



25           Concerns over the sustainability of the soil ecosystem that provides food, fiber, and fuel to  
26 the ever-increasing world population have helped coalesce efforts around soil health and  
27 conservation. Soil health is the continued capacity of soil to function as a vital living ecosystem  
28 that sustains plants, animals, and humans (NRCS - USDA, 2021) and is measured via indicators  
29 that are measurable soil properties that can provide inferences about soil functions. Numerous  
30 research report soil health in terms of soil physicochemical and biological indicators and identify  
31 different management practices that can improve it.

32           There is a growing consensus that soil health in cropland needs to be compared to a  
33 reference state to understand its status and how much it can be improved (Morgan and  
34 Cappellazzi, 2021). For example, Maharjan et al. (2020) proposed a term “Soil Health Gap” that  
35 compares soil health in cropland and native undisturbed land, providing a measure of decline in  
36 soil health in croplands since cultivation began and simultaneously setting potential soil health  
37 management goals in croplands. Many other researchers have suggested using native virgin or  
38 undisturbed land as the reference state, considering the theoretical prime health status. However,  
39 defining and selecting a benchmark reference site comparable to cropland of interest is intricate  
40 and should account for the heterogeneity in soil and climate.

41           Significant changes in soil bio-physicochemical properties can be observed across  
42 different soil series and associations (Caudle, 2019). Climate, especially precipitation, defines soil  
43 biological functions and the biogeochemistry of nutrients. Precipitation gradient and individual  
44 soil entities based on pedogenetic differences can create differences in soil health potential. For  
45 that reason, the response of soil health indicators to different management practices is site-specific  
46 (Nunes et al., 2021). Therefore, compared soils should belong to an ecologically discrete unit that  
47 accounts for soil and climate variability and have similar soil health potential.

48 In this paper, we propose a landmass unit, *Reference Ecological Unit (REU)*, that  
49 accounts for agroecological variability and wherein croplands can be compared among  
50 themselves and with native lands for their soil health statuses. If measured in the same REU, soil  
51 health indicators in different soils will provide true differences due to land use or management  
52 practices. The REU will provide a leveled platform for comparative studies where soil health can  
53 be assessed and compared for a group of soils with similar soil health potential.

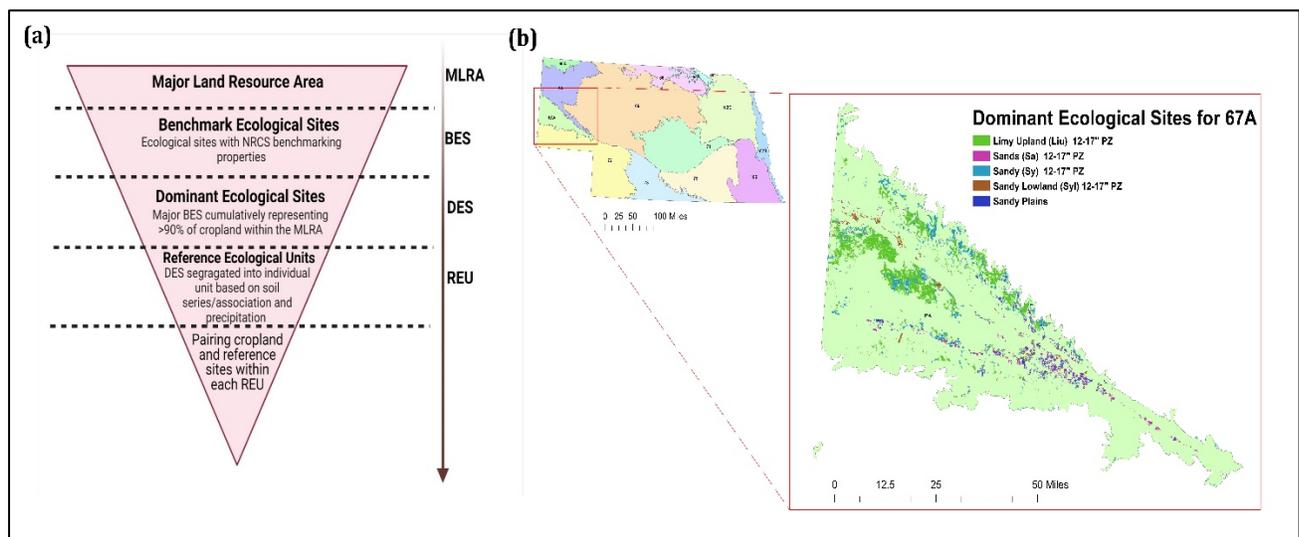
#### 54 **Definition of Reference Ecological Unit**

