Rhizosphere Organisms:
An Indispensable Biotic Component of the Northern Mixed Grass Prairie

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Prairie ecosystems are complex; exceedingly more complex than the most complicated machines ever built by humans. The long-standing standard process to understand complex systems is to initially investigate the separate component parts. The gained knowledge of each part combined with the synergistic effects resulting when the parts work together provide the information needed to develop an understanding of the whole ecosystem. This classical concept of biological systems was developed by the Greek philosopher/scientist Aristotle (384-322 BC) who taught that "the whole is greater than the sum of its parts".

The goals of this study were developed by Dr. Warren C. Whitman (c. 1950) and Dr. Harold Goetz (1963) which were to gain quantitative knowledge of each component part and to provide a pathway essential for the understanding of the whole prairie ecosystem that would result in the development and establishment of scientific standards for proper management of native rangelands of the Northern Plains. The introduction to this study can be found in report DREC 16-1093 (Manske 2016).

Grass vegetation, rhizosphere organisms, and domesticated graminivores are indispensable biotic components of a functional rangeland ecosystem. Grazing graminivores depend on grass plants for nutritious forage. Grass plants depend on rhizosphere organisms for mineralization of essential elements from the soil organic matter. Rhizosphere organisms, which are achlorophyllous, depend on grass plants for short carbon chain energy that is exudated through the roots of lead tillers at vegetative growth stages following partial defoliation by grazing graminivores. Grass plants produce double the leaf biomass than is needed for photosynthesis in order to attract the vital partial defoliation by grazing graminivores on which they depend.

The three indispensable biotic components of rangeland ecosystems: Grass Vegetation, Rhizosphere Organisms, and Domesticated Graminivores will each be quantitatively described in separate companion reports. This report will provide scientific information on rhizosphere organisms as indispensable biotic components of grassland ecosystems.

Elucidation of the Microbial Rhizosphere

The scientific research and resulting general information on the cropland (mixed soil) microbial rhizosphere and the grassland (intact soil) microbial rhizosphere have unwittingly been intermingled causing serious confusion. All of the microbes in cropland are free-living in the soil edaphosphere (earth sector or zone). A small proportion of microbes in grassland soils are free-living in the edaphosphere, with the greatest amount of microbes living in the rhizosphere (root sector or zone).

The majority of the basic research of grassland rhizosphere microbes was conducted mainly in the United States from the late 1970's to the early 1990's with no program for followup applied research. The majority of the basic research on microbial processes in cropland soils for assessing soil quality was primarily conducted in European Union countries after the adoption of the Treaty on Biological Diversity of Rio de Janeiro in 1992 until around 2006 with no program for followup applied research.

The microbes in cropland mixed soils in which annual plants grow are all free-living. Some of these microbes do not associate with plants. Two types of free-living microbes associate with plants, some of these microbes are plant-detrimental (pathogenic) and some are plant-beneficial. The free-living plant-beneficial microbes live in a zone of unaltered soil surrounding active annual roots. This volume of loose soil is called a rhizosphere which exists only as long as the root remains active.

The microbes in intact soils of grasslands in which perennial plants grow are symbiotic with the plants and inhabit a cylinder of soil particles bonded by fungal secreted adhesive polysaccharides that surrounds perennial roots are also called a rhizosphere. The numerous types of rhizosphere

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organisms are organized along a trophic hierarchy with a means of "communication" among the microbes and with the plant.

Confusion has been created by using the same term to describe these different microbial structures. The rhizosphere in croplands is a zone of loose soil around an annual root in which bunches of free-living plant-beneficial independent microbes congregate. The rhizosphere in grasslands is a constructed chamber of bonded soil particles around a perennial root that is the summer quarters for numerous trophic levels of interacting symbiotic microbes. The latter description of the microbial rhizosphere will be used in this report.

Indispensable Rhizosphere Organisms

The microbial rhizosphere is a cylinder of bonded soil around active roots of perennial grassland plants and is comprised of resident organisms and frequent regular visiting organisms. The resident organisms are bacteria, protozoa, and endomycorrhizal fungi and the visiting organisms are nematodes, springtails, and mites (Elliot 1978, Anderson et al. 1981, Harley and Smith 1983, Curl and Truelove 1986, Whipps 1990, Campbell and Greaves 1990) and another resident organism is ectomycorrhizal fungi (Caesar-TonThat et al. 2001b, Manske and Caesar-TonThat 2003, Manske 2007b). The activity of rhizosphere organisms increases along the trophic hierarchy, starting with the resident bacteria. The numerous types of bacteria lack chlorophyll and have low carbon content. Bacteria are microscopic single celled saprophytic organisms that collectively consume large quantities of soil organic matter and are major primary producers of the rhizosphere. Increases in biomass and activity of the bacteria trophic level elevates the concentration of respiratory carbon dioxide (CO_2) resulting in stimulation of activity in the other rhizosphere organisms. Protozoa are single celled microorganisms that are mainly small amoeba in grassland soils and feed primarily on bacteria.

The slightly larger rhizosphere organisms are mobile and move among various rhizosphere structures. Nematodes are a diverse group of small nonsegmented worms. Most nematodes feed primarily on bacteria or fungi, some feed on protozoa, and some eat other nematodes. Springtails are among the most abundant insect in grassland soils that travel among rhizosphere structures. Minute springtails ingest considerable quantities of soil organic matter in order to eat fungi and bacteria. Mites are small eight-legged arachnids that travel among rhizosphere

structures and feed on fungi, nematodes, small insects, and other mites. Mites help distribute fungus spores and bacteria through the soil by carrying them on their exoskeleton.

Two types of fungi are resident organisms of the rhizosphere; Endomycorrhizal fungi and Ectomycorrhizal fungi. Endomycorrhizal fungi are also major primary producers of the rhizosphere and are achlorophyllous saprophytes that live on dead organic matter and cannot fix carbon for energy. Endomycorrhizal fungi develop arbuscules, vesicles, and hyphae within root tissue of the host plant (Harley and Smith 1983) and secrete adhesive polysaccharides that bond soil particles around grass roots forming the structural environment for all rhizosphere organisms. The adhesive polysaccharides also bind soil into aggregates resulting in increased soil pore spaces, increased water holding capacity, and increased rooting depth. Endomycorrhizal fungi also move phosphorus, other macro and micro mineral nutrients, and water through the hyphae to the grass roots for absorption (Moorman and Reeves 1979, Harley and Smith 1983, Allen and Allen 1990, Box and Hammond 1990, Marschner 1992, Koide 1993, Marschner and Dell 1994, Smith and Reed 1997).

Ectomycorrhizal fungi develop a sheath around the grass root with hyphae that do not enter the tissue of the host plant (Harley and Smith 1983) and secrete large amounts of adhesive polysaccharides forming water-stable aggregates in soil that are water permeable but not water soluable. The increased soil aggregation improves soil quality, increases soil oxygenation, increases water infiltration, and decreases erodibility (Caesar-TonThat and Cochran 2000, Caesar-TonThat et al. 2001a, Caesar-TonThat et al. 2001b, Caesar-TonThat 2002, Manske and Caesar-TonThat 2003, Manske 2007b).

The bacteria and fungi are the microflora saprotrophic organisms at the bottom of the food chain and makeup the greatest biomass of the rhizosphere. Both bacteria and fungi contain high proportions of nitrogen in relation to their carbon content. The microfauna trophic level organisms with normal ratios of carbon to nitrogen, graze on bacteria or fungi and ingest greater quantities of nitrogen than they need for a balanced diet based on energy (carbon); the excess nitrogen is excreted as ammonium (NH_4). The endomycorrhizal fungi can nitrify the excreted ammonium into nitrate (NO_3) and pass either form of mineral nitrogen into the grass plant through its endophytic vesicles and arbuscules.

The elevated rhizosphere organism activity caused by the increase in available short carbon chain energy exudated from the grass plant following partial defoliation by graminivores results in a greater quantity of organic nitrogen mineralized into inorganic nitrogen (Coleman et al. 1983, Klein et al. 1988, Burrows and Pfleger 2002, Rillig et al. 2002, Bird et al. 2002, Driver et al. 2005).

Biogeochemical Processes

Biogeochemical processes performed by rhizosphere microorganisms renew the nutrient flow activities in ecosystem soils of renewable natural resources. Biogeochemical processes transform stored essential elements from organic forms into plant usable inorganic forms. Biogeochemical processes capture replacement quantities of lost or removed major essential elements of carbon, hydrogen, nitrogen, and oxygen with assistance from active live plants and transform the replacement essential elements into storage as organic forms for later use. Biogeochemical processes decompose complex unusable organic material into compounds and then into reusable essential elements (McNaughton 1979, 1983; Coleman et al. 1983; Ingham et al. 1985; Mueller and Richards 1986; Richards et al. 1988; Briske 1991; Murphy and Briske 1992; Briske and Richards 1994, 1995).

The quantity of biogeochemical processes conducted in grassland ecosystems is dependent on the rhizosphere volume and microorganism biomass (Coleman et al. 1983). Rhizosphere volume and microorganism biomass are limited by access to simple carbohydrate energy (Curl and Truelove 1986). Healthy grass plants produce double the quantity of leaf biomass (Crider 1955, Coyne et al. 1995), capture and fix large amounts of carbon during photosynthesis, and produce carbohydrates in quantities greater than the amount needed for normal growth and maintenance (Coyne et al. 1995). Partial defoliation of grass tillers at vegetative phenological growth stages by large grazing graminivores causes greater quantities of exudates containing simple carbohydrates to be released from the grass tillers through the roots into the rhizosphere (Hamilton and Frank 2001). With the increase in availability of carbon compounds in the rhizosphere, the biomass and activity of the microorganisms increases (Anderson et al. 1981, Curl and Truelove 1986, Whipps 1990). The increase in rhizosphere organism biomass and activity results in greater quantities of biogeochemical cycling of essential elements (Coleman et al. 1983, Biondini et al. 1988, Klein et

al. 1988, Burrows and Pfleger 2002, Rillig et al. 2002, Bird et al. 2002, Driver et al. 2005).

Rhizosphere Biomass effects Essential Elements

The essential elements are required for life to exist by ensuring growth and development of organisms and the maintenance of all life functions. Microbes and plants require seventeen elements and animals require twenty one elements. Sixteen of these essential elements are required by all grassland organisms. Microbes, plants, and animals require very large amounts of the same four major essential elements: carbon (C), hydrogen (H), nitrogen (N), and oxygen (O). Grassland organisms also require large amounts of the same five macronutrients: calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S). Animals require one additional macronutrient: sodium (Na). Warm season plants and cacti use sodium (Na). All grassland organisms require very small amounts of the same seven micronutrients or trace elements: iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), chlorine (Cl), molybdenum (Mo), and nickel (Ni). Plants require one additional micronutrient: boron (B). A few plants and rhizobia use cobalt (Co). Animals require four additional micronutrients: iodine (I), selenium (Se), chromium (Cr), and cobalt (Co) (tables 1 and 2).

Table 1. Essential Elements Required by Microbes and Plants.

Major Essential Elements

Carbon (C), Hydrogen (H), Nitrogen (N), and Oxygen (O).

Minor Essential Elements

Macronutrients

Phosphorus (P), Potassium (K),

Calcium (Ca), Magnesium (Mg), Sulfur (S)

Micronutrients

Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu),

Boron (B), Chlorine (Cl), Molybdenum (Mo), Nickel (Ni)

Table 2. Essential Elements Required by Animals.

Essential Nutrients

Crude Protein, Energy, Water, Vitamins

Major Essential Elements

Carbon (C), Hydrogen (H), Nitrogen (N), and Oxygen (O).

Minor Essential Elements

Macrominerals

Calcium (Ca), Phosphorus (P), Potassium (K),

Magnesium (Mg), Sulfur (S), Sodium (Na), Chlorine (Cl)

Microminerals

Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu),

Iodine (I), Cobalt (Co), Selenium (Se),

Molybdenum (Mo), Chromium (Cr), Nickel (Ni)

Recycling Essential Elements

Perpetuation of life on earth requires that the major and minor essential elements be reused over and over. Recycling of the essential elements is performed by microorganisms associated with renewable natural resources (rangelands, grasslands, croplands, forestlands, and fisheries). When greater quantities of essential elements are used or lost than the quantities accumulated, the ecosystem degrades (declines). When greater quantities of essential elements are accumulated than the quantities used, the ecosystem aggrades (improves).

Some loss of essential elements from a grassland ecosystem occurs naturally. The metabolic processes of respiration in soil organisms, plants, livestock, wildlife, and insects results in a loss of essential elements as carbon dioxide, water vapor, and heat energy. Essential elements are removed from a grassland ecosystem as weight biomass produced by insects and wildlife. The essential elements transferred from grass plants to grazing livestock and used for animal growth are removed from the ecosystem (Gibson 2009).

The small proportion of the ecosystem essential elements that are lost or removed annually need to be replenished by capturing input essential elements from the surrounding environment through ecosystem processes. The biogeochemical processes associated with active live plants and soil microorganisms can capture replacement quantities for the lost major essential elements of carbon, hydrogen, nitrogen and oxygen.

The ecosystem input source for energy is radiant light from the sun. Radiant energy from the sun is necessary for photosynthesis. In the Northern Plains, sunlight reaching the plants has a small reduction resulting from about 30% cloud cover. The intensity of sunlight can be greatly reduced by shading from taller grasses and shrubs. Nondefoliated live and standing dead leaves of grasses reduce light penetration to a similar degree as shrubs, even though shrub leaves are flat and wide and grass leaves are erect and linear (Kochy 1999). The light levels penetrating the leaf canopy can be about 20% of the light levels above the canopy (Peltzer and Kochy 2001).

