An Integrated Framework for Reference Site Selection and Benchmarking Soil Health Studies in the United States

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Method Article

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Abstract

Comparative soil health studies are critical in soil conservation and gauging the success of different management practices in soil health improvement. A primary challenge in these studies is the selection of a consistent natural reference site. Current choices vary widely, from minimally disturbed areas to pristine prairies. This methodological paper underscores the need for deliberate and thoughtful reference site selection for benchmark soil properties. Utilizing the State & Transition models, the study introduces a framework for this selection, drawing upon the ecological site (ES) and reference plant communities detailed in an ecological site description (ESD) within a respective Major Land Resource Area (MLRA). This study advocates for a localized classification within the framework of the Cropland Reference Ecological Unit (CREU), emphasizing the significance of local precipitation and soil data to ensure unbiased comparisons. Soil samples from eastern (MLRA 106) and western (MLRA 67A) Nebraska were collected, representing distinct pedogenetic and climatic differences. Analysis of soil organic matter between MLRAs displayed substantial variations, suggesting potential biases and complexities in soil health gap calculations when using reference sites and croplands not in the same MLRA, soil types (texture class), or precipitation zones. However, refining the comparisons by delineating the MLRA, soil, and precipitation zones within the framework of CREU yielded more consistent and realistic comparative data. Integrating the MLRA and ES, complemented by granular soil and precipitation data, provides a robust method for establishing soil health benchmark data.

1.0 Introduction

In recent scientific deliberations, soil health has gained significant attention due to its integral role in ecological stability, food security, and a broader perspective of soil security (McBratney et al., 2014). Concurrently, there is an evident inclination within the scientific community towards the development and adoption of methodologies that allow for quantitative determinations of both aggrading and degrading soil properties and functions to facilitate the formulation of valid local and continental scale monitoring strategies to support restorative conservation management (Maharjan et al., 2020; Román Dobarco et al., 2023, 2021; Seybold et al., 1997).

Many researchers advocate that to understand the health status of soil, given measurements of soil health need to be compared to an expected value for that soil in a prime health state or a reference state, at which soil is functioning at full potential (Maharjan et al., 2020; Morgan and Cappellazzi, 2021; Román Dobarco et al., 2023; Wuepper et al., 2021). Despite its common usage, the definition of a reference site for soil property comparison remains ambiguous, ranging from basic fence lines to entirely undisturbed conditions (Maharjan et al., 2020; Romig et al., 1995). This paper posits that field borders or areas adjacent to a field may not serve as accurate reference sites to provide benchmark data. Often, they don’t fully capture the local dynamic soil properties and ecosystem attributes of the study site, especially in terms of primary production, as the fence line can get compromised based on the practices carried out on neighboring fields and in the region (Maharjan et al., 2020; Romig et al., 1995). This work underscores the importance of careful reference site selection to achieve relevant soil health gap evaluations. The study mostly focuses on using ecologically managed land in its native state (Vaughn et al., 2010) as a reference for comparative analysis, creating a standard to assess ecosystem changes and compute variances in dynamic soil properties. The proposed approach integrates, Major Land Resource Area (MLRA) (NRCS-USDA, 2022a), ecological site concepts (Brischke et al., 2018; NRCS-USDA, 2022b), the State & Transition Model (Bestelmeyer et al., 2017), soil pedogenic attributes, climatic data (precipitation) (Das and Maharjan, 2022), and the space-for-time substitution principle (Wills et al., 2017). The objective of this research is
to establish a framework for selecting reference sites for comparative soil health studies. The study utilizes sampling data from two different MLRAs in Nebraska to establish and validate the importance of categorizing sites based on MLRA, soil texture class, and precipitation. Additionally, the study explores the potential development of a hierarchical land categorization as a future direction for comparative soil studies by integrating management aspects and land-use factors for monitoring and measuring the degree of soil changes resulting from different land uses and management practices.

Emphasizing the complex process of reference site selection in the pursuit of relevant soil health gap evaluations, this work highlights the reference site within the State & Transition Model concept (Bestelmeyer et al., 2017). A State & Transition Model outlines the ecosystem response to different disturbances and management, especially in terms of vegetation change. Here, the state refers to a particular condition or stage of an ecosystem characterized by specific vegetation types, which are responsive to soil conditions, climate, and management. Transitions are the changes that move an ecosystem from one state to another. These changes can be natural, such as those caused by fire, flood, disease, drought, and succession or they can be caused by human activities like land management practices, grazing, agriculture crop production, or urban development. Each reference site delineated herein is typified by the prevalence and proportional dominance of the reference plant community (RPC) mentioned in the ecological site description for that soil of importance and site (NRCS-USDA, 2022b) (Briskie et al., 2005; Moseley et al., 2010). The RPC represents a historical consortium of plant species native to an area, which have evolved with natural disturbances and minimal anthropogenic influence. This consortium depicts a likely native plant composition, aligning with native soil characteristics under local climatic conditions. Such a setup typically reflects an ecosystem's optimal stability and resilience (NRCS-USDA, 2022b). Here, "optimally stable" and "optimally resilient" describe an ecosystem's ideal state, where it maintains its structure and functions amidst disturbances and recovers effectively. In native plant communities, this implies resistance to environmental changes while preserving biodiversity and ecological roles.