55 Reference ecological unit is defined as a landmass unit with uniform pedogenetic and  
56 climatic properties in a hierarchical land classification system. Below, we present how to carve  
57 out REUs within ecological sites (ES) in each major land resource area (MLRA) in the USDA-  
58 NRCS Hierarchical Land Classification System (HLCS) (**Figure 1(a)**). The REU can be created  
59 to achieve the desired resolution by adjusting boundary conditions of pedogenetic and climatic  
60 factors.

61 In the USDA-NRCS HLCS, MLRA is a broad classification of geographically associated  
62 land considering the geology (parental material), climate (precipitation, temperature), water, soils  
63 (dominant soil orders), biological resources (plants and animals), and land-use types (NRCS-  
64 USDA, 2021). The MLRA is then divided into ecological sites (ES), which are distinctive lands  
65 with specific soil and physical characteristics (climate, geology, hydrology) that differ from each  
66 other to produce distinct kinds of vegetation and respond to management practices and natural  
67 disturbances. From ES, Benchmark Ecological Sites (BES) are selected for their potential to yield  
68 data and information about ecological functions, processes, and climate change which are  
69 important to characterize an area or critical ecological zones.

70 **Methodology to determine REU**

71 To determine REU, BES were categorized based on their crop cover area, and the top BES  
72 cumulatively representing >90% crop covers were selected and are referred to as Dominant  
73 Ecological Sites (DES) (**Figure 1(b)**). Individual DES was divided into discrete landmass units as  
74 a function of soil associations and precipitation range ( $\geq 3$  in; 7.6 cm) to determine the REU.  
75 Thus, theoretically, REU represents uniformity from perspectives of soil genesis (geology), biotic  
76 community (plant community), physical properties (topology and hydrology), and climate  
77 (precipitation). Selecting a group of soils within each REU will provide a leveled platform for  
78 comparison as they all would have similar soil health potential.