The ecosystem input source for carbon is atmospheric carbon dioxide (CO_2). Atmospheric carbon dioxide composes about 0.03% of the gasses in the atmosphere at concentrations of around 370 to 385 mg/kg. The carbon dioxide is fixed with

hydrogen from soil water during the plant process of photosynthesis which converts energy from sunlight into chemical energy and assimilates simple carbohydrates. Capturing energy by fixing carbon has a relatively low impact on the plant organisms that posses chlorophyll and has low biological costs to the ecosystem resources (Manske 2011b). Some of the short carbon chain energy is exuded into the rhizosphere to help increase the soil microorganism biomass. When the dead plant material is decomposed by the soil microbes, some of the carbon is combined with oxygen to form carbon dioxide gas which is volatilized and released back into the atmosphere.

The ecosystem input source of hydrogen is soil water (H_2O) which is infiltrated precipitation water. Soil water is absorbed through the roots and distributed throughout the plant within the xylem vascular tissue. When the rate of water absorption by the roots is less than the rate of water loss from transpiration through stomata openings, plant tissue develops water stress (Brown 1995). Plant water stress limits growth. In western North Dakota, the six month perennial plant growing season has a long-term periodicity rate of water deficiency conditions at 32.7% for the mean of 2.0 months with water deficiency per growing season (Manske et al. 2010). Hydrogen is released into the soil during the early stages of soil organic matter decomposition.

The ecosystem input source for nitrogen is wet deposition of nitrogen oxides following lightning. Lightning discharges cause atmospheric nitrogen (N_2) and oxygen (O_2) to combine and produce nitrogen oxides, mainly nitric acid (NO) and dinitrogen oxide (N_2O), that are deposited in precipitation (Manske 2009). The ambient amount of nitrogen deposition in temperate regions from natural sources is around 5 to 6 pounds per acre per year (Brady 1974). The source of nitrogen for grass growth is mineral nitrogen (NO_3, NH_4) mineralized from the soil organic nitrogen by rhizosphere organisms. Low quantities of available soil mineral nitrogen below 100 lbs/ac (112 kg/ha) is the major limiting factor of herbage growth on rangelands (Wight and Black 1979). However, rangeland soils are not deficient of nitrogen. Most of the nitrogen is immobilized in the soil as organic nitrogen. Soil organic nitrogen must be mineralized by rhizosphere organisms to become plant usable mineral nitrogen. The quantity of rhizosphere organisms is the limiting factor in rangeland ecosystems low in mineral nitrogen. Biomass and activity of organisms in the rhizosphere are limited by access to energy from simple carbon chains which can be exuded from grass lead tillers with partial

defoliation by grazing graminivores when grass tillers are at vegetative growth stages. Transformation of nitrogen from organic nitrogen to mineral nitrogen and back to organic nitrogen is complex, and has a great impact on many organisms at multiple trophic levels and has high biological costs on the ecosystem resources (Manske 2011a, b).

The ecosystem input source for oxygen is carbon dioxide, nitrogen oxides, and water. Atmospheric oxygen composes about 20.95% of the gasses in the atmosphere. Oxygen content in soil air is at a much lower concentration. The oxygen cycle between the biotic and abiotic components of the ecosystem is closely linked to the carbon cycle and the water, or hydrological cycle. Oxygen is vital for all organisms that carry out aerobic respiration. Oxygen is not known to be limiting on rangeland ecosystems.

The ecosystem input source for the six macronutrients and 12 micronutrients required by microbes, plants, and animals is weathered parent material. The elemental content of the parent material greatly influence the quantity of macro- and micronutrients in the soil. It is possible to remove macro- and micronutrients from soil at a faster rate than the rate of parent material weathering. The other natural mechanisms of transporting additional macro- and micronutrients into a region are glacial, flood, earthquake, and volcanic deposition.

Management practices that permit the use or loss of greater quantities of essential elements than the quantities of replacement essential elements captured cause grassland ecosystems to degrade. The single most important factor that permits the capture of greater quantities of replacement essential elements than the quantities used or lost is a large biomass of active soil microorganisms (Coleman et al. 1983, Schimel, Coleman, and Horton 1985, Cheng and Johnson 1998). The soil microorganisms are the renewable portion of grassland natural resources.

Aggradation of grassland ecosystems occurs when beneficial management practices cause increased quantities of labile (readily available) simple carbon chain energy from grass lead tillers at vegetative growth stages to be exuded (released) through the roots into the rhizosphere providing the limiting nutrient necessary for microorganism biomass to increase (Anderson et al. 1981, Curl and Truelove 1986, Whipps 1990, Hamilton and Frank 2001, Manske 2011b). The resulting increased biomass of soil microorganisms are able to decompose and mineralize greater quantities of the

soil organic matter producing greater quantities of essential elements in plant available mineral (inorganic) forms (Coleman et al. 1983, Klein et al. 1988, Bird et al. 2002, Burrows and Pfleger 2002, Rillig et al. 2002, Driver et al. 2005). The increased quantities of available essential elements provide for increased production of plant herbage biomass and increased livestock weight production (Manske 2008b).

This report includes a compilation of studies that evaluated the effects of management treatments on the quantity (weight or volume) of the Rhizosphere complex of organisms, active root segments, and adhered soil particles, and then evaluated the secondary effects that resulted from the rhizosphere quantities at various levels on the ecosystem biogeochemical processes. Each study will be treated separately.

Studies, Rhizosphere Organisms Affect on Grassland Ecosystems

A.   2006-2011 Study, Initial Effects from Twice-over Rotation Grazing

B.   1937-2011 Study, Seventy Five Years of Nongrazing

C.   1978-1990 Study, Effects of Prescribed Burning on Degraded Ecosystems

D.   1983-2018 Study, Evaluation of Biologically Effective Management

D1   1999-2000 Study, Finding Ectomycorrhizal Fungi in the Mixed Grass Prairie

D2   2002 Study, Determination of Rhizosphere Volume Around Grass Roots

D3   2006 Study, Determination of the Standard Reference Rhizosphere Weight

D4   1989-2018 Study, Availability of Soil Mineral Nitrogen

D5   2013-2018 Study, Soil Organic Matter (SOM) Affects Soil Quality

D6   2016-2018 Study, Evaluation of Soil Microbe Biomass and Activity

E.   Summary of the Indispensable Rhizosphere Organisms

A. 2006-2011 Study, Initial Effects from Twice-over Rotation Grazing

The twice-over rotation grazing strategy was the biologically effective treatment used for the initial restoration of a degraded ecosystem. The degraded mixed grass prairie grassland comprised 1,988 acres (804.5 hectors) and was a working cattle ranch prior to 1993 managed by traditional seasonlong practices based on use as forage for livestock grazed at moderate to heavy rates maintaining low production native grassland ecosystems. Management by the USDI Bureau of Land Management (BLM), after the purchase of this land, was based on use for recreation and wildlife habitat and all cattle grazing was removed for 13 years between 1993 and 2005 resulting in severely degraded plant communities dominated with undesirable cool season domesticated grasses, primarily Kentucky bluegrass, smooth bromegrass, and crested wheatgrass (Manske 2012c).

This 6 year project was conducted during 2006 to 2011 in order to describe and evaluate development of the initial changes of the biological restoration of degraded intact grassland ecosystems through implementation of the biologically effective three pasture twice-over rotation management strategy compared to a control of nongrazing (Manske 2012c). A nongrazed control treatment was used to manage one native rangeland pasture (NR 4) during this study. The biologically effective concept was used to manage three native rangeland pastures (NR 1, 2, & 3) with a twice-over rotation system grazed for 4.5 months from 1 June to 15 October (137 days) with commercial crossbred cattle grazed at a mean 78% of the potential 176 AUE beef cows with a total combined weight on 1 June of 175,533 pounds stocked at 1.92 ac/AUM (Manske 2012c).

Procedure

Permanent sample plots organized in a paired-plot design of grazed and ungrazed treatments with a 16' X 32' (4.88 m X 9.75 m) stock panel exclosures preventing livestock access to the ungrazed plots was established on silty ecological sites of each pasture. Rhizosphere biomass was collected during three periods per growing season on the grazed and nongrazed treatments by three replicated soil cores (7.6 cm X 10.2 cm) using a humane soil beastie catcher (Manske and Urban 2012a). The fresh rhizosphere material, which included the rhizosphere organisms, the active plant roots, and the adhered soil particles, was separated from matrix soil by meticulous excavation with fine hand tools and reported in kg/m^3. Soil mineral nitrogen, nitrate and ammonium, was sampled monthly (May to October) on the grazed and nongrazed treatments of the silty ecological sites by two replicated soil cores collected in incremental depths to 24 inches using a Veihmeyer soil tube with each sample air dried. Analysis of soil core samples for available mineral nitrogen (NO_3, NH_4) was conducted by the North Dakota State University Soil Testing Laboratory using wet chemistry methods. Aboveground herbage biomass was collected monthly (May to October) by the standard clipping method (Cook and Stubbendieck 1986) sorted in the field into domesticated grasses, cool season grasses, warm season grasses, upland sedges, forbs, standing dead, and litter and oven dried. Plant species basal cover was determined by the ten-pin point frame method (Cook and Stubbendieck 1986) and sorted into domesticated grasses, cool season grasses, warm season grasses, upland sedges, forbs, and litter.

Interpretation of treatment effects on plant community characteristics assumes only minor differences in the ecosystem status on the grazed treatment and nongrazed treatment at the time of the paired-plot exclosure constuction on each silty ecological reference site.

Results

Long-term perennial plant growing season precipitation (April to October) in the region near Richardton, ND, was 14.79 inches. Growing season precipitation during the first three years had a mean of 10.54 inches (71.26% of LTM). During the next three years, growing season presipitation had a mean of 14.07 inches (95.13% of LTM) (table 3).

The rhizosphere weights on the nongrazed and grazed treatments responded dissimilarly to the different treatments. During years 1 to 5, the small changes in rhizosphere weight on the nongrazed pasture at a mean of 5.7 kg/m^2 per year appeared to be related to the small changes in growing season precipitation at a mean of 1.00 inch (2.54 cm) per year. During year 6, there was a 28% increase in growing season precipitation at 3.72 inches (9.45 cm) per year and the rhizosphere weight on the nongrazed pasture increased 50% to 130.56 kg/m^3 (figure 1 and table 4).

The rhizosphere weights on the grazed pastures did not change during years 1 and 2 and they were not significantly different from the rhizosphere weights on the nongrazed pasture (table 4). The rhizosphere weights increased 33% during the third

year on the grazed pastures and continued to increase at a mean rate of 30.5 kg/m^3 per year from year 3 to year 6, reaching a mean weight of 214.3 kg/m^3, which was 64.2% greater than the year 6 rhizosphere weight on the nongrazed pasture (figure 1 and table 4). The increase in rhizosphere weights during years three to six on the grazed pastures appeared to be related to increases in carbon energy exudates released from grass lead tillers at vegetative growth stages between the three and a half new leaf stage and the flower stage following partial defoliation by grazing graminvores that removed 25% to 33% of the aboveground tiller material.

The partial defoliation of vegetative lead tillers resulting from the twice-over grazing treatment progressively decreased the rates of leaf senescence during the first two years and slowly increased the photosynthetic rates causing greater quantities of carbon to be fixed that became available for elevated grass growth and for exudation into the rhizosphere. During year 3, the increased quantities of exudated short chain carbon energy caused the rhizosphere weight on the grazed pastures to increase 73.5% greater than the weight of the rhizosphere on the nongrazed pasture. This greater rhizosphere weight and microorganism activity, resulted in mineralization of greater quantities of nitrogen and other essential elements from soil organic matter, which in turn, permitted greater grass growth and increased vegetative reproduction by tillering.

Mineral nitrogen available at low quantities below the threshold of 100 lbs/ac (112 kg/ha) is the major cause for less than potential levels of grass herbage production and calf weight gains and is a primary factor for ecosystem degradation (Manske 1999). The quantity of available mineral nitrogen is dependent on the biomass and activity levels of the microorganisms in the rhizosphere. The mean rhizosphere weight reached during year 6 on the nongrazed treatment was 130.56 kg/m^3 (figure 1 and table 4) which was only 32.1% of the standard reference rhizosphere weight of 406.44 kg/m^3. The quantity of available mineral nitrogen during the growing season was low on the nongrazed treatment (table 5). Nitrate and ammonium at the 0 to 12 inch depths peaked during May and was at decreased quantities the remainder of the growing season. The total quantity of available mineral nitrogen at the 0 to 24 inch depths peaked during July because both nitrate and ammonium levels peaked during July at the 12 to 24 inch depth. The quantity of available nitrate and ammonium remained relatively high at the 12-24 inch depth during June to September indicating

very little grass root growth and activity at that soil depth (table 5).

The mean rhizosphere weight reached during year 6 on the grazed pastures was 214.34 kg/m^3 (figure 1 and table 4) which was 52.7% of the standard reference rhizosphere weight of 406.44 kg/m^3. The total quantity of available mineral nitrogen at the 0 to 24 inch depths peaked during May at 99.35 lbs/ac which was just a little shy of the threshold quantity of 100 lbs/ac (table 6). The values of available mineral nitrate and ammonium at each incremental depth from 0 to 24 inches were lower during the growing season months June to October than the peak values during May indicated robust grass root growth and activity at each incremental depth from 0 to 24 inches during all of the growing season months June to October (table 6). A rhizosphere weight of just over 50% of the standard reference rhizosphere weight on the twice-over rotation grazed pastures was able to mineralize nearly 100 lbs/ac of total available mineral nitrogen.

