. The β-1,4-N-acetylglucosaminidase enzyme is involved in chitin degradation and serves as a proxy indicator of N cycling. Detecting improvements in soil carbon cycling processes requires long-term periods owing to the heterogenous nature of soil, the relatively large inherent pools of soil organic matter, and the slow-acting processes leading to carbon buildup. It is possible that the enzyme assays for alkaline phosphates and arylsulfatase may serve as earlier indicators of soil health change.

One problem with simply assessing the size of organic matter pools to draw interpretations about soil health is that pool size provides an incomplete picture of soil functioning. Pool size does not provide information on the degree of soil organic matter degradation, and perhaps soil health assessments would be improved if indicators of degradation status, such as C:N:P:S stoichiometry of soil organic matter, were more widely considered as an indicator of soil health (Tipping et al., 2016). It is possible that our results indicate a higher degree of organic matter degradation in the more southern soil classes (Brown soils) and when soils are under more conventional crop production, either via soil organic carbon depletion or by the enrichment of nutrients via fertilization practices. Soil organic matter stoichiometry might be a useful metric to explore in future soil health assessments, warranting more research to elucidate meaningful patterns and expression.

While soil *functioning* is frequently claimed as a defining feature of soil health (and regularly mentioned in introductory paragraphs of soil health literature), adequately capturing soil functioning in soil health scoring efforts is debatable. Measuring concentrations, quantities, and ratios—as done for most soil health scoring systems—

does not directly indicate 'functioning'. In the present study, our goal was to focus specifically on the function of soil carbon and nutrient cycling; this goal guided the selection of soil attributes that govern this function. Through the measurement of soil carbon and nitrogen pools alongside *processes* like CO_2 mineralization and enzyme activity, and soil *life* such as microbial biomass and biomarkers we aim to approach a better understanding of soil functioning and encourage others to follow suit. Of course, there are other important functions provided by soils that were not included in our study (water cycling, filtering and buffering, physical stability, and supporting plant and human systems), and each function deserves an in-depth analysis as we collectively move towards more holistic soil health scoring tools.

10. Conclusions

Soil health assessments can help improve our understanding of the relational mechanisms between and among soil, ecosystems, and society—guiding us to nurture soil attributes that promote the functions we value. However, soil health assessments need to improve on three important aspects: better representing soil biology, appropriately contextualizing soil health scores, and better targeting soil *functioning*. Our study demonstrates that, despite being dynamic and changeable, biological indicators of soil health (such as the ones presented herein) are useful for illustrating soil health, can be adequately contextualized by soil classification, and managed in agricultural systems to indicate improved soil functioning. As policymakers begin to use soil health metrics more widely to monitor soil stewardship progress and create incentive programs, it is important to account for soil class differences. Doing so will help avoid penalizing soils with lower inherent thresholds of biological soil health or inadvertently rewarding poor soil health management decisions in soils that are naturally high in organic matter and biological activity. We recommend, for Saskatchewan, soil classification by great group as an important contextualizing factor to adjust soil health scores, thereby improving the meaningfulness of soil health assessments. We also suggest the exploration of soil element to carbon ratios as a potentially useful indicator of organic matter quality, indicating soil health. By measuring *pools* of soil carbon and nitrogen alongside soil *processes* such as soil CO_2 mineralization and enzyme activity, and other indicators of vitality such as *soil life* via microbial biomass and biomarkers, we hope to move closer to capturing soil functioning.

11. List any technology transfer activities undertaken in relation to this project:

Interviews

Congreves, K.A. Farrell, R.E. (2023). Interview with Joanne Paulson for text article featuring Congreves' and Farrell's research. "Seeking a holistic understanding of soil health". Campus News.

Magazine Articles

Congreves, K.A. Otchere, O. Wu, A. 2023. Gardening with cover crops. Article in the Canadian Gardener Magazine.

Publications

- Congreves, K.A. and Wu, Q. (2024). Using soil classification to improve interpretation of biological soil health indicators. *Submitted to Geoderma.*
- Wu, Q., Congreves, K.A. (2024). Soil health benefits associated with urban horticulture. *Science of the Total Environment, 912 doi:10.1016/j.scitotenv.2023.168852.*
- Wu, Q., Lawley, Y., Congreves, K.A. (2024). Soil health indicator response to three years of cover crop and crop rotation in a northern semi-arid region, the Canadian prairies. Agriculture, Ecosystems and Environment. 359*, doi:10.1016/j.agee.2023.108755*
- Wu, Q., Congreves, K.A., Farrell, R.E. 2023. Microwave-assisted citrate extraction (*MaCE*) as an alternative to autoclave citrate extraction (*ACE*) of a soil protein fraction. *Canadian Journal of Soil Science. https://doi.org/10.1139/CJSS-2023-001*
- Council of Canadian Academies. Cultivating Diversity. Report on Plant Health Risks in Canada (2022). (Congreves is a co-author)
- Van Eerd, L.L., Congreves, K.A., Arcand, M.M., Lawley, Y., Halde, C. 2021. Chapter 15: Soil Health and Management. In M. Krzic, F. Walley, A. Diochon, M. Paré & R. Farrell (Eds.), Digging Into Canadian Soils: An Introduction to Soil Science. Pinawa, MB: CSSS. https://openpress.usask.ca/soilsciencetest/chapter/soil-health-and-management/.