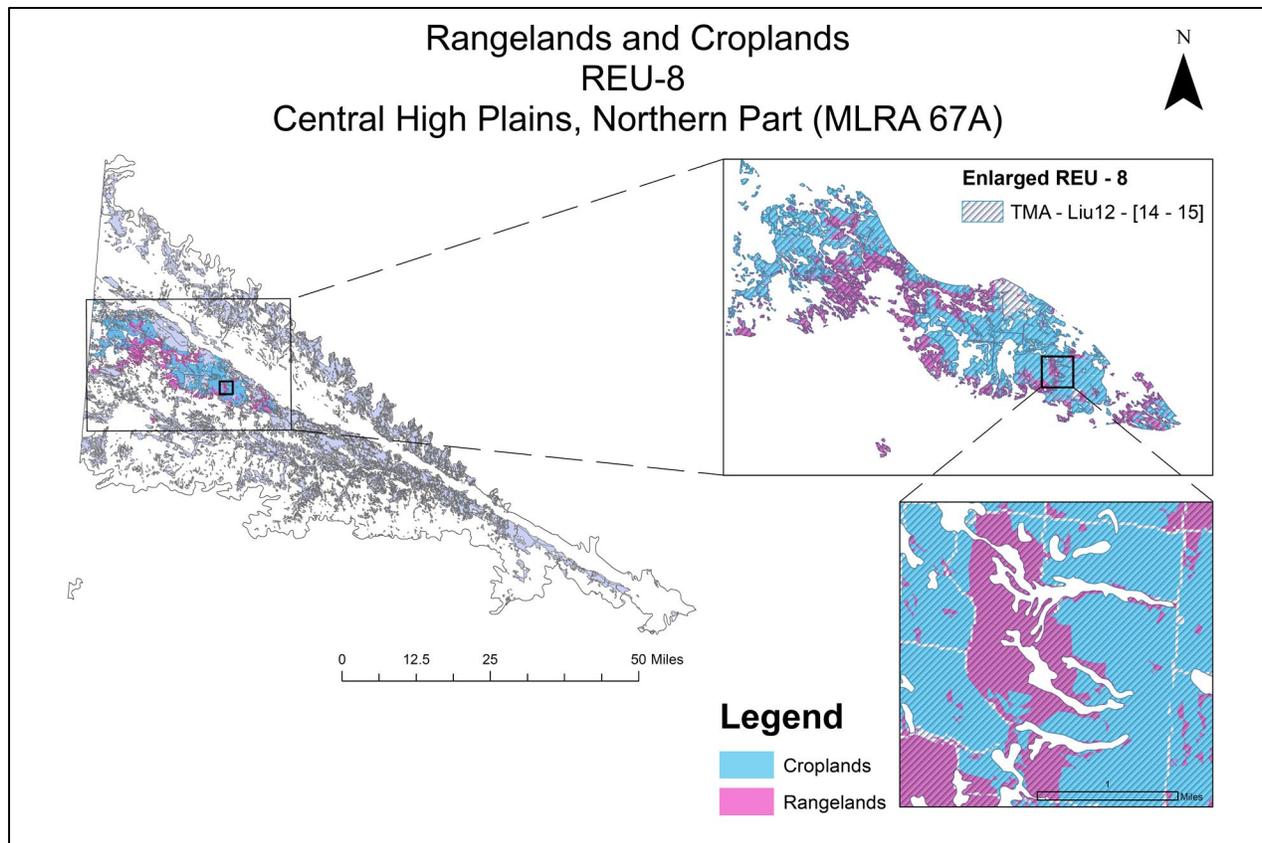
80 **Figure 1.** (a) Hierarchical land classification leading to Reference Ecological Units where soils  
81 can be compared, (b) Dominant ecological sites identified based on >90% crop cover for the  
82 MLRA 67A, one of 13 MLRAs in Nebraska.

83  
84 Geo-spatial analyses were conducted to determine REUs in ArcGIS 10.8 (Esri, CA). The  
85 USA Contiguous Albers Equal Area Conic was used as a reference projection model for geo-  
86 spatial analysis and methodology development. Available shapefiles and layer files such as  
87 MLRA, Land Cover and Land Use were downloaded from the USDA - Geospatial Data Gateway  
88 (USDA – GDW) by generating a request form from the website. In this study, the MLRA 67A in

89 Nebraska is used for an example to demonstrate the determination of REUs. The REU selection  
90 methodology was performed in the following order:

- 91 i. A layer of MLRA for Nebraska was created from USA – MLRA shapefile obtained from  
92 USDA - GDW by clipping with NE state shapefile.
- 93 ii. A layer of the Land-Cover-Land-Use was created from NASS land cover data. All the  
94 land use properties were removed from the layer attributes except for the cropland to  
95 create the cropland layer file.
- 96 iii. A layer of BES was created from the polygon data of ES as provided by the USDA –  
97 NRCS based on the benchmarking attributes determined by NRCS.
- 98 iv. MLRA layer was intersected with the cropland layer (from step ii) and BES (from step iii)  
99 using ArcGIS intersect function of geo-spatial analysis. Output shapefile was split into 13  
100 discrete MLRA units for Nebraska using intersect layer as the primary input and MLRA  
101 layer as the split unit.
- 102 v. Each MLRA at this point has multiple polygons for each BES as they are segregated based  
103 on the cropland cover. Dissolve function from the generalized tool of ArcMap was used to  
104 aggregate the attributes of each BES feature class to represent each BES as single unit in  
105 the MLRA attribute.
- 106 vi. Using field value calculator, area of cropland for each BES was calculated for individual  
107 MLRA. Percentage of land cover for each BES was calculated by dividing the individual  
108 area of cropland for each BES to the total area of cropland for the MLRA.
- 109 vii. A cumulative percentage of cropland cover was calculated. Top BES cumulatively  
110 covering > 90% of the cropland was selected as the DES. For MLRA 67A, there were 5  
111 DES.

- 112 viii. The layer of DES for each MLRA was intersected with the *Soil associations Layer* and  
113 *Precipitation Layer*. Each intersected map unit was grouped by unique DES and Soil  
114 associations and then discretized over two or three inches (5.1 or 7.6 cm) of precipitation  
115 gradient to determine the ***Reference Ecological Unit*** for each MLRA (**Figure 2**). For  
116 MLRA 67A, there were 45 REUs.
- 117 ix. For soil health comparative studies, the native grassland site (~rangeland) and cropland  
118 should be present in the same REU for determination of true differences in soil health  
119 statuses or the Soil Health Gap in croplands. **Figure 2** has an enlarged section of reference  
120 ecological unit (REU-8) from the MLRA 67A to illustrate the concept. Here, in **Figure 2**,  
121 the shaded background in the enlarged section is the REU, and the blue and pink shared  
122 area represents the croplands and rangelands, respectively. Croplands in the blue-shaded  
123 area should be comparable to determine soil health differences due to management  
124 practices. Soils from croplands and rangelands in the REU can be compared to determine  
125 the Soil Health Gap and set potential soil health management goals in those croplands.



127 **Figure 2.** Reference ecological units for MLRA 67A (left) and one of 45 identified REUs (REU-8)  
 128 is enlarged and layered with cropland and rangeland (right). The REU identifier consists of soil  
 129 association- DES- [pz; precipitation zone in inches]. TMA = Trip-Mitchell-Alice soil association,  
 130 Liu12 = Limy upland 12 – 17 pz ecological site, and [14 – 15] is the precipitation zone.  
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132 **Relevance and Future Prospect of REU in Soil (Health) Research**

133 Determining REU based off the existing USDA NRCS Hierarchical Land Classification  
 134 System allows to find some levels of homogeneity in a land mass that otherwise has dynamic soil  
 135 pedogenetic properties and climatic variability. As majority of the soil health researches attempt  
 136 at understanding and determining management effects on soil health and the degree of gain in soil  
 137 health over time, it is essential to be able to compare cropland soil health to an soil in a prime  
 138 health state, or a reference state (Morgan and Cappellazzi, 2021). It is equally important, if not  
 139 more, to select the soils from the same REU that would have similar soil health potential.  
 140 Otherwise, the differences in pedology and climate among comparing sites create confounding

141 effects on soil health indicators. In such cases, measurement of soil health indicators and  
142 comparative studies of soils with different potentials do not represent the true like-for-like  
143 comparison. Thus, REU will provide a unique leveled platform in soil science research for its  
144 functional attributes like:

- 145 • It will provide a land unit for comparative study with similar soil health potential as it  
146 accounts for site-specificity.
- 147 • A true quantitative difference in soil health for different land use and agronomic  
148 management practices can be determined by comparing sites within an individual REU.  
149 Evaluation of management effects on soil health properties in REU will provide the true  
150 understanding of beneficial effect of such practices, unconfounded by agroecological  
151 variations.
- 152 • Implementation of REU will help in comprehensive correlative understanding for soil  
153 health matrices for different agroecological regions.

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