Native grass herbage biomass on the nongrazed pasture increased 59.6% and basal cover increased 33.3% during 6 years (tables 7 and 8). From year 1 to year 6, native grass herbage biomass production increased 175.2% and basal cover increased 153.3% on the grazed pastures (tables 9 and 10). During 6 years on the nongrazed pasture, domesticated grass herbage biomass increased 27.3% and basal cover increased 24.3% (tables 7 and 8). Domesticated grasses on the grazed pastures decreased herbage biomass 28.2% during year 5 but during year 6 herbage biomass increased 32.1% because of the reduced stocking rate. Basal cover of domesticated grasses on the grazed pastures increased 43.3% (tables 9 and 10). The increase in herbage biomass and basal cover of native grasses and domesticated grasses on the nongrazed treatment from year 3 to year 6 was primarily the increase in precipitation. The increase in domesticated grass herbage biomass and basal cover on the grazed pastures was the increase in precipitation and the decrease in stocking pressure. The huge increase in native grass herbage biomass and basal cover on the grazed pastures was caused by the great increase in available mineral nitrogen mineralized by the increased biomass of rhizosphere organisms.

The biologically effective twice-over rotation grazing treatment coordinated partial defoliation by grazing graminivores with phenological growth stages of grass plants which provided the biological requirements of all the above and belowground components of the grassland

ecosystem activating the biogeochemcial processes performed by the rhizosphere organisms and the physiological mechanisms within the grass plants. After six growing seasons, the rhizosphere weight had increased 175% which was 53% of the long-term standard reference weight of 406.4 kg/m^3, and the plant community composition of native grass herbage biomass increased 99% and basal cover increased 69%. Neither the weight of the rhizosphere nor the composition of the plant community had been fully restored in 6 years. However, the necessary biogeochemical processes and physiological mechanisms had been activated in order for the ecosystem restoration to continue if the protocol for the twice-over rotation strategy is followed at a stocking rate of 80% to 100% of the assessed level (Manske 2012c)

Discussion

The mixed grass prairie ecosystems on the study area degraded because the previous management with no grazing was designed for an intended "use" that did not meet the biological requirements of the perennial native grass plants and the rhizosphere organisms and was detrimental to the biogeochemical processes. The use of rangeland natural resources should not be the objective of management. The management should be the means to accomplish the uses.

Ecosystem processes functioned at regressive degrees less than potential level each growing season that the rangeland was managed with the traditional concept that assumes one use can be substituted for another use. Soon after the first ecosystem process failed to function properly, the other belowground processes and mechanisms began to deteriorate. The native grass live root biomass decreased (Whitman 1974), the physiological mechanisms within grass plants diminished, the ecosystem biogeochemical processes declined, and the competitiveness of grass plant resource uptake deteriorated (Manske 2011b).

The reduction of live root surface area caused a decrease in active root length for interaction with symbiotic rhizosphere organisms and caused a decrease in absorption of water and essential nutrients from the soil. Reduction of active root biomass and diminishment of grass plant health vigor resulted in a loss of resource uptake efficiency and a suppression of the competitiveness of grass plants to take up mineral nitrogen, essential elements, and soil water (Li and Wilson 1998, Kochy 1999, Kochy and Wilson 2000, Peltzer and Kochy 2001). The loss of

active root length was a contributing factor in the reduction of rhizosphere biomass. The primary cause for the reduction in rhizosphere biomass was, however, the great reduction in the quantity of carbohydrates exuded from the grass roots into the rhizosphere zone. The antagonistic traditional practices of no grazing greatly reduced the quantity of short carbon chain energy exuded from the grass roots into the rhizosphere; the low amount of simple carbon compounds from leakage was not enough to sustain an adequate rhizosphere biomass. The small biomass of rhizosphere organisms only could mineralized small quantities of nitrogen and other essential elements (Coleman et al. 1983, Klein et al. 1988).

The decreased amounts of available mineral nitrogen below 100 lbs/ac in the ecosystem caused reductions in native grass herbage biomass production (Wight and Black 1972, 1979) and caused decreases in native grass density (basal cover). As degradation continued, numerous bare spaces between native grass plants were created in the plant communities. The open spaces were ideal habitat for growth of opportunistic domesticated grass species. The composition of grass species changed with decreases in the desirable native species and increases in the less desirable domesticated species.

Standing dead leaves accumulated (Brand and Goetz 1986) as ecosystem deterioration progressed. The accumulation of live and standing dead leaves of domesticated grasses reduced light penetration greatly. Reduced sunlight to native grasses caused reduced rates of photosynthesis, decreased rates of herbage production, and increased rates of leaf senescence (Langer 1972, Briske and Richards 1995) decreasing native grass composition further. Great quantities of standing dead material did not make contact with soil preventing decomposition through microbial activity and causing litter to build up into a thick mulch layer. The thick mulch modified soil temperatures, inhibited water infiltration, and tied up carbon and nitrogen (Wright and Bailey 1982; Manske 2000, 2011b). Native grasses were further inhibited by deficiencies of soil water, cool soil temperatures during spring, and reduced ecosystem nutrients caused by thick mulch.

The change in plant composition from desirable native grasses to less desirable domesticated grasses was the visible symptom of ecosystem degradation; the fundamental degradation of the ecosystem was the reduction of rhizosphere biomass, the reduction of biogeochemical processes, the reduction of available mineral nitrogen below 100

lbs/ac, and the reduction in availability of all the other essential elements. The degree of the aboveground plant species deterioration lagged behind the degree of degradation of the belowground ecosystem processes and mechanisms (Manske 2011b).

There is a major fundamental problem with traditional concepts that manage renewable natural resources from the perspective of their use or for the product removed. Management of renewable resources for a use narrowly considers only a few factors directly related to that use or product, and neglects to address the needs of all the other components required for the ecosystems to function at potential levels. The renewable natural resources (rangelands, grasslands, croplands, forestlands, and fisheries) have all been managed traditionally for their use. The ecosystem processes that renew the renewable natural resources have regressed and are functioning at subpotential levels. The declining production from the worlds renewable resources is a symptom of degraded ecosystem processes that have resulted from management for a use.

Table 3. Precipitation in inches for growing season months for 2006-2011, Richardton, North Dakota.

	Apr	May	Jun	Jul	Aug	Sep	Oct	Growing Season
Long-term mean (1971-2000)	1.75	2.49	3.39	2.27	1.88	1.60	1.41	14.79
2006	2.53	0.60	0.37	0.79	1.40	2.33	1.40	9.42
% of LTM	144.57	24.10	10.91	34.80	74.47	145.63	99.29	63.69
2007	1.04	3.57	2.22	0.44	1.57	1.29	0.62	10.75
% of LTM	59.43	143.37	65.49	19.38	83.51	80.63	43.97	72.68
2008	0.45	1.32	3.93	2.04	0.56	1.70	1.45	11.45
% of LTM	25.71	53.01	115.93	89.87	29.79	106.25	102.84	77.42
2009	0.59	0.85	3.09	2.82	0.53	1.67	2.08	11.63
% of LTM	33.71	34.14	91.15	124.23	28.19	104.38	147.52	78.63
2010	0.71	3.29	4.35	1.42	0.90	2.30	0.46	13.43
% of LTM	40.57	132.13	128.32	62.56	47.87	143.75	32.62	90.80
2011	2.01	169.56	1.76	4.06	2.07	0.96	1.35	17.15
% of LTM	114.86	6809.44	51.92	178.85	110.11	60.00	95.74	115.96
2006-2011	1.22	2.43	2.62	1.93	1.17	1.71	1.23	12.31
% of LTM	69.71	97.59	77.28	85.02	62.23	106.88	87.23	83.23
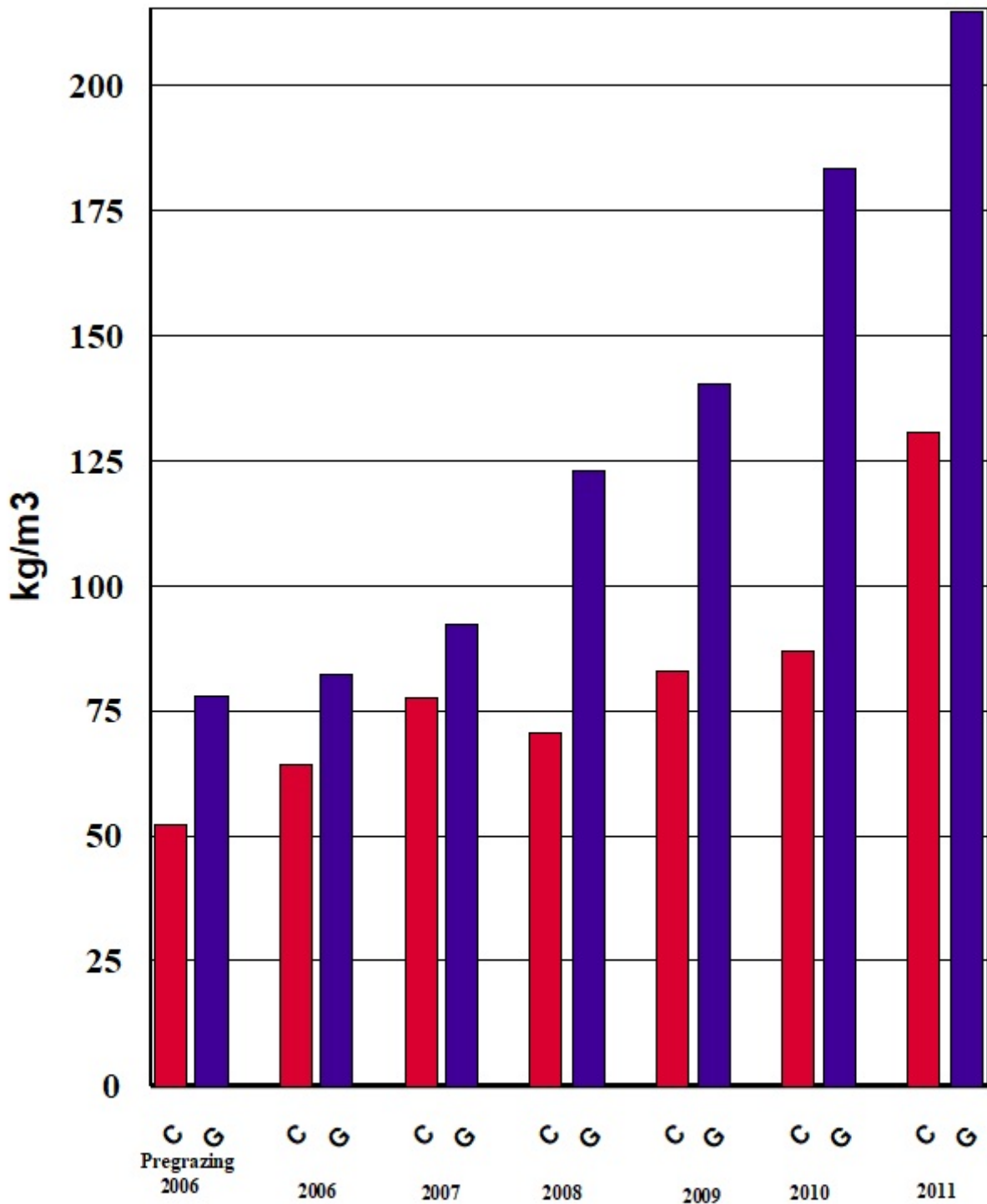
Figure 1. Rhizosphere weight (kg/m3) for the control pasture (red) and grazed pastures (blue) during six years of twice-over rotation management, 2006-2011.

Table 4. Rhizosphere weight (kg/m^3) for the nongrazed control pasture and grazed pastures during six years of twice-over rotation management.

	Control Pasture kg/m^3	Grazed Pastures kg/m^3	% Difference
Pregrazing	52.23	77.99	49.32
Year 1	64.24x	83.28x	29.64
Year 2	77.82x	92.22x	18.50
Year 3	70.67y	122.61x	73.50
Year 4	82.88y	140.32x	69.31
Year 5	86.85y	183.00x	110.71
Year 6	130.56y	214.34x	64.17

Means in the same row and followed by the same letter (x, y) are not significantly different (P<0.05).

Table 5. Mean mineral nitrogen, nitrate (NO$_3$) and ammonium (NH$_4$), in lbs/ac in incremental depths to 24 inches during growing season months on silty ecological sites of the nongrazed control, 2013-2014.

Soil Depth (inches)	May	Jun	Jul	Aug	Sep	Oct
NO$_3$ nitrate						
0-6	13.25	13.00	8.75	6.50	5.25	7.50
6-12	9.75	6.75	6.00	5.00	4.25	4.25
12-24	7.69	10.00	19.00	11.00	4.00	4.00
0-24	30.69	29.75	33.75	22.50	13.50	15.75
NH$_4$ ammonium						
0-6	19.99	11.83	16.24	12.40	13.79	13.63
6-12	12.32	11.18	12.24	11.26	10.85	12.16
12-24	8.24	13.14	16.16	12.07	12.40	3.65
0-24	40.55	36.15	44.64	35.73	37.04	29.44
NO$_3$ + NH$_4$						
0-6	33.24	24.83	24.99	18.90	19.04	21.13
6-12	22.07	17.93	18.24	16.26	15.10	16.41
12-24	15.93	23.14	35.16	23.07	16.40	7.65
0-24	71.24	65.90	78.39	58.23	50.54	45.19

Table 6. Mean mineral nitrogen, nitrate (NO_3) and ammonium (NH_4), in lbs/ac in incremental depths to 24 inches during growing season months on silty ecological sites of the twice-over rotation grazed treatment, 2013-2014.