Integrating the Soil Health Gap (SHG) (Maharjan et al., 2020) concept with a reference site identified by the reference plant community, specifically within a defined cropland reference ecological unit (CREU) (Das and Maharjan, 2022), provides a robust framework. The soil health gap is defined as the difference between soil health status (measured as different bio-physicochemical indicators) in an undisturbed native soil and a cropland in a given agroecosystem (Maharjan et al., 2020). This allows for a consistent benchmark, aiding in evaluating ecosystem health and the potential outcomes of management strategies, ecological shifts, and climatic changes. The CREU framework integrates pedogenic and climatic covariates to group the soils with relatively homogenous characteristics (Das and Maharjan, 2022). The CREU is defined as a land area with presumably uniform pedogenetic (soil formation) and climatic properties (precipitation), allowing more accurate and less confounded comparisons across different agro-ecological settings. By referencing the soil health data from a CREU-defined site with a particular RPC, it becomes practical to measure soil health variations due to management changes and varying degrees of degradation. The Cropland Reference Ecological Unit concept also highlights the use of a more practical approach of developing a reference for a group of soils (texture class) that functions similarly or are genetically similar as defined by the National Cooperative Soil Survey (Das and Maharjan, 2022; Seybold et al., 1997).

As our environment changes rapidly, a proactive approach to measure and monitor soil health becomes imperative. Combining reference sites with the space-for-time substitution method helps in measuring and monitoring soil changes resulting from different land use and management practices. Furthermore, as global
efforts to increase carbon sequestration grow, the importance of reference sites becomes more critical, as reference sites can help in evaluating the success of different soil carbon management practices. This study lays the foundation for developing strategies for soil (health) change measurement, monitoring, and prediction.

2.0 Methods and Materials

2.1 Cropland Reference Ecological Unit

The CREU framework divides ecological sites within an MLRA by a function of soil (texture class) and precipitation units (range) (Das and Maharjan, 2022), as outlined in Equation I. The framework emphasizes the need for regional adjustments made in the selection of precipitation ranges for delineation. Such adjustments are crucial because the impact of the same amount of precipitation can vary across different regions – from hydric to mesic to arid – due to differences in potential evapotranspiration (PET) (Altieri et al., 2015; Robinson and Nielsen, 2015). For instance, in semi-arid regions where precipitation accounts for 20–35% of PET and where dryland farming prevails, narrower precipitation ranges are recommended compared to regions with rainfed farming (precipitation > 35% of PET) (Altieri et al., 2015; Das and Maharjan, 2022). The selected precipitation range for each CREU should result in an area with a relatively uniform agroecological response to precipitation. For this study, we have selected a 76 mm (~ 3 inches) precipitation range. The CREU provides a leveled platform for comparative studies where soil health can be assessed and compared for a group of sites with presumably similar soil health potential. Thus, if measured in the same CREU, the changes in soil properties will be reflective of management practices.

\[
\text{CREU} = f (\text{MLRA, ES, Precipitation, Soil}) \quad \text{--- Equation I}
\]

- \text{CREU} represents the Cropland Reference Ecological Unit for the MLRA.
- \text{MLRA} is the Major Land Resource Area.
- \text{ES} represents the Ecological Site within the MLRA.
- \text{Precipitation} and \text{Soil} are the precipitation unit (a range of precipitation) and soil (texture class) in the Ecological Site within the MLRA.

2.2 Step-by-Step Guide in Reference Site Selection

For reference site selection and soil health gap analysis, follow the steps from I to VIII. Here, for the purpose of illustration, the Mitchell Soil Series will be highlighted as a part of the CREU of the TRIPP-MITCELL-ALICE soil association within the precipitation range of 356–432 mm (14–17 inches) in MLRA 67A.

1. \textbf{Ecological site identification}: The first step in the selection process is to identify the soil of importance and the associated ecological site within the respective MLRA under the CREU framework. We selected soils dominantly representing the croplands in MLRA 67A and MLRA 106. After selection, use the SSURGO Web Soil Survey (https://websoilsurvey.nrcs.usda.gov/app/) to determine the map unit and Ecological Site ID (Refer to Figs. 1a and supplementary file S1 for guidance).

