Technical appendix:

Soil Analysis

Bulk density was measured following Throop and Archer (2012)’s hybrid method: the full core was passed through a 2 mm sieve, weighed, and 30 cm³ of < 2 mm soil subsample was weighed before and after drying at 105 °C for 12 hours to correct for moisture. Bulk density was calculated as the dry mass of < 2 mm soil (coarse roots and rocks removed) per unit volume. We included the < 2mm fraction and excluded the > 2 mm fraction to reduce the chance of inflated SOC values in rocky soils (Throop and Archer 2012). Approximately 100 g of the < 2 mm soil from each plot were sent to the UC Davis Analytical Lab for plant available PO₄³⁻ (extraction by sodium bicarbonate solution; Olsen et al. 1954), plant available SO₄²⁻ (extraction by monocalcium phosphate solution; Schulte and Eik 1988), and exchangeable K⁺, Na⁺, Ca²⁺, and Mg²⁺ (displacement with ammonium acetate solution; Thomas 1982). To measure soil pH, 15 g of < 2 mm soil was mixed with 30 mL deionized water and readings were taken using a handheld pH meter (Robertson et al. 1999). Soil texture was estimated by the hydrometer method using 50 g of soil and 100 mL of dispersing solution (Sheldrick and Wang 1993). An index for soil organic matter was measured by the loss-on-ignition (LOI) method: 5 g of < 2 mm soil were placed in porcelain crucibles in a furnace at 105 °C for 12 hours, weighed, and reweighed after the samples were ignited at 550 °C for 4 hours (Robertson 2011). Concentration of SOM (%) was calculated from the percent weight loss between 105 and 550 °C. Amount of SOM (Mg/ha) was calculated from concentration of SOM multiplied by bulk density and depth. The LOI method tends to overestimate organic matter in soils with significant proportions of clay because of water bound to clay minerals (Howard and Howard 1990). The amount of water bound to clay minerals depends on the clay type but we do not have minerology data for our soil samples, which creates uncertainties around our SOM estimates. However, we still chose to use LOI because it is a simple and inexpensive method that is most accessible to range managers. To measure total soil C percentage, < 2 mm soil were pulverized with a ball grinder (SPEX Sample Prep Mixer Mill 8000D, Metuchen, New Jersey, USA), then analyzed using a flash combustion method with atropine as a standard (Carlo Erba NA 1500 Elemental Analyzer, Lakewood, New Jersey, USA). For each tray we analyzed, we calibrated the instrument and checked for accuracy of measurements with atropine every 10 samples. Concentration of SOC was converted to SOC stocks (Mg/ha) by multiplying it by bulk density and depth. Finally, SOM and SOC stocks in 0-15 cm and 15-30 cm were summed to estimate cumulative SOM and SOC stocks in 0-30 cm. According to the USDA Soil Survey Geographic (SSURGO) Database, the study area has negligible inorganic carbon (0-4% calcium carbonate content), so we assumed that the concentration of total soil C to primarily reflect the concentration of SOC (%). Our measured pH values fall within the endpoints for potential carbonate presence (pH range between 10.3 and 6.3 – USGS) and is possible that some inorganic carbon could be found in our study area, which should be investigated in future studies. However, this possibility would not affect the interpretation of soil C in ESDs, because all three ecological sites have similar pH. Moreover, our estimated concentration and stocks of SOC are similar to those observed in previous studies in the region (Carey et al. 2020b; Eastburn 2017).
Data Analysis

All statistical analyses were done in R version 3.5.1 (R Development Core Team 2018). All soil and environmental data were standardized to z-scores using the function “decostand” in the R package “vegan” (Oksanen et al. 2019). Mean of SOM and SOC stocks were calculated to collapse the variation from samples collected four times. To test how physical patterns of the landscape and vegetation affected SOM and SOC by depth increment (0-15 cm, 15-30 cm, 0-30 cm), a linear mixed effect model was fitted with ecological site, vegetation state, and ecological site by vegetation state interaction as fixed effects, and plot as a random effect using the function “lme” in the R package “nlme” (Pinheiro et al. 2019). To address the interactive group differences, post-hoc Tukey test was conducted using the function “glht” in the R package “multcomp” (Hothorn et al. 2008). A regression analysis was performed on SOM and SOC to estimate the relationship between SOM and SOC.

To compare the spatial variability in SOM and SOC at the plot level, the coefficient of variation (CV) was calculated by dividing the standard deviation by the mean, as in Kelsey et al. (2012). Estimating spatial variation as CVs by each spatial scale is helpful in partitioning the spatial patterns of landscapes (Hammond and Kolasa 2014). Plot-level, or vegetation type-level variability was determined as the average variation among replicates within each plot. To test the effect of microhabitats on SOC stocks, a linear mixed effect model was fitted with ecological site and microhabitat as fixed effects and plot as a random effect, followed by a post-hoc Tukey test.

To determine the relative influence of environmental variables on SOM and SOC stocks, a multi-model linear regression approach was used. First, multicollinearity was checked on 27 environmental variables, including climate, topography, geology, ground cover, and cattle using the function “vif” in the R package “car” (Fox and Weisberg 2019). Maximum and minimum air temperature, silt, sand, PO4³⁻, Na⁺, SO4²⁻, K⁺, fresh cow manure, herbivory, trailing, and cover estimates of herbaceous plants, litter, and bare ground were correlated with one other environmental variable, thus these environmental variables were removed (VIF > 5). Second, backward stepwise model selection by Akaike’s information criterion (AIC) was applied to choose the best predictive model for SOM and SOC stocks (depth 0-15 cm and 15-30 cm modeled separately) using the function “stepAIC” in the R package “MASS” (Venables and Ripley 2002). Finally, a Bayesian hierarchical mixed model was fitted to the models above to estimate the 95% credible intervals of coefficient estimates using the function “MCMCglmm” and family = “gaussian” in the R package “MCMCglmm” (Hadfield 2010). Values reported in the text are means plus or minus standard errors.


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