Soil Depth (inches)	May	Jun	Jul	Aug	Sep	Oct
NO_3 nitrate						
0-6	29.63	10.75	7.63	15.75	9.63	7.75
6-12	11.38	4.88	4.88	4.38	4.25	4.63
12-24	9.50	6.00	5.00	8.00	5.50	4.00
0-24	50.50	21.63	17.51	28.13	19.38	16.38
NH_4 ammonium						
0-6	20.21	14.44	14.77	16.88	19.00	14.81
6-12	14.20	10.32	13.96	15.46	12.57	12.52
12-24	14.45	11.67	14.44	11.99	13.30	3.88
0-24	48.85	36.42	43.17	44.33	44.87	31.21
$NO_3 + NH_4$						
0-6	49.83	25.19	22.39	32.63	28.62	22.56
6-12	25.57	15.20	18.83	19.84	16.82	17.15
12-24	23.95	17.67	19.44	19.99	18.80	7.88
0-24	99.35	58.06	60.66	72.46	64.24	47.59

Table 7. Mean herbage biomass (lbs/ac) for nongrazed silty native rangeland sites, 2006-2011.

Pastures NG Biotype	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Domesticated	1833.85	1791.31	1320.16	1779.62	1468.69	2333.98
Native Grasses	132.32	236.81	142.46	304.91	283.71	211.22
Upland Sedges	25.91	22.27	20.29	49.44	30.48	11.82
Forbs	128.75	75.97	27.02	116.81	238.65	185.43
Standing Dead	1381.12	708.48	928.70	499.32	432.54	1229.02
Litter	2452.03	2131.29	2521.86	1946.39	1476.03	3178.78

Table 8. Basal cover (%) for nongrazed silty native rangeland sites, 2006-2011.

Pastures NG Biotype	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Domesticated	12.35	19.95	11.20	15.30	23.60	15.35
Native Grasses	0.90	3.10	3.95	1.25	1.90	1.20
Upland Sedges	2.00	2.20	1.90	2.35	1.75	1.05
Forbs	0.80	0.30	0.10	0.10	1.15	0.20
Litter	83.95	74.45	82.85	81.00	71.60	82.20

Table 9. Mean herbage biomass (lbs/ac) for grazed silty native rangeland sites, 2006-2011.

Pastures TOR Biotype	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Domesticated	954.70	1156.08	310.77	547.59	685.72	1261.24
Native Grasses	206.09	342.40	211.74	444.27	346.76	567.07
Upland Sedges	287.58	264.50	266.99	382.80	334.68	245.17
Forbs	122.28	77.06	35.17	94.50	357.87	100.11
Standing Dead	853.06	491.99	420.37	107.40	363.68	509.77
Litter	1479.24	1030.31	1114.80	610.79	473.94	898.17

Table 10. Basal cover (%) for grazed silty native rangeland sites, 2006-2011.

Pastures TOR Biotype	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
Domesticated	4.80	5.35	4.08	6.20	6.88	6.88
Native Grasses	2.55	8.66	6.81	7.61	7.66	6.46
Upland Sedges	7.75	10.83	10.75	11.05	12.70	9.55
Forbs	0.45	0.43	0.20	0.45	3.05	0.50
Litter	84.43	74.75	78.18	74.10	69.73	76.63

B. 1937-2011 Study, Seventy Five Years of Nongrazing

The human population of western North Dakota greatly increased during 1898 to 1915 primarily because of the Homestead Act of 1862 transferred title for 160 acres (64.8 hectors) of surveyed public domain land from the US Government to private citizens. However, during the late 1920's and early 1930's economic depression, severe drought conditions, and low agricultural commodity prices created extreme hardships for homesteaders. The Land Utilization Project and resettlement plan of 1935 permitted the US Government to repurchase 1,104,789 acres (405,000 hectors) of submarginal homestead land in North Dakota (Hibbard 1965, Carstensen 1968, Manske 1994b, 2008a) for three designated specific purposes: for grazing use, for recreation use, and for wildlife use on three different identified land areas. The Bankhead-Jones Farm Tenant Act of 1937 provided for the implementation of followup conservation and utilization programs and development of improved practices of management of the repurchased grasslands. The USDA Agriculture Resettlement Administration authorized the establishment of experimental rangeland management laboratory areas by North Dakota Agricultural Experiment Station on the Little Missouri River Badlands (Whitman 1953). In 1936, Dr. Warren C. Whitman established four two-way rangeland reference areas that included a livestock exclosure and a similar area exposed to livestock grazing on sandy, shallow, silty, and overflow ecological sites (Hanson and Whitman 1938) and initiated the study to evaluate the effects to rangeland ecosystems caused by grazing and nongrazing.

This ongoing long-term project monitors changes in rhizosphere biomass, plant root biomass, soil mineral nitrogen, herbage biomass production, and plant species composition. During the growing season of 2011, the effects from long-term nongrazing after 75 years was compared to the effects from moderately stocked, 7 to 8 month from 1 May through 31 December, seasonlong grazing treatments, with the grazing season shortened because of inclement weather conditions during most years (Manske 2013).

Procedure

Rhizosphere biomass was collected on the grazed and nongrazed treatments of each ecological site by three replicated soil cores (7.6 cm X 10.2 cm) using a humane soil beastie catcher (Manske and Urban 2012a). The fresh rhizosphere material, which included the rhizosphere organisms, the active plant roots, and the adhered soil particles, was separated from matrix soil by meticulous excavation with fine hand tools and reported in kg/m^3. Belowground plant root biomass was collected on the grazed and nongrazed treatments of each ecological site by two replicated soil cores 7.6 cm (3 in) in diameter and 10.2 cm (4 in) in depth. Root material was separated from soil in a water bath assisted with gentle manual agitation, placed in labeled paper bags of known weight, oven dried at 140° F (60° C), and weighed. Soil mineral nitrogen, nitrate and ammonium, was sampled on the grazed and nongrazed treatments of each ecological site by three replicated soil cores collected using a Veihmeyer soil tube and frozen. Analysis of soil core samples for available mineral nitrogen (NO_3, NH_4) was conducted by the North Dakota State University Soil Testing Laboratory using wet chemistry methods. Changes in vegetation composition over time were described using the 'range condition index'. Range condition index is the percent similarity of the percent composition of the dry weights of major plant species and categories of minor species on a current ecological site compared to the hypothetically determined standards of the percent composition of the dry weights of the major and minor species for that same plant community at its best biological potential. Index values of 80% and greater are considered to be similar. Index values greater than 50% are degrees of similarity. Index values of less than 50% are degrees of dissimilarity. And index values of 20% and less are considered to be dissimilar. Aboveground herbage biomass was collected by the standard clipping method (Cook and Stubbendieck 1986) sorted in the field into domesticated grasses, cool season grasses, warm season grasses, upland sedges, forbs, standing dead, and litter, and oven dried. Plant species basal cover was determined by the ten-pin point frame method (Cook and Stubbendieck 1986) and sorted into domesticated grasses, cool season grasses, warm season grasses, upland sedges, forbs, and litter. Density of forbs was determined by counting individual stems of each forb species rooted inside twenty five $0.1m^2$ quadrats. Density of shrubs were collected by counting individual plants of each shrub species rooted inside twenty five $1.0 \, m^2$ quadrats. A species present list of shrubs, cacti, and trees were also completed. These procedures adequately represented the shrub component of the grazed plant communities, however, because of the great extent and high number of woody species growing inside the exclosures, these methods greatly undersampled the woody species growing inside the exclosures.

Surface area of the woody infected shrub and tree map units and the nonwoody grass map units were determined in acres as digital data in ArcGIS by occular assessment of USDA National Agriculture Imagery Program 2009 orthoimages as displayed by Google Earth was conducted by the Dickinson State University Department of Agriculture and Technical Studies (Manske 2013).

Results

The standard reference rhizosphere weight of 406.4 kg/m^3 was recorded on silty ecological sites managed long-term (24 years) with a twice-over rotation grazing strategy. All of the measured rhizosphere weights on the grazed seasonlong and nongrazed treatments from the four ecological sites of this study were lower than 45% of the standard reference rhizosphere weight after 75 years of management indicating that all of these treatments were detrimental, but not lethal, to rhizosphere organisms (figure 2 and table 11). The rhizosphere weights on the nongrazed treatments of the sandy and overflow ecological sites were substantially lower than the rhizosphere weights on the grazed seasonlong treatments indicating that the nongrazed treatments were more detrimental to rhizosphere organisms than the grazed seasonlong treatments. The lowest rhizosphere weight on a seasonlong treatment was on the silty ecological site which was similar to the rhizosphere weight on the nongrazed treatment of the silty ecological site indicating that the long-term stocking rate on the grazed seasonlong treatment was a little heavier or the grazing season was longer by starting earlier and coming off later than the grazed seasonlong treatments of the sandy and overflow ecological sites and that the heavily grazed treatment and nongrazed treatment of the silty ecological site had similar detrimental effects on the rhizosphere organisms. The rhizosphere weight on the grazed seasonlong treatment of the shallow ecological site was lower than the rhizosphere weight on the nongrazed treatment indicating that a relatively recent change in the grazing management was more detrimental to the rhizosphere organisms than the previous grazing treatment and the nongrazed treatment of the shallow ecological site (figure 2 and table 11).

The weight of the grass roots on the nongrazed treatments of all four ecological sites were substantially lower than the weight of the grass roots on the grazed seasonlong treatments. When grazing graminivores are removed from native rangeland ecosystems, the stimulation effects from partial defoliation of aboveground leaf material stops, causing the ecosystem biogeochemical processes and physiological mechanisms within grass plants to decrease resulting in great reductions in above and belowground grass biomass growth, hence, extremely low root weights on the nongrazed treatments (table 12).

Available soil mineral nitrogen is the major limiting factor of herbage growth on native rangeland ecosystems (Wight and Black 1979). Deficiencies in mineral nitrogen limit herbage production more often than water in temperate grasslands (Tilman 1990). A minimum rate of mineralization that supplies 100 lbs/ac (112 kg/ha) of mineral nitrogen is required to sustain herbage production at biological potential levels on rangelands (Wight and Black 1972). Wight and Black (1972, 1979) determined that the processes associated with precipitation (water) use efficiency in grass plants were not fully activated unless 100 lbs/ac of mineral nitrogen was available. When less than 100 lbs/ac of available mineral nitrogen occurred in rangeland ecosystems, the weight of herbage produced per inch of precipitation received was reduced an average of 49.6% below the weight of herbage produced per inch of precipitation on rangeland ecosystems that had 100 lbs/ac of mineral nitrogen or greater (Wight and Black 1979). Manske (2010a, b) found two major plant mechanisms that performed the compensatory physiological processes within grass plants and the processes for vegetative reproduction by tillering could not be activated when less than 100 lbs/ac of mineral nitrogen was available and were fully activated when greater than 100 lbs/ac of mineral nitrogen was available.

The quantity of organic nitrogen mineralized in an ecosystem is dependent on the biomass and activity levels of the microorganisms in the rhizosphere. The quantity of mineral nitrogen immobilized in an ecosystem is dependent on the rate of plant growth. The quantity of available soil mineral nitrogen, nitrate and ammonium, varies with changes in rhizosphere microorganism biomass and the changes in plant growth rate during the growing season (Whitman 1975). The quantity of available mineral nitrogen, nitrate and ammonium, on the grazed seasonlong and nongrazed treatments were at deficiency levels on all four ecological sites (table 13). Available mineral nitrogen was lower on the nongrazed treatments than on the grazed treatments of the sandy, silty, and overflow ecological sites as a result of the lower rhizosphere weight. Available mineral nitrogen was lower on the grazed seasonlong treatment than on the nongrazed treatment of the shallow ecological site as a result of a recent detrimental change in grazing management (table 13).

The ecosystem deterioration of the aboveground vegetation was severe on the nongrazed treatments of the four ecological sites. The native graminoids greatly decreased. The decrease in herbage biomass ranged from 32% to 96% with a mean of 58.7% for the cool season grasses, from 63% to 99% with a mean of 86.5% for the warm season grasses, and from 24% to 95% with a mean of 55.6% for the upland sedges (tables 14, 15, 16, 17, and 22). The decrease in basal cover ranged from 49% to 100% with a mean of 80.2% for the cool season grasses, from 85% to 100% with a mean of 92.7% for the warm season grasses, and from an increase of 2% to decrease of 92% with a mean decrease of 33.5% for the upland sedges (tables 14, 15, 16, 17, and 22).

As a result of the ecosystem deterioration on the nongrazed treatments, the domesticated grasses, mainly Kentucky bluegrass, increased, with increases of herbage biomass ranging from 100% to 1,647% with a mean of 481.3% and with increases of basal cover from a decrease of 33% to an increase of 3,700% with a mean increase of 56.2% (tables 14, 15, 16, 17, and 22). The herbage biomass of the standing dead greatly increased that ranged from a small decrease of 35% to an increase of 10,434% with a mean increase of 2,772.7% (tables 14, 15, 16, 17, and 22). The herbage biomass of the litter increased ranging from 131% to 811% with a mean of 470.1% and litter basal cover increase ranged from 19% to 32% with a mean of 23.2% (tables 14, 15, 16, 17, and 22).

The change in forbs, as one category, was negative and positive as a result of the ecosystem deterioration of the nongrazed treatments. The changes in herbage biomass ranged from a decrease of 58% to an increase of 72% with a mean of a 4.0% increase. The changes in basal cover ranged from a decrease of 59% to an increase of 167% with a mean of 4.9% increase (tables 14, 15, 16, 17, and 22). The number of forb species present greatly decreased on the nongrazed treatments of all four ecological sites with a mean decrease of 53.1% (table 22).