2. \textbf{Understand State & Transition Model}: When available, review the State & Transition Model (STM) for the identified ecological site (ES) described by the Ecological Site Description (ESD) (Briske et al., 2005). The STM will provide a description of potential plant communities (states) and the transitions that may occur
between them (Fig. 1b & Supplementary file S2). The ESD can be downloaded from the Ecosystem Dynamics Interpretive Tool (EDIT) website (https://edit.jornada.nmsu.edu/) or directly from SSURGO (connecting to the EDIT website) (Supplementary file S1). In regions where ESD and STM are not available, a collaborative effort with local experts can be established to identify areas that can serve as potential reference sites based on regional knowledge of undisturbed or minimally disturbed ecosystems.

3. **Identify the plant community to describe native reference state**: Within the State-and-Transition Model (STM), one state is outlined to describe the natural or intact native plant community. This is referred to as the "Reference Plant Community" (RPC) or "Potential Natural Vegetation" (Fig. 1b). The RPC is the plant community that would exist in the absence of significant agronomic disturbance and is maintained by natural or conservation disturbance regimes, such as fire or grazing. Changes in management, including overgrazing, can affect the RPC, making it a valuable tool for assessing the impact of human activities on the ecosystem. The RPC serves as a guide for the final reference site selection.

4. **Potential Reference Site Selection**: For reference site selection, we analyzed the distribution of soil series using the "Soil Series Extent Explorer" tool: https://casoilresource.lawr.ucdavis.edu/see/. After identifying the distribution area, we selected grassland areas with the identified soil, then communicated with local conservation professionals to survey these sites and assist in gaining access permission. Once a potential reference site is identified and established, we scout the area of the priority soil map unit to find portions exhibiting vegetation characteristics similar to those described in the Ecological Site Descriptions (ESD). Depending on the location and access, these areas can be identified through local knowledge (communication with landowners) or by conservation partners (in our case, NRCS professionals).

5. **On-Site Identification**: Outline the access permissions and limitations of activities and disturbances as mandated by the land manager or owner. Negotiate any necessary compromises before accessing the site and adhere to the outlined restrictions. Efficiently enter the site to access the preferred sampling area. On foot, navigate to the sampling area. While completing this initial transect of the site, conduct a plant census, cross-referencing the RPC plant community list. The key species listed in the STM should serve as the vegetative diagnostic characteristic for visually delineating the key sampling area. Note any deviations and site characteristics, such as the dominance of invasive species or percent bare ground. For example, in the Great Plains, Cheatgrass, Kentucky Bluegrass, Smooth Bromegrass, and Crested Wheatgrass are considered invasive species. Note the percentage of bare ground, which often correlates with overgrazing or drought and an increase in invasive species. While working across or through the target soil map unit and landform position, identify and outline the extent of the RPC. Use pin flags, a GPS unit, or a pen and map to document the extent.

6. **Soil description match**: Once the site has been identified by plant community characteristics, it is important to ensure the soil type aligns with the Official Soil Series Description (OSD) (USDA-NRCS, 2023). Extract several soil cores from the selected site using a soil auger and observe the soil profile's morphology (mostly superficial layers). This is to confirm the morphological attributes of the soil align with the range of characteristics mentioned in the OSD (updated soil characteristics can also be downloaded from SSURGO). For example, the OSD for the Mitchell soil series (https://soilseries.sc.egov.usda.gov/OSD_Docs/M/MITCHELL.html) describes the top 0–23 cm as dark greyish brown when moist, with a weak, medium granular structure, and slight effervescence (presence of carbonate). To test for soil effervescence, apply a few drops of 1M hydrochloric acid (HCl) to the soil. Fizzing indicates the presence of carbonate.
7. **Sampling and Data Collection**: Establish a sampling transect that best fits within the extent of the selected site. Vegetation and soil sampling procedures, including prescribed sampling depths and locations, should be used, which best correspond to other standard protocols and should align with the goals and requirements of your study.

8. **“Soil Health Gap” assessment**: Comparative analysis of any and all applicable soil health properties between the established reference site and the cropland within the outlined CREU is now possible. Differences in soil health properties between the reference site and managed land will indicate the soil health gap (Equation II).

\[
(SH)_n - (SH)_m = \text{SH}_x \quad \text{(Equation II)}
\]

*Here, (SH)$_n$ and (SH)$_m$ refer to soil health in $n$; native soil and $m$; managed cropland soil. \text{SH}_x = \text{Soil Health Gap with the subscript } x \text{ indicating a general or specific property,}*

### 2.3 Soil sampling

The procedural explanation in this manuscript was developed while conducting a study that established a comprehensive set of reference sites from MLRA 106 (Eastern Nebraska, USA) and MLRA 67A (Western Nebraska, USA) (Fig. 2). A reference site was established for each soil of interest (Table 1) based on the method mentioned in section 2.2. Management information, including history of land use, fertilizer application, irrigation, and crop rotation, was collected by communicating with landowners (Table 1).