- Wu, Q., Congreves, K.A. (2021). A soil health scoring framework for arable cropping systems in Saskatchewan. Canadian Journal of Soil Science, https://doi.org/10.1139/CJSS-2021-0045
- Audette, Y, Congreves, K.A., Schneider, K., Zhang, H., Geovanna C. Z., Nunes, A., Zhang, H., Voroney, R.P. (2021). The effect of agroecosystem management on the distribution of C functional groups in soil organic matter: A review. Biology and Fertility of Soils, https://doi.org/10.1007/s00374-021-01580-2.
- Farzadfar, S., Knight, J.D., Congreves, K.A. (2021). Organic nitrogen: an overlooked but potentially significant contribution to crop nutrition. Plant and Soil, 462: 7-23.

Presentations

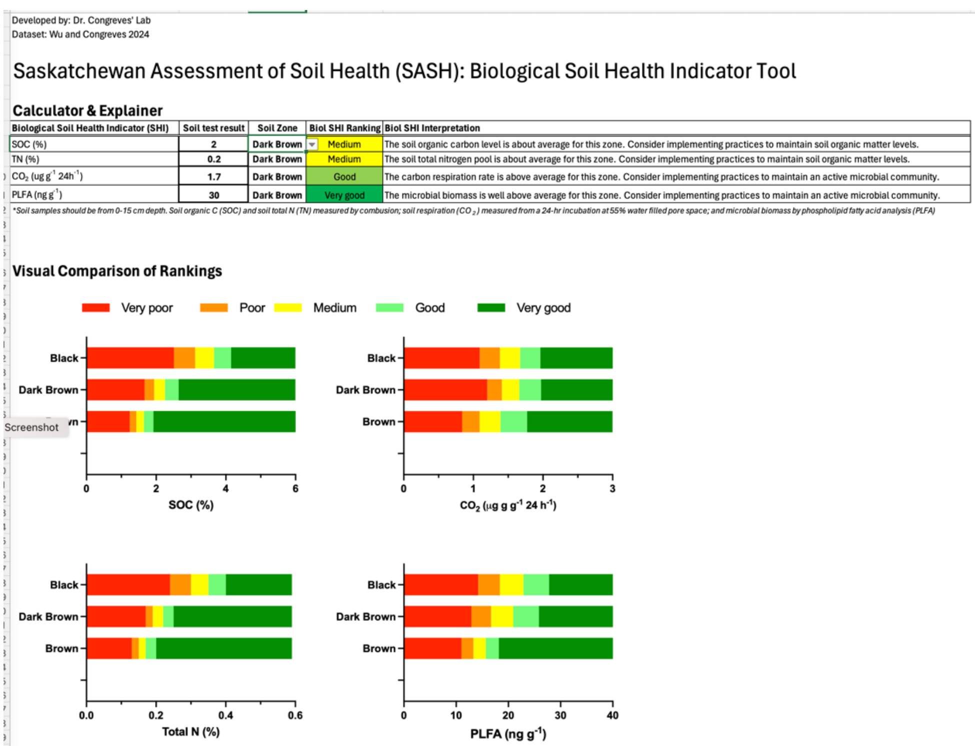
- Congreves, K.A. (2023). Turning over a new leaf for nitrogen management. Canadian Society of Soil Science, Session: Potentially Mineralizable Nitrogen. Truro, Nova Scotia, Canada. June 26-29, 2023. Invited Keynote.
- Wu, Qianyi (2023). Microwave-assisted extraction (MaCE) as an alternative to autoclave citrate extraction (ACE) of soil protein. Soils and Crops. March 7, 2023. Poster Presentation.
- Wu, Athena (2021). Developing a soil health test for arable cropping systems in Saskatchewan. 2021 CSSS Annual Meeting of the Canadian Society of Soil Science. June 7, 2021. Oral presentation (Virtual).
- Congreves, K.A. (2021). Balancing acts for a sustainable food future. Café Science. Sept 28, 2021.
- Congreves, K.A. (2021). Moving towards sustainable agriculture by nurturing soil ecosystem services. 36th Plant Sciences Graduate Student Symposium, Saskatoon (Virtual), Mar 4, 2021. Invited Keynote.

Graduate Student Thesis

- Wu, Athena (2021). Developing a soil health test for arable cropping systems in Saskatchewan. Master's Thesis, Department of Plant Sciences, University of Saskatchewan.

Soil Health Scoring Tool

- Beta version of a scoring tool and interpretation aid for growers. Users input their soil test result, and select the soil zone from a drop-down menu. Once selected, the score ranking and interpretation is outputted for growers.



This tool currently functions in Excel, and is being transformed into a web-based tool, to be hosted on Congreves' lab website. This Beta version is currently being vetted by peers before being released to public.