The similarity index of range condition on the four grazed ecological reference areas ranged from 52.7% to 68.4% with a mean of 58.6%, low good condition, indicating that the composition of the current grazed plant communities were slightly similar to the composition of the hypothetical standard historical plant communities (table 18). The composition of the aboveground vegetation on the grazed shallow ecological site has not yet deteriorated to the extent of the deterioration of the belowground components. The similarity index of range condition

on the four nongrazed ecological reference areas ranged from 19.1% to 36.8% with a mean of 28.0%, low fair condition, indicating that the composition of the current nongrazed plant communities were nearly dissimilar, with the nongrazed areas on the shallow and silty ecological sites dissimilar, to the composition of the hypothetical standard historical plant communities (table 18). The current plant communities on the nongrazed areas had degraded from the hypothetical standard plant communities 51.3% greater than the amount of degradation that had ocurred on the current plant communities on the grazed areas after 75 years (table 22).

With forbs separated into three categories of late stage, mid stage, and early stage of succession, the results of the ecosystem deterioration of the nongrazed treatments on the four ecological sites showed different effects. The late stage forb density decreased on the sandy, shallow, and overflow sites and increased on the silty site (table 19) with a mean decrease of 52.0% (table 22). The mid stage forb density increased on the sandy and shallow sites and decreased on the silty and overflow sites (table 19) with a mean increase of 35.9% (table 22). The early stage forb density decreased on the sandy, shallow and silty sites and increased on the overflow site (table 19) with a mean decrease of 60.2% (table 22). The late stage forbs decreased as a result of the reduced ecosystem biogeochemical processes. The early stage forbs decreased as a result of the reduced amount of sunlight reaching the soil level because of the increased biomass of standing dead and litter. The mid stage forbs increased because they were able to survive without the benefits of the ecosystem biogeochemical processes.

The greatest visual impact occurring as a result of the ecosystem deterioration of the nongrazed treatment on all four ecological sites was the huge increase of woody shrubs, cacti, and trees with an increase of 254.2% in species present (tables 20 and 22) and an increase of 62.4% of the land area occupied by woody plants (tables 21 and 22).

The nongrazed and grazed seasonlong areas had not been burned for at least 75 years and most likely for a great deal of time longer. Both the nongrazed and grazed treatments had a lack of fire for a long time, however, the woody plant infestation only occurred on the nongrazed treatments. The basal cover of native graminoids was 24.2% on the grazed treatments and 7.0% on the nongrazed treatments for a reduction of 71.1%. This reduction in basal cover along with the reduction in above and belowground plant biomass has caused a great

reduction in competitiveness in the native graminoids. The belowground resources of nutrients and soil water that had previously been used by the healthy robust graminoids, but no longer consumed by the smaller, less vigorous degraded graminoids, are taken up by the encroaching woody species resulting in proportional increases in woody plant biomass production (Kochy and Wilson 2000). As woody stem density increased, the competition shifted to primarily the aboveground resources of light; under these different degraded conditions, woody species have the advantage and the graminoids are strongly suppressed (Kochy and Wilson 2000). The great increase of woody plants into grassland ecosystems has not been the result of fire suppression. The increased woody plant infestation has been caused by the greatly reduced competitiveness of the graminoids and the degradation of the biogeochemical processes in the grass plant communities caused by the removal of grazing by large graminivores.

Grassland ecosystem deterioration occurs first in the belowground components. The rhizosphere microbes decrease in biomass, the biogeochemical processes cycle less essential elements and the quantity of available mineral nitrogen decreases. Consequently, the aboveground components deteriorate. The native grasses and forbs decrease in herbage biomass and density followed by increases in herbaceous invader species and then by increases in woody invader species.

Discussion

Removal of cattle grazing does not promote development of stable climax plant communities and does not preserve prairie grasslands in perpetuity. Grassland communities deprived of large grazing graminivores decline steadily into unhealthy disfunctional ecosystems with severe reductions of native grasses, considerable decreases of desirable forbs, enormous increases of introduced domesticated grasses, remarkable increases of woody shrubs and trees, and excessive increases of standing dead and litter.

Grass plants have four major mechanisms: compensatory physiological mechanisms, efficient water use mechanisms, mechanisms for vegetative reproduction by tillering, and competitive resource uptake mechanism that permit growth and functionality. The ecosystem has numerous biogeochemical processes performed by achlorophyllous soil microorganisms that provide available essential elements needed for growth of all ecosystem organisms and these important microbes

depend on grass plants to fix their energy. The grazing graminivores activate plant mechanisms and ecosystem processes with partial defoliation and depend on nutritious forage produced by the grass plants. All of there complex mechanisms and processes are interconnected and interdependent that require an adequate supply of essential elements with a threshold of mineral nitrogen to be available at 100 lbs/ac and require activation by removal of 25% to 33% of lead tiller material at phenological growth stages between the three and a half new leaf stage and the flower stage by large grazing graminivores. If any of the numerous processes are not functioning at potential level, the ecosystem does not function at potential level. Management of grassland ecosystem must meet the biological and physiological requirements of all the biotic components and also stimulate the biogeochemical processes that cycle and recycle the abiotic components.

The key to control of undesirable woody shrub and tree and herbaceous plant encroachment into grassland ecosystems is to regain the competitive advantage of the native grasses by restoration of the mechanisms and processes in the prairie ecosystem that results from proper biologically effective partial defoliation coordinated with grass phenological growth stages by large grazing graminivores which are an indispensable biotic component of grasslands.
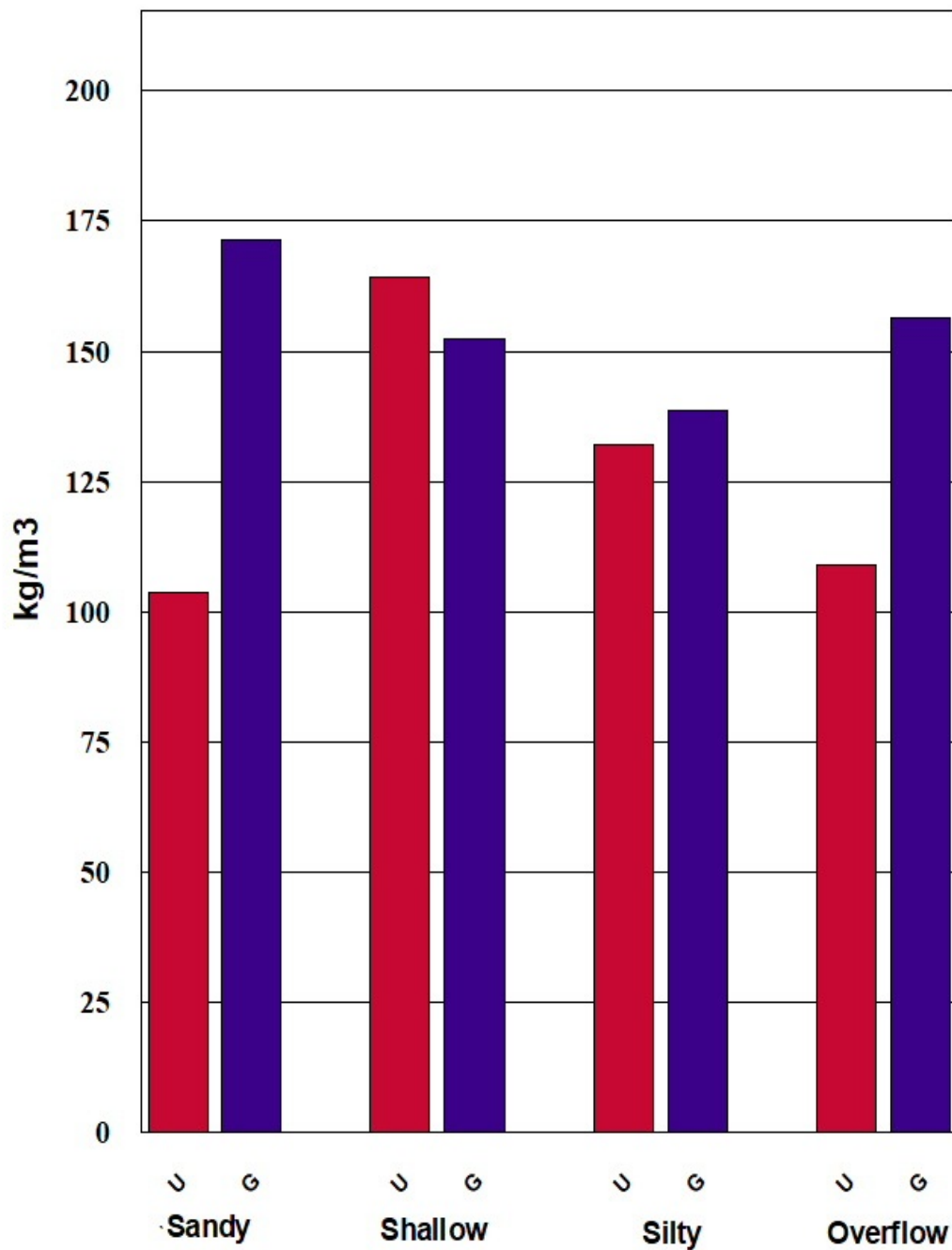
Figure 2. Rhizosphere weight (kg/m3) for the ungrazed
exclosure (red) and grazed area (blue) on the
ecological site reference areas in the Little Missouri
River Badlands, 1936-2011.

Table 11. Rhizosphere biomass (kg/m^3) for native rangeland on the four reference areas after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

	Rhizosphere Biomass (kg/m^3)		
Ecological Sites	Grazed	Exclosure	% Difference
Sandy	171.01	103.74	-39.34
Shallow	152.59	163.88	7.40
Silty	138.63	132.08	-4.72
Overflow	156.55	109.21	-30.24

Table 12. Belowground biomass (kg/m^3) of roots for native rangeland on the four reference areas after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

	Belowground Biomass (kg/m^3)		
Ecological Sites Component	Grazed	Exclosure	% Difference
Sandy			
Root	32.48	14.35	-55.82
Shallow			
Root	34.53	26.87	-22.18
Silty			
Root	24.82	16.73	-32.72
Overflow			
Root	14.35	11.12	-22.51

Table 13. Soil available mineral nitrogen (nitrate NO_3 and ammonium NH_4) for native rangeland on the four reference areas after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

Ecological Sites Mineral Nitrogen	Available Mineral Nitrogen (lbs/ac)		
	Grazed	Exclosure	% Difference
Sandy			
Nitrate NO_3	6.33	7.33	15.80
Ammonium NH_4	25.19	16.19	-35.73
$NO_3 + NH_4$	31.52	23.52	-25.38
Shallow			
Nitrate NO_3	6.00	6.00	0.00
Ammonium NH_4	27.12	35.26	30.01
$NO_3 + NH_4$	33.12	41.26	24.58
Silty			
Nitrate NO_3	11.00	10.00	-9.09
Ammonium NH_4	35.62	32.02	-10.11
$NO_3 + NH_4$	46.62	42.02	-9.87
Overflow			
Nitrate NO_3	9.67	14.33	48.19
Ammonium NH_4	48.56	36.70	-24.42
$NO_3 + NH_4$	58.23	51.03	-12.36

Table 14. Herbage biomass (lbs/ac) and Basal cover (%) for native rangeland on the sandy reference area after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

Sandy Ecological Site	Herbage Biomass (lbs/ac)			Basal Cover %		
Biotype	Grazed	Exclosure	% Difference	Grazed	Exclosure	% Difference
Domesticated	0.00	1158.89	100.00	0.15	5.70	3700.00
Cool Season	803.51	239.77	-70.16	3.25	1.65	-49.23
Warm Season	468.84	102.04	-78.24	14.25	2.20	-84.56
Upland Sedges	505.23	203.38	-59.75	11.60	7.05	-39.22
Forbs	147.72	156.99	6.28	0.85	0.35	-58.82
Standing Dead	353.23	230.49	-34.75	-	-	-
Litter	104.90	791.38	654.41	69.85	83.05	18.90

Table 15. Herbage biomass (lbs/ac) and Basal cover (%) for native rangeland on the shallow reference area after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

Shallow Ecological Site	Herbage Biomass (lbs/ac)			Basal Cover %		
Biotype	Grazed	Exclosure	% Difference	Grazed	Exclosure	% Difference
Domesticated	0.00	1299.47	100.00	0.00	5.80	100.00
Cool Season	583.01	22.84	-96.08	7.95	0.90	-88.68
Warm Season	215.51	3.57	-98.34	13.90	0.25	-98.20
Upland Sedges	287.58	154.85	-46.15	10.30	10.50	1.94
Forbs	183.40	316.12	72.37	2.70	1.55	-42.59
Standing Dead	3.57	376.07	10434.17	-	-	-
Litter	70.65	643.67	811.07	62.65	75.90	21.15

Table 16. Herbage biomass (lbs/ac) and Basal cover (%) for native rangeland on the silty reference area after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

Silty Ecological Site	Herbage Biomass (lbs/ac)			Basal Cover %		
Biotype	Grazed	Exclosure	% Difference	Grazed	Exclosure	% Difference
Domesticated	692.91	1427.91	106.07	17.45	11.65	-33.24
Cool Season	506.66	101.33	-80.00	3.25	0.00	-100.00
Warm Season	201.24	0.71	-99.65	2.75	0.00	-100.00
Upland Sedges	149.14	113.46	-23.93	5.70	1.15	-79.82
Forbs	124.88	149.86	20.00	0.90	2.40	166.67
Standing Dead	112.04	494.52	341.38	-	-	-
Litter	563.03	2162.21	284.03	64.25	84.80	31.98