At each reference site, three to five replicated soil samples were collected based on the resources and site accessibility. For sampling, a random primary (centroid) sampling point was first established within the best representative area, and then two to four secondary (satellite) points were established, which were >15m apart from each other (Fig. 3). For this study soil samples were collected at each sampling point from 0–15 cm depth. Similarly for comparative purposes, cropland samples were collected in a paired manner from the same CREU. Soil samples collected in this study, especially in terms of soil series, represent a pseudo-replication with few exceptions (Table 1). However, when focusing on soil texture, the study incorporates multiple sites with similar texture groups, providing both within-site and cross-site replication and extending to the climatic regions (Table 1).

The discussion here will focus on soil organic matter (SOM) data from the analyzed soil samples using the Loss on Ignition method (Hoogsteen et al., 2015). Soil organic matter serves as an important metric of soil health and soil functions, as the SOM can determine water-holding capacity (Bissonnais, 1995; Lal, 2020), aggregate stability (Bissonnais, 1995; Chaney and Swift, 1984; Chenu et al., 2000), microbial activity (Das et al., 2023), and nutrient dynamics (Rice, 2005; Tiessen et al., 1994). The Soil Health Gap framework accommodates the inclusion of any other bio-physicochemical soil health indicators, contingent on research objectives and goals (Maharjan et al., 2020). A key rationale for choosing SOM is its stability; unless there's a significant change in soil texture, climate, land use, or management, SOM variability should remain minimal within its range of natural variation (Johnson, 1995; Wortman et al., 2017). The data were analyzed using analysis of variance (ANOVA), and significant effects were tested with the Tukey HSD test. All of the tests and analyses were done in R, using the agricolae package (de Mendiburu and de Mendiburu, 2019).
Table 1
Metadata of the reference sites and cropland spanning eastern (MLRA 106) and western Nebraska (67A).

<table>
<thead>
<tr>
<th>Sampling sites</th>
<th>MLRA Code</th>
<th>Ecological sites</th>
<th>Soil Series</th>
<th>Texture</th>
<th>Precipitation (mm)</th>
<th>Land groups</th>
<th>Site &amp; Management information</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td>106</td>
<td>Clayey Upland</td>
<td>Pawnee</td>
<td>Clay loam</td>
<td>762–838</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>DP</td>
<td>106</td>
<td>Clayey Upland</td>
<td>Wymore</td>
<td>Clay loam</td>
<td>762–838</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>PTP*</td>
<td>106</td>
<td>Limy Upland</td>
<td>Steinauer</td>
<td>Clay loam</td>
<td>762–838</td>
<td>Altered reference</td>
<td>Hayland and fertilizer was applied intermittently (&gt;10 years)</td>
</tr>
<tr>
<td>BTP</td>
<td>106</td>
<td>Wet Floodplain Prairie</td>
<td>Zook</td>
<td>Silty clay loam</td>
<td>762–838</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>WOLT*</td>
<td>106</td>
<td>Clayey Upland</td>
<td>Mayberry</td>
<td>Clay loam</td>
<td>762–838</td>
<td>Altered reference</td>
<td>Reseeded with native grasses, was cropland in past (approximately &gt;30 years ago)</td>
</tr>
<tr>
<td>DAR</td>
<td>67A</td>
<td>Loamy (Ly)</td>
<td>Tripp</td>
<td>Sandy loam</td>
<td>356–432</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>CR-M</td>
<td>67A</td>
<td>Limy Upland (LiU)</td>
<td>Mitchell</td>
<td>Sandy loam</td>
<td>356–432</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>CR-O</td>
<td>67A</td>
<td>Sandy (Sy)</td>
<td>Otero</td>
<td>Sandy loam</td>
<td>356–432</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>SX</td>
<td>67A</td>
<td>Sands (Sa)</td>
<td>Valent</td>
<td>Sand</td>
<td>356–432</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>CC-1</td>
<td>67A</td>
<td>Sands (Sa)</td>
<td>Valentine</td>
<td>Sand</td>
<td>356–432</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>CC-2</td>
<td>67A</td>
<td>Shallow (Sw)</td>
<td>Tassel</td>
<td>Fine sandy loam</td>
<td>356–432</td>
<td>Reference</td>
<td>-</td>
</tr>
<tr>
<td>BM</td>
<td>67A</td>
<td>Limy Upland (LiU)</td>
<td>Mitchell</td>
<td>Sandy loam</td>
<td>356–432</td>
<td>Reference</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: "-" in the absence of any specific management history; otherwise, the management history is mentioned in the table.

