

Soil sample collection:

Soil samples were collected during the growing season. From each plot, litter and vegetation were removed from the soil surface then four to five soil cores (~2.5 x 10 cm) were collected from the 2.5 x 2.5 m core subplots. The sub-samples were homogenized into a single sample for each 5 x 5 m plot. Samples were allowed to air dry in paper bags and then sieved (2mm) to remove rocks, plant root fragments, and break up large clumps of soil. Samples were then shipped to the University of Minnesota (St.Paul, MN, USA) for further processing.

Soil sample preparation and processing:

Soils were prepared for carbon, nitrogen, micronutrient, and texture analysis in the Borer/Seabloom Laboratory at the University of Minnesota. A subsample (~80-100 g) of sieved soil from each plot was homogenized by grinding the soil with two steel BBs (Daisy Premium 3/8" steel slingshot ammo) with 90 minutes of vigorous shaking. Approximately 18 - 25 mg of ground, homogenized soils were then packed into 5 x 9 mm tin capsules for carbon and nitrogen analysis in the Borer/Seabloom Laboratory using dry combustion gas chromatography on an Elemental Analyzer (Costech ECS 4010 CHNSO Analyzer, Valencia, California, USA) calibrated with the analytical standard, atropine (C₁₇H₂₃NO₃). Percent total carbon and total nitrogen are measured on the Elemental Analyzer when gases separated by a gas chromatographic separation column flow through a thermal conductivity detector to generate a detectable electric signal. The electrical signal is then interpreted as proportional to the concentration of carbon or nitrogen in the total sample.

20 g per sample of the ground, homogenized soils were sent to Waypoint Analytical (Memphis, TN, USA) to measure major nutrients, micronutrients, cation exchange capacity, soil pH, and organic matter. Phosphorus, potassium, calcium, magnesium, sulfur, boron, copper, iron, manganese, zinc, and sodium were measured and reported in parts per million using the Mehlich-3 method (Mehlich A. 1984. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. *Commun Soil Sci Plant Anal* 15(12):1409–16), a method considered to effectively measure a wide range of physical and chemical properties of soils. Cation exchange capacity (CEC), reported here as meq/100 g (milliequivalents of charge per 100 g of dry soil), is a measure of the capacity of soil surfaces to retain cations and is used as an indicator of quality and productivity of the soil. CEC ($CEC = (\text{ppm Ca} / 200) + (\text{ppm Mg} / 120) + (\text{ppm K} / 390)$) was calculated using the ppm of Ca, Mg, and K reported from the Mehlich-3 method (Ross, Donald S., and Quirine Ketterings. "Recommended methods for determining soil cation exchange capacity." *Recommended soil testing procedures for the northeastern United States* 2 (1995): 62-70.). Soil pH was measured with a water pH meter on a 1:1 suspension. Organic matter of the soil, the percent organic matter content in soil as measured using the Loss on Ignition (LOI) method, was determined by combustion for two hours at 400 C. Values reported here were not treated with acid prior to combustion. Waypoint Analytical includes laboratory control samples for every batch of soils processed. For every 105 samples, the run begins and ends with a laboratory control sample and includes a laboratory control sample every 11 samples. A laboratory reagent blank is included in each batch, as well.

At experiment year zero, texture was measured from each control, no-fence plot. Approximately 75 g of soil was sent to Waypoint Analytical Texture and measured using the hydrometer method (Klute, Arnold & W. (Richard W.) Weaver, R & H. Mickelson, S & , Sparks & L, Donald & M. Bartels, J. (1986). Methods of soil analysis. SERBIULA (sistema Librum 2.0)) (*FYI – I believe this is the correct citation for the following reference that WAYPOINT gave to me “Methods of Soil Analysis, Part 1 - Chemical Methods. 1986”*) using a sodium-hexametaphosphate solution (HMP). Briefly, the soil sample was shaken with the HMP solution to complex cations that bind clay and silt particles, and then transferred to a settling cylinder and mixed. The percent- sand, silt, and clay particles are calculated from hydrometer density readings taken at 40 seconds and two hours.