Table 17. Herbage biomass (lbs/ac) and Basal cover (%) for native rangeland on the overflow reference area after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

Overflow Ecological Site	Herbage Biomass (lbs/ac)			Basal Cover %		
Biotype	Grazed	Exclosure	% Difference	Grazed	Exclosure	% Difference
Domesticated	12.13	211.94	1647.24	0.20	4.65	2225.00
Cool Season	1559.93	1061.84	-31.93	18.60	4.00	-78.49
Warm Season	57.80	21.41	-62.96	4.50	0.15	-96.67
Upland Sedges	135.58	7.14	-94.73	0.60	0.05	-91.67
Forbs	239.06	99.90	-58.21	1.20	0.65	-45.83
Standing Dead	300.43	1351.56	349.88	-	-	-
Litter	648.66	1497.85	130.91	74.90	90.50	20.83

Table 18. Similarity index of range condition for the grazed areas and nongrazed areas on the four reference areas after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

Ecological Reference Area	Similarity Index of Range Condition		
	Grazed Area	Nongrazed Area	% Difference
Sandy	59.8	36.8	-38.4
	Low Good	Low Fair	
Shallow	68.4	20.1	-70.6
	Good	Poor	
Silty	53.6	19.1	-64.4
	Low Good	Poor	
Overflow	52.7	36.1	-31.6
	Low Good	Low Fair	
Mean of Four	58.6	28.0	-51.3
	Low Good	Low Fair	

Table 19. Forb density (#/0.10 m^2) related to succession stage for native rangeland on the four reference areas after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

Ecological Sites Treatments	Late Stage Forbs	Mid Stage Forbs	Early Stage Forbs	Total Live Forbs
Sandy				
Grazed	5.28	0.20	0.36	5.88
Exclosure	1.28	2.16	0.04	3.48
% Difference	-75.76	980.00	-88.89	-40.82
Shallow				
Grazed	22.52	0.36	0.88	23.80
Exclosure	3.92	5.88	0.08	9.88
% Difference	-82.59	1533.33	-90.91	-58.49
Silty				
Grazed	5.12	3.20	2.16	10.48
Exclosure	12.56	0.24	0.00	12.80
% Difference	145.31	-92.50	-100.00	22.14
Overflow				
Grazed	6.48	3.92	1.52	11.92
Exclosure	1.16	2.16	1.84	5.16
% Difference	-82.10	-44.90	21.05	-56.71

Table 20. Shrubs, cacti, and trees present on the reference areas after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

Woody Plants	Sandy Graze	Sandy Exclosure	Shallow Grazed	Shallow Exclosure	Silty Grazed	Silty Exclosure	Overflow Grazed	Overflow Exclosure
Silver sagebrush				X		X	X	X
Common juniper						X		
Creeping juniper						X		
Chokecherry		X		X		X		
Skunkbush		X		X		X		
Prairie wild rose	X	X	X	X	X	X		
Buffalo berry						X		
Western snowberry		X	X	X	X	X	X	X
Great Plains yucca		X				X		
Ball cactus	X	X						
Prickly pear	X	X				X		
Green ash				X		X		X
Rocky Mtn juniper		X		X		X		
Plains cottonwood								X
Number Present	3	8	2	7	2	12	2	4

Table 21. Acreage and percent land area occupied by nonwoody grass and woody shrub and tree infested plant communities on the four reference areas after 75 years in the Little Missouri River Badlands, ND, 1936-2011.

	Exclosure	Major Plant Communities				
	Total Area	Nonwoody Plants			Woody Plants	
Ecological Sites	acres	acres	%		acres	%
Sandy	6.27	2.93	46.67		3.34	53.33
Shallow	4.90	2.15	43.90		2.75	56.10
Silty	14.10	6.52	46.23		7.58	53.77
Overflow	2.90	0.40	13.80		2.50	86.20

Determined by Arc GIS mapping procedures.

Table 22. Percent difference in plant community characteristics on the nongrazed exclosure area compared to the grazed area after 75 years on the four reference areas in the Little Missouri River Badlands, ND, 1936-2011.

Biotype	Sandy Ecological Sites	Shallow Ecological Sites	Silty Ecological Sites	Overflow Ecological Sites	Mean of Four Ecological Sites
Herbage Biomass					
Domesticated	100.00	100.00	106.07	1647.24	481.3
Cool Season	-70.16	-96.08	-80.00	-31.93	-58.7
Warm Season	-78.24	-98.34	-99.65	-62.96	-86.5
Upland Sedges	-59.75	-46.15	-23.93	-94.73	-55.6
Forbs	6.28	72.37	20.00	-58.21	4.0
Standing Dead	-34.75	10434.17	341.38	349.88	2772.7
Litter	654.41	811.07	284.03	130.91	470.1
Basal Cover					
Domesticated	3700.00	100.00	-33.24	2225.00	56.2
Cool Season	-49.23	-88.68	-100.00	-78.49	-80.2
Warm Season	-84.56	-98.20	-100.00	-96.67	-92.7
Upland Sedges	-39.22	1.94	-79.82	-91.67	-33.5
Forbs	-58.82	-42.59	166.67	-45.83	4.9
Litter	18.90	21.15	31.98	20.83	23.2
Forb Density					
Late Stage	-75.76	-82.59	145.31	-82.10	-52.0
Mid Stage	980.00	1533.33	-92.50	-44.90	35.9
Early Stage	-88.89	-90.91	-100.00	21.05	-60.2
Total Live	-40.82	-58.49	22.14	-56.71	-39.8
Species Present	-53.9	-50.0	-56.3	-52.9	-53.1
Shrub Density					
Species Present	166.7	250.0	500.0	100.0	254.2
% Woody Area	53.33	56.10	53.77	86.20	62.35
Range Condition					
Similarity Index	-38.4	-70.6	-64.4	-31.6	-51.3
Rank	Low Fair	Poor	Poor	Low Fair	Low Fair

C. 1978-1990 Study, Effects of Prescribed Burning on Degraded Ecosystems

Many grassland ecologists have accepted the observational concept that the occurrence of fire was the force that prevented intrusion of shrubs and trees into grasslands. However, the processes of how fire prevents woody species encroachment into prairie has not been clearly explained. Total plant kill caused by fire occurs only to species of evergreen conifers and one deciduous shrub, big sagebrush. All other deciduous trees and shrubs are temporarily set back with partial damage or top kill of aerial stems by fire. Deciduous woody species recover and replace the damaged stems with sprout growth from vegetative buds (Manske 2014d). A study of the effects from repeated prescribed burning once to four times during a thirteen year period on degraded mixed grass prairie was conducted.

The US Government repurchased 26,904 acres (10,896 ha) of submarginal homestead land in Burke and Mountrail counties of North Dakota as permitted by the Land Utilization Project and resettlement plan of 1935 for wildlife use which later became the USDI Fish and Wildlife Service, Lostwood National Wildlife Refuge (Manske 1994).

Management of Lostwood Refuge has been based on the concept of preserving wildlife habitat with little or no disturbance (idle). All grazing on the refuge was stopped between 1935 and 1940. After 1940, all of the upland acreage (about 59% of the refuge), except the areas of wetlands (20%) and the land designated as wilderness (21%), was grazed periodically using deferred seasonlong management. Some parcels were grazed only one time while other parcels were grazed as many as 22 times in the 35 year period between 1940 and 1975. Also, during this time period, a parcel of about 7,000 acre (2,833 ha) (26% of the refuge) was grazed annually with deferred seasonlong management for 4.5 to 5.0 months at low to moderate stocking rates during July through November (Smith 1988).

About 23% of the refuge land area had previously been used as cropland by homesteaders. About 35% of the cropland acres were allowed to revegetate through natural secondary succession as "go back" land. The remaining cropland parcels were managed as cropland to provide winter food for resident wildlife. In the mid 1950's, these existing cropland acres were seeded with domesticated cool season grasses, primarily smooth bromegrass and crested wheatgrass (Smith 1988).

The changes in vegetation cover was described from a series of black and white aerial photographs by Smith (1988) and summarized here. The Lostwood Wildlife Refuge increased in shrub cover from about 5% during the mid 1930's to greater than 50% in 1979. This change, however, did not occur at a uniform rate. The shrub composition in the plant community did not change much during the first 20 years. A substantial increase in shrub cover occurred between 1953 and 1969, and then, between 1969 and 1979, the western snowberry colonies expanded rapidly and invaded extensive areas of degraded grassland. As a result, over half of the refuge upland was transformed into shrubland (Smith 1988). Few trees existed on the refuge during the 1930's and 1940's, but by 1985, there were over 540 expanding aspen groves covering about 475 acres (192 ha) interspersed across the landscape located at the edges of seasonal wetlands. Over 55%, about 300 of the aspen groves had completely occupied previous wetland basins (Smith 1997).

Domesticated cool season grasses had greatly increased over five decades of vegetation change. Kentucky bluegrass was the dominant grass associated with the western snowberry colonies. Large portions of the western snowberry colonies were extremely dense and had no herbaceous understory. Decadent centers of old western snowberry colonies had been reinvaded by smooth bromegrass, quackgrass (Smith 1985a), and Canada thistle (Smith 1985b).

Remnant native grasses and forbs were present in low quantities with greatly suppressed distribution as a result of the previous long-term management concepts of no disturbance and deferred seasonlong grazing. The deferred-type management that delays grazing until after the flowering stage of grasses is known to decrease grass tiller density and grass plant competitiveness (Sarvis 1941, Manske et al. 1988).

The historical records for the region indicate that the local homesteaders had been able to suppress all fires from sometime in the late 1800's and indirectly implied that the land area that became the wildlife refuge had not been burned by wildfire or prescribed fire during the 80 year period prior to 1978 (Smith 1985b).

In 1978, refuge manager Karen Smith initiated an every-other-year prescribed burning strategy. This burning regime was designed to reduce

the invading western snowberry and the exotic domesticated cool season grasses with the intent to renovate the prairie ecosystem. Annual burns were not possible because of insufficient production of plant biomass for fuel (Smith 1985a). The refuge, except the wilderness area, was subdivided into prescribed burn management units that used trails or mowed swaths as fire breaks. Several parcels of the refuge were designated to receive no burning treatments for use as reference control areas. The prescibed burn management units received 1, 2, 3, or 4 repeated every-other-year burns during the 13 year period between 1978 and 1990. During the growing season of 1990, Manske (1992) evaluated the effects of every-other-year prescribed burning after thirteen years of treatments. This report is a summary of that study.

Procedure

Field data were collected along permanent landscape transects that included the plant communities on the summit, shoulder, back, foot, and toe slopes from 15 prescribed burn management units with an average size of 530.5 acres (214.85 ha) and 6 control management units of no burning with an average size of 436.8 acres (176.90 ha) (Manske 1992).

Endomycorrhizal fungal infection in roots was evaluated for blue grama, western wheatgrass, smooth bromegrass, and western snowberry. Three replicated soil cores were collected for each species from nearly level loam soils along the permanent landscape transects of each control and prescribed burn treatment using a golf cup cutter. In the laboratory, root samples were prepared using procedures described by Phillips and Hayman (1970) and modified by Kormanik and McGraw (1982). Percent fungal infection was assessed using a nonsystematic modification of the grid-intersect method (Giovannetti and Mosse 1980), with presence or absence (P/A) of fungal structures recorded for 100 intersected root segments viewed through a Nikon microscope.

Changes in soil microorganism activity were monitored by changes in the quantity of soil inorganic (mineral) nitrogen. Soil mineral nitrogen, nitrate (NO_3) and ammonium (NH_4), was determined from five replicated soil core samples 0-6 inches in depth, collected from nearly level loam soil sites along the permanent landscape transects using the one inch Veihmeyer soil tube during mid June and mid August from the no burn control treatment and each burn treatment with 1 to 4 burns with each sample air

dried. Analysis of soil core samples for available mineral nitrogen (NO_3 and NH_4) was conducted by the North Dakota State University Soil Testing Laboratory using wet chemistry methods (Manske 1992).

Gravimetric soil water data (Cook and Stubbendieck 1986) were collected on both the summit slopes and foot slopes of each landscape transect during June, July, and August using a Veihmeyer soil tube to 24 inches in depth.

Aboveground herbage biomass was collected during peak growth in mid July by the standard clipping method (Cook and Stubbendieck 1986), sorted in the field into grasses, sedges, forbs, shrubs, and standing dead, and oven dried. Plant species composition was determined during mid July to mid August by the plant shoot cover method (% shoot frequency) (Cook and Stubbendieck 1986), with one hundred 0.1 m^2 quadrats per landscape transect.

Results

Annual precipitation during the 13 year (1978-1990) study period averaged 15.36 inches (390.14 mm) (93.10% of LTM). Precipitation during the perennial plant growing season (April through October) averaged 12.35 inches (313.69 mm) (89.48% of LTM). A wet growing season occurred during 1984. Normal growing season precipitation occurred during 8 years. Water deficiencies occurred during 1979, 1983, 1987, and 1988 causing water stress in perennial plants resulting in restricted herbage biomass production (Manske 2007a). The inches of soil water during the growing season to 24 inches in depth on the summit slope (table 23) and the lower slope (table 24) were not significantly different among the no burn control treatment and the 1 to 4 every-other-year burn treatments (Manske 2007a).

Percent endomycorrhizal fungi infection of root segments of four major plant species, blue grama, western wheatgrass, smooth bromegrass, and western snowberry, was determined (table 25). There was no significant difference in percent fungal infection of each plant species root segments among the no burn control treatments and the 1 to 4 every-other-year burn treatments (Manske 2007a).