*Change in management and land use in the past, termed as altered reference sites (for more information on the states and transition: supplementary file S4)
### 3.0 Results and Discussion

#### 3.1 Differences in reference sites between and within MLRA

Analyses comparing average SOM values from reference sites in MLRA 106 versus MLRA 67A showed a significant difference ($p < 0.001$) of 2.8% (Fig. 4A). It’s important to note that this difference is an average across both true and altered (disturbed) reference sites (Table 1). When comparing average SOM solely between true reference sites from MLRA 106 and MLRA 67A, the gap widens to 3.4%. This finding highlights the risk of drawing comparisons between reference sites and croplands from different MLRAs, as it could potentially lead to establishing unattainable and unrealistic benchmarks. For instance, the average SOM value for true reference sites from MLRA 106 is 6.2%, while the average SOM value from reference site SX in MLRA 67A, characterized by sandy soils, is only 1.5%, resulting in a gap of 4.7%. Similarly, comparing the average SOM from conventionally managed cropland (KH-C) in MLRA 67A with reference sites from MLRA 106 yields a gap of 4.6%. Given the
Inherent characteristics of lands in MLRA 67A (lower precipitation and sandy loam to sandy soil), achieving over 4% organic matter is not a realistic target within a human timescale, factoring in the slow and gradual nature of SOM accumulation. Therefore, MLRA boundaries should be the primary level of classification. An MLRA is a geographically associated land resource unit classified based on physiography, geology (parent material), climate (precipitation, temperature), water sources, soils, biological resources (plant species), and land use (NRCS, 2023; NRCS-USDA, 2022a). This is supported by several studies that have observed significant variations in soil biophysicochemical properties between different MLRAs with respect to different management practices and land use (Brejda et al., 2000; Chatterjee and Lal, 2009).

In a comprehensive analysis, where sites were categorized based on their MLRA affiliations and subsequently filtered by soil types, the similarities and differences among the reference sites were reasonable (Fig. 4B). From MLRA 106, soil series such as Zook, Wymore, and Pawnee—predominantly clay loam in texture—displayed minimal differences in their average SOM levels (Fig. 4B). However, within the same MLRA, the Mayberry and Steinauer soil series exhibited significant variation in their mean SOM values compared to other clay loam textured soil series. Notably, both inventoried sites have experienced significant management and land-use changes. The Mayberry site was previously cropland (over 30 years ago) and was later seeded with native grasses (Table 1). The Steinauer site, a hayland field for more than 10 years, has a history of fertilizer application (Table 1). These changes from the natural reference state have created a gap in the soil characteristics due to the management history. These disturbed and altered reference sites are documented along with their inventoried ecological phase or state and identified vegetation characteristics in supplementary file S4. This suggests that while soil texture significantly influences SOM levels, it is also imperative to consider the historical land management practices that have shaped these soils over time.

From MLRA 67A, soil series like Tripp, Mitchell, Otero, and Tassel, characterized by their sandy loam texture, showed less variation in SOM metrics. In contrast, the Valentine and Valent soil series, known for their sandy texture, exhibited significant differences in SOM compared to the Mitchell, Tassel, and Tripp series (Fig. 4B). This underlines the importance of categorizing soils based on soil textures and highlights a significant challenge in drawing conclusions about reference sites from different soil texture classes. For instance, the difference in average SOM between the Mitchell soil series (3.5%) and the Valent soil series (1.5%) is 2%. Given that sandy soils typically have less than 2% soil organic matter in this region, aiming for a potential of 3.5% would create an unattainable benchmark for human-time scale (Osman, 2018). These findings are consistent with studies that emphasize the importance of texture class in determining soil health outcomes (Amsili et al., 2021; Chahal et al., 2023; Nunes et al., 2021).

### 3.2 Differences in reference sites across Ecological Sites

Incorporating ecological sites (ES) as a factor in the ANOVA showed that the resulting SOM was significantly different across ecological sites. It should be noted that there were replications within a site for ES Wet Floodplain and Shallow, but there were no cross-site replications (Table 1). A large set of data with a cross-site replication can provide better statistical confidence in such differences. However, based on the current data and findings, these variations can be primarily tied to soil textural differences rather than intrinsic ecological site attributes (Fig. 5). For example, samples from ecological sites such as Wet Floodplain and Clayey Upland showed similar SOM content despite originating from two different ecological sites. This similarity was due to their soil textural similarities, specifically clay loam. Samples from the ecological site Sands ranked the lowest in SOM (Fig. 5).
There was no notable difference between the ecological sites categorized as Loamy, Shallow, and Sandy (Fig. 5). This highlights the notion that while ecological sites represent the differences in potential vegetation influenced by the site's soil and water factors and management (a top-down approach), they might not fully encapsulate the soil's heterogeneity and homogeneity (a bottom-up approach). Hence, while ecological sites and descriptions are crucial for pinpointing soil sampling reference points for specific soil series and locations or determining the states of transition, the division of land for comparative soil analysis could potentially be broader, detouring detailed ecological site theories. The soils can be grouped based on the texture classes, and each class of texture can be used as a unit for measuring soil changes. A study by Amsili et al., (2021) analyzed 1750 samples to assess soil health across New York State and found that SOM, permanganate oxidizable carbon, soil respiration, and water available capacity were significantly affected by soil texture. The study also mentioned the potential development of a soil health scoring function by texture group (Amsili et al., 2021). Similarly, a study by Nunes et al., (2021) developed a Soil Health Assessment Protocol and Evaluation (SHAPE) scoring system for soil organic carbon, using soil texture divided into five classes (T1 – T5). The CREU framework could potentially be updated to consider soil texture classes; however, this would require a large dataset to establish and analyze sensitivity, and correlation with other dynamic soil properties, including biological characteristics, should be considered.