A problem developed with the technique in determining percent infection. All of the roots of each species collected were included in the evaluation samples. Endomycorrhizal fungi, however, do not colonize the entire root. Fungal colonization occurs

at the portions of current years' roots that are biologically active. Previous years' roots, mature root portions, and young growing root portions do not host fungal structures. Identification of biologically active root portions from mature root portions is difficult with the naked eye or low power hand lens. Therefore, the reported (table 25) percent fungal infection of root segments is primarily a factor of the proportion of biologically active root portions in relation to the amount of mature and young root portions included in the sample. Basically, the less than 100% fungal infection of the blue grama, western wheatgrass, and western snowberry root samples in table 25 should be considered to indicate the percentage of biologically active root portions within the root sample. The percent fungal infection in the root segments of blue grama, western wheatgrass, and western snowberry were not changed significantly by the no burn or the number of repeated every-other-year prescribed burn treatments. The quantity of endomycorrhizal fungal colonization in plant roots was not stimulated by the prescribed burning treatments.

The percent fungal infection data for the smooth bromegrass root segments were not applicable to standard evaluation. The smooth bromegrass root samples contained a small amount of young and mature root portions, with most of the samples appearing to consist of biologically active root portions. The large proportion of seemingly ideal biologically active root segments had effectually no fungal infection. The observed fungal infections were restricted to the root hairs. Under a microscope, the root hairs had a swollen bulb-like part where the hair attached to the root which was the site for the fungal infection. Smooth bromegrass had relatively low fungal infection in the root hairs (table 25).

The escaped smooth bromegrass plants present on the no burn and burn treatments at the Lostwood Wildlife Refuge had long aggressive rhizomes and were nonmycorrhizal which would fit the description of the Southern Type. The Southern Type smooth bromegrass germ plasm originated from the Hungarian Plains and France that have both basal leaves and stem leaves, strongly rhizomatous producing mostly rhizome tillers resulting in sod forming plants with high seed viability, strong seedling vigor, that grows rapidly early in the growing season for a short period producing greater peak aboveground herbage biomass (Manske 2017).

The quantities of available mineral nitrogen, nitrate (NO_3) and ammonium (NH_4), were unusually low on the no burn controls and the 1 to 4 every-

other-year burn treatments (table 26). Most of the nitrogen in grassland soils is in the organic form and unavailable for direct use by plants. The soil organic nitrogen must be mineralized by rhizosphere microorganisms to provide plant usable mineral nitrogen (Ingham et al. 1985). Grassland ecosystems with low biomass of rhizosphere organisms mineralize low quantities of organic nitrogen into mineral nitrogen (Coleman et al. 1983). The low quantities of mineral nitrogen (NO_3 & NH_4) available in the soils of the 1 to 4 repeated every-other-year prescribed burn treatments were not significantly changed from the low quantities of mineral nitrogen available in the soils of the control no burn treatments (Manske 2007a). The rhizosphere microorganism biomass and activity levels were not stimulated by any of the prescribed burn treatments, and the quantity of organic nitrogen mineralized into mineral nitrogen was not increased by any of the prescribed burn treatments. The low available mineral nitrogen was a primary cause for low herbage production and the reason annual burns were not possible.

The low total peak live herbage production was not different after one, two, three, and four repeated prescribed burns compared to the low live biomass produced on the no burn treatments (table 27). However, the composition of the aboveground biomass did change.

The shrub biomass contribution was 47.5% on the no burn treatments, shrub biomass decreased 83.1% after one burn and decreased 95.1% after four burns. Shrub biomass contribution was only 3.0% after four burns (table 27).

The forb biomass increased 78.0% after one burn and the contribution to total aboveground biomass was 139.7% greater than that on the no burn treatments. After two and three burns, the weedy forbs decreased and the prairie perennial forbs improved. Forb biomass increased 4.4% after four burns and the biomass contribution to the total biomass was 35.3% greater after four burns than that on the no burn treatments. The contribution of forbs to the total biomass was 15.0% on the no burn treatments and increased to 20.3% after four burns (table 27).

Upland sedge biomass increased 61.6% after one burn and decreased 35.1% after four burns. The contribution of upland sedges to the total biomass was 13.2% on the no burn treatments and decreased to 11.1% after four burns (table 27).

Native grass biomass decreased 24.7% after one burn and increased 109.3% after four burns. The contribution of native grass to the total biomass was 24.2% on the no burn treatments and increased to 65.6% after four burns (table 27) as a result of reduced shading and increased sunlight.

Shoot frequency of native grasses increased as a result of repeated burning. The average increase in shoot frequency was 79.6% after one, two, and three burns and increased 94.7% after four burns (table 28). However, the actual basal cover of native grasses was not well developed after four burns (Manske 2007a) because the physiological mechanisms were not activated within the grass plants.

Upland sedge shoot frequency increased an average of 58.4% after repeated burns (table 28).

Shoot frequency of the domesticated grasses decreased an average of 49.4% after one, two, and three burns and decreased 65.1% after four burns (table 28). Four burns were required to temporarily reduce domesticated grasses significantly (Manske 2007a). Shoot frequency of Kentucky bluegrass decreased an average of 36.2% after one, two, three, and four burns. Shoot frequency of Quackgrass decreased an average of 84.0% after one and two burns and decreased an average of 90.9% after three and four burns. Shoot frequency of Smooth bromegrass decreased an average of 90.0% after one and two burns and decreased an average of 96.7% after three and four burns.

Shoot frequency of perennial prairie forbs increased 39.3% after one burn (table 28) then shoot frequency averaged 7.5% above the shoot frequency on the no burn treatments after two, three, and four burns. Early stage forbs and weedy forbs shoot frequency increased 8.2% after one burn, decreased an average of 7.5% after two and three burns, and decreased 50.9% after four burns (table 28). Four burns were required to reduce early stage and weedy forbs significantly (Manske 2007a).

Shoot frequency of shrubs decreased 36.4% after one burn, decreased an average of 46.1% after two and three burns, and decreased 58.2% after four burns (table 28). Four burns were required to reduce shrubs significantly (Manske 2007a).

Shoot frequency of western snowberry decreased 68.3% after one burn, decreased 40.0% after two burns, decreased 67.1% after three burns, and decreased 58.4% after four burns. The greatest

reduction in shoot frequency occurred from early spring (mid-late April) burns. The repeated burns of 2 to 4 times caused no significant reduction in shoot frequency after one burn, however, the aboveground biomass produced by shrubs was greatly reduced after the third and fourth burns (Manske 2007a). Western snowberry reproduced vegetatively from buds on rhizomes and crowns. Removal of all or most of the aboveground shoots with fire does not stop the vegetative reproductive processes. Burning cannot remove deciduous shrubs and trees that produce vegetative sprouts from grassland communities.

Burning treatments did not increase water infiltration or soil water holding capacity. Burning treatments did not increase endomycorrhizal fungal colonization. Burning treatments did not increase rhizosphere microbe biomass and activity levels. Burning treatments did not increase mineralization of organic nitrogen into inorganic nitrogen. Burning treatments did not increase total herbage biomass production. Burning treatments did not restore functionality to the degraded grassland ecosystem.

Burning treatments did modify the composition of the aboveground plant community. The composition of introduced cool season grasses, early stage and weedy forbs, and shrub aerial stems decreased temporarily after four repeated prescribed burns. However, the fundamental problems of weak nutrient resource uptake, reduced water use efficiency, nonfunctional compensatory physiological mechanisms and vegetative reproduction by tillering remained within the plants and diminished biogeochemical processes remained in the degraded ecosystems following repeated burning treatments. None of the biological, physiological, or asexual mechanisms within grass plants and none of the rhizosphere microbes or the biogeochemical processes they perform were activated by burning treatments. Burning treatments did not and cannot improve grassland ecosystems biologically or ecologically.

Discussion

The existence of a woody plant component in a grassland is not an ecologically beneficial relationship as woody plants and grasses are adversarial inhibitive competitors. They compete for sunlight, mineral nitrogen, other essential elements, and soil water.

Seedlings of trees, shrubs, weedy forbs, and introduced grasses cannot become established in grasslands containing healthy grasses with full

nutrient resource uptake competitiveness (Peltzer and Kochy 2001). Fire in grasslands cannot prevent the invasion of, or cause the removal of, shrubs, and trees that are able to reproduce by vegetative secondary suckers (Wright and Bailey 1982, Manske 2006a, b). Almost all deciduous woody plants reproduce vegetatively, except big sagebrush (Artemisia tridentata) (Manske 2014d). Intrusive seedlings can only become established in a grassland after the ecosystem has been degraded by poor management practices.

Burning of grasslands exacerbates ecosystem degradation. When the losses of essential elements are greater than the quantity of captured essential elements, the result is degradation of the grassland ecosystem (McGill and Cole 1981). Almost all of the essential elements in the aboveground herbage are volatilized when a grassland is burned, with no active processes to recapture the lost essential elements. When burning occurs during dry soil periods, some of the belowground essential elements are also lost (Russelle 1992). Burning grasslands does not restore degraded ecosystems. Degraded grasslands continue to have the same fundamental problems following the repeated prescribed burning regime, and when the burning sequence stops, the undesirable replacement plants return to dominate the communities.

The presence of fire does not prove that grasslands need or are caused by fire (Heady 1975). Burning does not improve grassland ecosystems biologically or ecologically and fire cannot replace the contribution that partial defoliation by grazing graminivores achieves in managing healthy and productive grassland ecosystems.

Table 23. Inches of soil water by sample depth from summit slope position with deep loam soils after 13 years of no burns and 1 to 4 every-other-year burns on the Lostwood Wildlife Refuge, ND, 1978-1990.

Summit Slope Treatment	Soil Depth Inches	Jun	Jul	Aug
No Burns	0-6	-	0.88	0.68
	6-12	-	0.65	0.53
	12-24	-	1.48	1.10
	0-24	-	3.01a	2.31b
1 Burn	0-6	1.53	1.07	0.84
	6-12	1.00	0.75	0.67
	12-24	2.43	1.66	1.28
	0-24	4.97	3.48a	2.79b
2 Burns	0-6	-	0.91	0.67
	6-12	-	0.64	0.56
	12-24	-	1.37	0.95
	0-24	-	2.92a	2.18b
3 Burns	0-6	1.09	0.74	0.74
	6-12	0.63	0.59	0.54
	12-24	1.43	0.99	1.02
	0-24	3.15	2.32a	2.30b
4 Burns	0-6	0.82	0.89	0.72
	6-12	0.65	0.76	0.51
	12-24	1.15	1.10	1.13
	0-24	2.62	2.75a	2.36b

Data from Manske 1992.
Means in the same column and followed by the same letter are not significantly different (P<0.05).

Table 24. Inches of soil water by sample depth from lower slope position with deep loam soils after 13 years of no burns and 1 to 4 every-other-year burns on the Lostwood Wildlife Refuge, ND, 1978-1990.

Lower Slope Treatment	Soil Depth Inches	Jun	Jul	Aug
No Burns	0-6	1.46	1.12	0.73
	6-12	0.84	0.72	0.61
	12-24	1.84	1.64	1.26
	0-24	4.14	3.48c	2.60d
1 Burn	0-6	1.55	1.02	0.83
	6-12	1.18	0.96	0.66
	12-24	1.94	1.50	1.53
	0-24	4.67	3.48c	3.02d
2 Burns	0-6	2.00	1.06	0.67
	6-12	1.13	0.91	0.55
	12-24	1.54	1.27	1.27
	0-24	4.67	3.24c	2.49d
3 Burns	0-6	-	0.87	0.97
	6-12	-	0.68	0.56
	12-24	-	1.33	1.18
	0-24	-	2.88c	2.71d
4 Burns	0-6	1.77	1.00	0.74
	6-12	1.22	1.25	0.65
	12-24	2.54	2.19	1.88
	0-24	5.53	4.44c	3.27d

Data from Manske 1992.
Means in the same column and followed by the same letter are not significantly different (P<0.05).

Table 25. Mycorrhizal fungal infection of plant roots on the silty ecological sites after 13 years of no burns and 1 to 4 every-other-year burn treatments on the Lostwood Wildlife Refuge, ND, 1978-1990.

Biotype		No Burns 6 reps	1 Burn 4 reps	2 Burns 4 reps	3 Burns 4 reps	4 Burns 3 reps
Blue grama Fungi infection	(%)	78.8a	77.1a	84.9a	79.9a	73.5a
Western wheatgrass Fungi infection	(%)	65.7b	67.0b	61.3b	76.8b	63.8b
Smooth bromegrass Fungi infection	(%)	32.4c	41.3c	50.0c	31.4c	40.1c
Western snowberry Fungi infection	(%)	93.8d	84.7d	84.3d	85.2d	85.9d

Data from Manske 1992.
Means in the same row and followed by the same letter are not significantly different (P<0.05).

Table 26. Available mineral nitrogen (NO_3-N and NH_4-N) in lbs/ac-ft during June and August on the silty ecological sites after 13 years of no burns and 1 to 4 every-other-year burn treatment on the Lostwood Wildlife Refuge, ND, 1978-1990.

Mineral Nitrogen lbs/ac-ft	No Burns 6 reps	1 Burn 4 reps	2 Burns 4 reps	3 Burns 4 reps	4 Burns 3 reps
June					
NO_3-N	25.88	19.94	30.74	10.25	11.88
NH_4-N	17.85	21.63	13.57	8.58	15.11
Total N	43.73a	41.57a	44.31a	18.83a	26.99a
August					
NO_3-N	6.72	4.18	5.61	4.44	11.49
NH_4-N	11.94	17.29	11.49	12.86	11.23
Total N	18.66b	21.47b	17.10b	17.29b	22.71b

Data from Manske 1992.
Means in the same row and followed by the same letter are not significantly different (P<0.05).