3.3 Differences between samples and state of reference

Variations in SOM may potentially be linked to the intrinsic characteristics of the soil (p < 0.001) and the regional precipitation patterns (p < 0.001). Reference sites CR – M and BM (Table 1), with the Mitchell soil series, and DAR with Tripp soil series (Table 1), exhibited similar SOM values. Likewise, the CC-2 site, characterized by the Tassel soil series (Table 1), mirrored these SOM values, with no statistically significant differences (Fig. 6). It's important to note that Tripp, Mitchell, and Tassel all classify as super-active calcareous mesic soil series. Such findings resonate with the perspective of Seybold et al. (1997) and CREU (Das and Maharjan, 2022), emphasizing the possibility of formulating reference points by grouping soils that exhibit similar pedogenetic attributes, thereby providing a practical framework for guiding consistent management practices across a particular agricultural landscape, where the soils and precipitation pattern are uniform. The Valent soil series (site: SX) from MLRA 67A, characterized by its sand texture (comprising 70–100% sand) and low precipitation zone, showed lowest SOM when compared with other soil series, especially clay loam textured soil from MLRA 106, with higher precipitation zone (Table 1, Fig. 6). This underscores the strategic importance of melding the MLRA framework with the CREU concept, which divides the MLRA based on soil and precipitation gradient. Such an integration facilitates a comprehensive landscape classification aiming to identify zones with similar soil health potential.

In MLRA 67, within the same CREU, with same soil type and precipitation zone (Tripp soil series; 356–432 mm precipitation range), an analysis of SOM variation among differently managed reference states revealed significant differences. These variations are largely due to human modification and management history, which have caused disturbances in the naturally managed reference conditions. The soil health gap between a true reference (DAR) and an altered reference (PREEC) was 0.7% (Table 1). The SOM content at the PREEC site, situated at the field's edge, was recorded at 2.7%, closely aligning with that of cropland under long-term manure (> 70 years) management (2.7%). This suggests that selecting a disturbed reference to set benchmarks can create a misleading impression that the soil health is on par with that of more naturally maintained conditions when, in fact, it may be reflecting the outcomes of prolonged and intensive management practices.

3.4 Soil Health Gap Assessment
The results from these comparative studies underscore the importance of judiciously selecting reference sites based on MLRA, soil (texture class), and precipitation gradients. The central role played by these reference sites, especially those that align with reference plant communities detailed in ecological site descriptions, is evident in generating accurate soil health gap assessments (Fig. 7). Significant variances were noted between the reference site and the paired croplands within MLRA 67A and MLRA 106. In the case of the Tripp soil series, within a precipitation range of 356–432 mm (14–17 inches), the soil health gap was 1.8% when compared to conventionally managed cropland (KH-C) and 0.8% against cropland with long-term manure application (KH-M: manure application for > 70 years). This finding emphasizes the benefits of manure application in improving SOM content and overall soil health. The Wymore reference site (DP) showed a 2.0% higher SOM content compared to its paired cropland (PD) (Fig. 7). While these differences are evident, they remain within a reasonable margin of improvement. Here, the reasonable margin of improvement refers to an achievable increase in SOM over a realistic human timescale (decades), considering the slow rate of organic matter improvement.

Contrastingly, comparing reference site data from eastern Nebraska with cropland site data in western Nebraska, which clearly exhibits distinct soil and climatic characteristics, yielded a significant 4.0% SOM gap. Such disparities would pose considerable challenges for farmers in western Nebraska due to the difference in the potential capacity of each soil. Thus, this extreme extrapolation reinforces the importance of prudent reference site selection based on the native potential of each site within the proposed framework.

4.0 Progression, Limitations, and Opportunities

Building upon our prior research, the primary objective of this study was to devise a framework for reference site selection and evaluate the framework’s applicability using data of SOM collected from eastern and western Nebraska and also put forward a future conceptual framework for soil health quantification. In this effort, the concept of a soil health gap uses native land to theorize and establish a benchmark for quantifying aggrading and degrading factors in cropland and also measures the success of different management practices for soil health improvement (Maharjan et al., 2020). The concept of the Cropland Reference Ecological Unit acknowledges the inherent variation in soil and climate and the difference in the native potential of each soil to reduce bias in comparative studies (Das and Maharjan, 2022). This paper extends those concepts and provides an easily reproducible framework for reference site selection to establish a benchmark by accounting for the inherent variability in soil and climate and then the subsequent use of the reference site in soil health gap calculation. In this context, reference plant communities were used as a tool, providing a means to assess the degree of human modification of the ecosystem (Supplementary file S4). Although the reference plant community may not offer the comprehensive robustness of more advanced techniques, it provides a reliable, straightforward, and contextually appropriate way to evaluate ecological states and transitions. Local knowledge of plant community structure and distribution and the degree of change on a spatio-temporal scale in response to agricultural intensification can be translated into a human modification index. This approach’s ease of replication makes it applicable in other parts of the world (Garnier et al., 2007; Laliberté et al., 2010; McIntyre and Lavorel, 2007). Sophisticated methods like remote sensing and extensive land-use change datasets, can be used to develop advanced human modification indices (Theobald et al., 2020) and habitat condition assessment system (Harwood et al., 2016) to measure human impact on ecosystem and subsequently for measuring soil change as explored in other studies (Román Dobarco et al., 2023), but also comes with their challenges (Román Dobarco et al., 2023). Our focus was on leveraging the practicality and relevance of reference plant communities to
understand soil health dynamics and set up a reference point within the parameters of our study's design and objectives.

The study acknowledges that completely reverting to the reference state may not be feasible due to extensive agricultural history (Powlson et al., 2022). However, the purpose of the reference is to understand the native potential of the land and guide improvement efforts. The reference concept can be adapted to include management and land-use factors, enabling the creation of hierarchical land classifications for comparative soil health studies (Table 2). These classifications can set multiple attainable targets, each representing a step towards improved soil health (Fig. 8). For instance, research by Krupek et al., (2022) illustrates how cover crops can narrow the soil health gap, fitting into a higher hierarchical order compared to conventional practices.

Similarly, Singh et al., (2023) demonstrate that conservation practices like no-till and cover crops are more effective than conventional methods, helping to close the gap with the reference state. Management practices such as crop residue management, manure application, and including periods of pasture within the rotation can improve the soil properties, often to 60% − 70% of the initial values (Powlson et al., 2022). These improvements, brought about by management changes, can be organized into different hierarchical orders (Table 2), providing a structure for setting various achievable targets (Fig. 8). For instance, an initial target (T1) could be set to be reached with cover crops (Krupek et al., 2022) or manure management (Das et al., 2022). Subsequently, a second target (T2) could be strategically set to advance beyond T1 by enhancing the existing management practices like cover crops and manure management and incorporating additional methods such as no-till farming (combining manure application with no-till techniques). This layered, hierarchical approach not only aids in deciphering the dynamics of soil health but also provides tangible, incremental steps for continuous improvement. In areas lacking true reference sites, this system offers an alternative reference framework. For instance, conventional practices can be compared with higher-order practices like cover crops. This comparison provides insights into the potential improvements achievable and the existing soil health gap, guiding targeted interventions. Moreover, a collaborative effort involving extensive sampling across various management practices on a spatio-temporal scale can further refine this classification system. Such an approach would enable a more accurate assessment of the impacts of different land management strategies on soil health, offering valuable insights into agricultural policy and practice.

Table 2

<table>
<thead>
<tr>
<th>Group – A</th>
<th>Group – B</th>
<th>Group – C</th>
<th>Group – D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmanaged Land</td>
<td>Minimally or optimally managed land</td>
<td>Moderately managed land</td>
<td>Intensively managed land</td>
</tr>
<tr>
<td>♣ Native Forest</td>
<td>♣ Cropland retired for &gt; 50 years</td>
<td>♣ Croplands with conservation practices</td>
<td>♣ Croplands with conventional practices</td>
</tr>
<tr>
<td>♣ Native prairie</td>
<td>♣ Rangeland*</td>
<td>♣ Rangeland with prescribed grazing</td>
<td>♣ Grazing lands with heavy grazing</td>
</tr>
<tr>
<td>♣ Natural grassland</td>
<td></td>
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Groupings are based on land management ranging from unmanaged land to intensively managed croplands.
Lands that never underwent any management

*Rangeland that is lightly grazed or under optimal grazing management.

†Croplands retired for > 50 years, reported achieving properties similar to those in native lands (Burke et al., 1995; Ihori et al., 1995)

§Cropland where known conservation practices, including no or minimal tillage, residue management, and cover crop, are practiced.

Rangeland where frequent management is introduced or practiced such as prescribed fire, reseeding with perennial grasses.

5.0 Conclusion

This study presents an examination of soil health gaps across various land resource areas and ecological sites, underscoring the importance of carefully selecting reference sites to set achievable soil health goals. Our research demonstrates that when reference sites are carefully selected, aligning with factors such as Major Land Resource Area (MLRA), soil type, precipitation gradients, and reference plant communities, the resulting soil health gaps are more practical and attainable in terms of soil health improvement. The analysis of soil organic matter variations across different MLRAs and soil types highlights the significant role of soil texture and climate in soil health. Our findings indicate that both texture class and precipitation gradient are key to dividing land for agroecological purposes, thereby establishing more accurate benchmarks for Soil Health Gap analysis. While this study primarily utilizes tools and resources available in the United States, the methodology has the potential for broader application and is adaptable based on local soil classifications, plant community knowledge, and land management information. Additionally, the study's scope can be expanded with advanced techniques such as remote sensing and various bio-physicochemical models.

Declarations

Acknowledgments

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Authors' Contributions

SD, AH, and BM conceptualized the study. SD, AH, and BM prepared the initial draft. All authors (SD, AH, BM, MS, LK) reviewed and revised the manuscript. Field data collection was undertaken by SD, AH and MS, with data analysis also performed by SD and AH.
References


**Figures**
Figure 1

(a) Soil map unit and ecological site map, Scottsbluff, Nebraska (MLRA 67A). Soil map units shaded red here represent Limy upland ecological site, and the number within the colored polygon represents the soil map unit (MU: 5845: Mitchell Soil Series) for a particular soil component. Refer to supplementary file S3 for the description of all the map unit keys and corresponding ecological sites. This map was created using the Web Soil Survey (USDA – NRCS) (b) The state and transition model for the ecological site Limy Upland (as described in ecological site description). Box-1 represents the reference state; phase 1.1 Reference Plant Community describes a short list of potential reference plant communities. Here, the reference plant community includes blue grama, little bluestem, and side oats grama. These key species should be used as a diagnostic characteristic of potential reference sites. This information is downloaded from the web soil survey (https://websoilsurvey.nrcs.usda.gov/app/) and the edit server (https://edit.jornada.nmsu.edu/).
Figure 2

Map depicting Nebraska’s major land resource areas (MLRAs) with designated sampled reference sites and cropland. In this map, the MLRAs of Nebraska are indicated in black font numbers, for example, 67A, 65, 64. The green, red, and orange circular dots represent the reference sites, cropland, and altered reference (which have land-use change in management history) within each MLRA, respectively.
Figure 3

*Representation of soil sampling methods: Five-Point and Three-Point Methods.* In this figure, the red points indicate the primary (centroid) sampling point, while the orange points represent the secondary (satellite) sampling points within a site.

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Figure 4

*Variation in soil organic matter across reference sites within (A) Averaged across Major Land Resource Areas (MLRA) and (B) specific soil series within MLRA 106 and MLRA 67A.* Lowercase annotations correspond to groupings from the Tukey HSD test, with distinct letters signifying statistically significant differences.
Figure 5

Variation in soil organic matter (SOM) across distinct ecological sites. Lowercase annotations represent groupings based on the Tukey HSD test; differing letters denote statistically significant differences in mean value. To note, there was only within-site replicative sample (n = 5) for Wet Floodplain and Shallow (n = 5) ecological sites; for other ecological sites, both within and cross-site replicative samples were present (Table 1).
Figure 6

Distribution of soil organic matter (SOM) across various reference sites and the difference in average SOM between MLRA 106 and MLRA 67A. Lowercase annotations indicate groupings from the Tukey HSD test; distinct letters highlight statistically significant differences. The blue line and red line represent the average SOM for the MLRA 106 (eastern Nebraska) and 67A (western Nebraska), respectively. The sites with *are altered reference states, meaning they had different land-use and management histories in the past (Table 1).
Figure 7

Assessment of the Soil Health Gap (SHG) between reference sites and cropland, focusing on two distinct soil groups: "tripp" and "wymore". Columns denote specific reference and cropland sites, identified by their respective abbreviations (full details provided in Table 1). The SHG, illustrated in terms of soil organic matter, is expressed as a difference in SOM percentage. SHGs are represented with an arrow, showing the sites among which the soil health gap was calculated. Here, KH-C is a control plot, and KH-M is a plot with manure management.
Multiple attainable targets. Here, $T_1$, $T_2$, and $T_3$ refer to different attainable targets. The potential target shown here is for representative purposes. Here $(SHG)_{SOM}$ represents the Soil Health Gap calculated in terms of Soil Organic Matter, and $SOM_{rf}$ and $SOM_{cp}$ represent soil organic matter content in reference state and cropland, respectively.