Table 27. Herbage biomass (lbs/ac) by biotype categories in mid July after 13 years of no burns and 1 to 4 every-other-year burn treatments on the Lostwood Wildlife Refuge, ND, 1978-1990.

Biotype	No Burns 6 reps	1 Burn 4 reps	2 Burns 4 reps	3 Burns 4 reps	4 Burns 3 reps
Native Grass	411.61	310.12	762.75	512.87	861.51
Upland Sedge	224.56	362.93	74.34	238.58	145.81
Forbs	242.83	454.35	445.14	587.41	266.49
Shrubs	806.83	136.00	237.09	52.00	39.57
Total Live	1698.35a	1263.39a	1519.19a	1390.87a	913.66a
Standing Dead	817.43	390.19	226.81	223.36	252.06

Data from Manske 1992.
Means in the same row and followed by the same letter are not significantly different (P<0.05).

Table 28. Shoot frequency (%) of plant biotypes during July to August after 13 years of no burns and 1 to 4 every- other-year burn treatments on the Lostwood Wildlife Refuge, ND, 1978-1990.

Biotype		No Burns 6 reps	1 Burn 4 reps	2 Burns 4 reps	3 Burns 4 reps	4 Burns 3 reps
Domesticated	%	86.7	46.3	31.8	53.5	30.3
Cool Season	%	89.2	169.8	152.8	179.0	175.7
Warm Season	%	17.8	24.5	30.5	19.8	32.7
Upland Sedges	%	56.7	95.5	97.0	77.8	89.0
Late Stage Forbs	%	120.5	167.8	125.5	137.5	125.7
Early Stage Forbs	%	59.3	74.3	54.3	48.3	19.7
Weedy Forbs	%	12.3	8.8	13.0	6.5	8.0
Shrubs	%	111.7	71.0	58.5	62.0	46.7
Fringed Sage	%	13.8	9.5	13.0	23.3	14.3
Total Shoot Frequency		568.0	667.5	576.4	607.7	542.1

Data from Manske 1992.

D. 1983-2018 Study, Evaluation of Biologically Effective Management

A 36 year study evaluated characteristics of various management practices to determine the factors related to biologically effective management. A long-term nongrazed control treatment replicated two times, was used as a nondefoliation practice for managing native rangeland during this study. The traditional concept managed the land for its use as forage for livestock and was used to manage one native rangeland pasture, replicated two and three times during various periods, with a seasonlong system stocked with 7 cows/80 acres at 11.43 ac/AU and 2.60 ac/AUM for 4.5 months from 1 June to 15 October (137 days). The biologically effective concept managed the land as an ecosystem that considers the biological requirements of the grass plants, soil microbes, and the livestock and was used to manage three native rangeland pastures, replicated two times, with a twice-over rotation system stocked with 8 cows/80 acres at 10.22 ac/AU and 2.30 ac/AUM for 4.5 months from 1 June to 15 October (137 days).

Commercial cow-calf pairs grazed the native rangeland pastures through this study. During 1983 to 1994, commercial Angus-Hereford cows with Charolais sired calves were used. These cows were assigned to separate herd pools for each grazing treatment. During 1995 to 2012, commercial crossbred cattle were used on all grazing treatments. During 2013 to 2018, cows with variable ratios of breed combinations with mixed breed sired calves were used. Before spring turn out cow-calf pairs were sorted by cow age, and calf age, with 50% steers or bulls and heifers.

D1. 1999-2000 Study, Finding Ectomycorrhizal Fungi in the Mixed Grass Prairie

Livestock producers have typically observed changes in the soil structure and quality in their native rangeland pastures after three to five years of management with the twice-over rotation strategy. They notice that shovels are easily pushed into the soil, grass density thickens, grass rooting depth increases 12 to 24 inches deeper, and watershed harvest dams fail to fill because of a decrease in water runoff. These commonly observed changes in native rangeland soils had to have a cause.

These observations continued for many growing seasons with no definitive answer available. Until a McKenzie county, ND, rancher observed a huge difference in his predominately clayey soils that had changed from a rooting depth and water holding soil profile of 2 to 3 inches to an aggregated soil of 18 to 24 inches in depth after seven years of management with a twice-over rotation system. Hence, soil samples from his ranch were collected and taken for analysis by Soil Microbiologist TheCan Caesar-TonThat PhD at her laboratory at USDA Agricultural Research Service, Sidney, MT. She detected the presence of ectomycorrhizal fungi. During the field seasons of 1999 and 2000 replicated soil cores were collected from the sandy and silty ecological sites of pastures managed with the seasonlong and twice-over rotation grazing strategies of the 1983-2018 Study at the Dickinson Research Extension Center ranch located in Dunn County in western North Dakota. The soil samples were analyzed by Dr. Caesar-TonThat in Sidney, MT. The absorbance readings determined the presence of ectomycorrhizal fungi from the Basidiomycota phylum (previous taxon:Homobasidiomycetes class and Russuloid clade order) current taxon: Agaricomycetes class and Russulales order in both the sandy and silty soil cores from the twice-over rotation system but not in the soil cores from the seasonlong system (Manske 2007b).

The effects from the twice-over rotation strategy had enhanced the development of ectomycorrhizal fungi in the rhizosphere of mixed grass prairie grasses. Active ectomycorrhizal fungi form water-stable aggregates in soil that are water permeable but not water soluble by secreting large amounts of insoluble extracellular polysaccharides that have adhesive qualities (Caesar-TonThat et al. 2001b). Adhesive polysaccharides act as binding agents for soil particles, causing aggregation of soil (Caesar-TonThat 2002) that range from about the size of air rifle pellets to the size of large marbles. Increases in soil aggregation enlarges soil pore size and improves distribution and stabilization of soil particles. These improvements in soil quality cause increases in soil oxygenation, increases in water infiltration, and decreases in erodibility (Caesar-TonThat and Cochran 2000, Caesar-TonThat et al. 2001a, Caesar-TonThat et al. 2001b, Caesar-TonThat 2002, Manske and Caesar-TonThat 2003). Increased soil aggregation contributes to improvement of grassland ecosystem soil structure and quality increasing productivity.
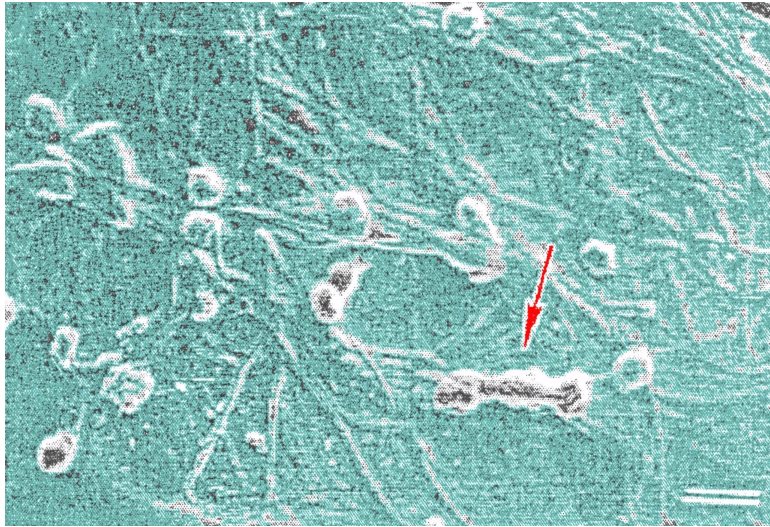
ECTOMYCORRHIZAL FUNGI

Photo from T.C. Caesar-TonThat

Ectomycorrhizal fungus with extracellular polysaccharides

D2. 2002 Study, Determination of Rhizosphere Volume Around Grass Roots.

The rhizosphere is the zone of bonded soil particles surrounding active perennial grass roots. The soil cylinder provides the living quarters for symbiotic microbes. The resident organisms are endomycorrhizal fungi, ectomycorrhizal fungi, bacteria, and protozoa and visiting organisms are nematodes, springtails, and mites. These rhizosphere organisms interact in a complex trophic web that is critical for nutrient flow in grassland ecosystems. The rhizosphere organisms are achlorophyllous and limited by access to energy. Healthy grass plants produce double the quantity of leaf biomass, capture and fix large amounts of carbon during photosynthesis, and produce carbohydrates in quantities greater than the amount needed for normal growth and maintenance. After the three and a half new leaf stage and before the flower stage, partial defoliation of grass tillers that removes 25% to 33% of the aboveground biomass by large grazing graminivores causes great quantities of the simple carbohydrates to be exudated through the roots into the rhizosphere. With this increase in available carbon compound energy in the rhizosphere, the biomass and activity of the microorganisms increase resulting in changes of the volume of the rhizosphere.

Measuring biomass of soil microbes is a perplexing difficulty. However, rhizosphere microbes reside in a bonded soil particle structure that changes in size and weight with increases or decreases in microorganism biomass and activity which can be measured.

Procedure

This one growing season project was conducted to evaluate rhizosphere volume from diameter and length measurements of each rhizosphere surrounding every western wheatgrass root contained within two replicated soil cores, 3 inches (7.62 cm) in diameter and 4 inches (10.16 cm) in depth, that were collected monthly from June to September and removed intact inside plastic PVC pipe that had been forced into soil of silty ecological sites managed for 20 years by three different treatments; a) long-term nongrazed control, b) traditional seasonlong grazed 4.5 months, and c) twice-over rotation strategy. The rhizosphere material, which included the rhizosphere organisms, the active plant roots extending from plant crown to tip, and the adhered soil particles, was separated from matrix soil by meticulous excavation with fine hand tools. Exposed rhizosphere segments were sprayed with a clear acrylic coating to prevent damage during further handling. The length and diameter of the rhizosphere around each root of every grass plant,

including associated vegetative tillers, were measured with a vernier caliper and used to determine total rhizosphere volume per cubic meter of soil for each sample period of each of the three treatments (Gorder, Manske, and Stroh 2004).

Results

Precipitation during the perennial plant growing season (April through October) of 2002 was 18.85 inches (129.78% of LTM) and considered to be above average. September experienced water deficiency conditions.

Rhizosphere volume was not different among the three management treatments during the early season month of June and was not different between the nongrazed and seasonlong treatments during August and September (figure 3 and table 29). The twice-over sample pasture #1 was grazed during the stimulation period from early July to mid July followed by a remarkable increase in rhizosphere volume of 123.45% from the starting June volume. The rhizosphere volume of 6885 cm^3/m^3 on the twice-over management treatment was significantly greater than the volume of 2415 cm^3/m^3 on the nongrazed and the volume of 1883 cm^3/m^3 on the seasonlong treatments as mean of August and September (figure 3 and table 29).

Rhizosphere volume is a proxy measurement for soil microorganism biomass. The significant increase of rhizosphere volume on the twice-over treatment indicates that a significant increase in microorganism biomass and activity levels also occurred and would indicate increased rates of mineralization of organic nitrogen into mineral nitrogen. Greater quantities of available mineral nitrogen would support a greater population of grass tillers.

The total western wheatgrass tiller density/m^2 on the twice-over management treatment was significantly greater than that on the seasonlong treatment during August and September (table 30). During June, the total tiller density on the twice-over treatment was greater than those on both the nongrazed and seasonlong treatments. The tiller numbers present in June would include the second year lead tillers, the surviving previous fall initiated secondary vegetative tillers, and the current spring initiated secondary vegetative tillers. The twice-over management treatment is the only grazing treatment that stimulates a significant quantity of spring initiated secondary tillers (Manske 2014a, b).

Discussion

Production of grassland herbage and livestock weight performance at potential biological levels requires all of the primary grass plant mechanisms and the ecosystem biogeochemical processes to be functioning at potential levels. Elevation of the rhizosphere microorganism biomass is the initial objective in order for mineralization of nitrogen to occur at or above the threshold rate of 100 lbs/ac (112 kg/ha) which permits the primary grass mechanisms to be activated. Rhizosphere organisms are limited by access to energy in the form of short carbon chains. Soil microbes are achlorophyllous and cannot fix carbon energy and must depend on grass plants for their source of energy. Exudation of carbon energy can be released from grass lead tillers through the roots into the rhizosphere by removal of 25% to 33% of the aboveground leaf biomass by large grazing graminivores when the lead tillers are at vegetative phenological growth between the three and a half new leaf stage and the flower (anthesis) stage during early June to mid July which is the stimulation period (Manske 1999, 2011b, 2014c). Depending on the degree of degradation of the grassland ecosystem, three to five or more growing seasons are required to increase the rhizosphere microorganism biomass to levels capable of mineralization of 100 lbs/ac (112 kg/ha) or greater of available mineral nitrogen. Full activation of the primary internal grass plant mechanisms requires mineral nitrogen to be available at the threshold level or greater and requires the quantity of available carbon fixed through photosynthesis from 75% to 67% of the leaf area of predefoliated lead tillers before the flower stage and from 50% of the leaf area after the flower stage (Manske 2010a, b). The increased rhizosphere volume and microorganism biomass permits cycling greater quantities of the major and minor essential elements. With increased provisions of essential elements, grass tillers are able to synthesize greater quantities of carbohydrates, proteins, and nucleic acids and to accelerate growth rates of replacement leaves and shoots, increase photosynthetic capacity of remaining mature leaves, increase secondary tiller development from axillary buds, enhance competitiveness of nutrient resource uptake, and improve water use efficiency. The combination of increased ecosystem biogeochemical processes and improved functioning of the internal grass plant mechanisms results in increases in grass herbage production and increases in plant density (basal cover) of the desirable native grass species. This increase in available forage quantity and improved quality through the entire growing season results in improved livestock weight grains on less land area.
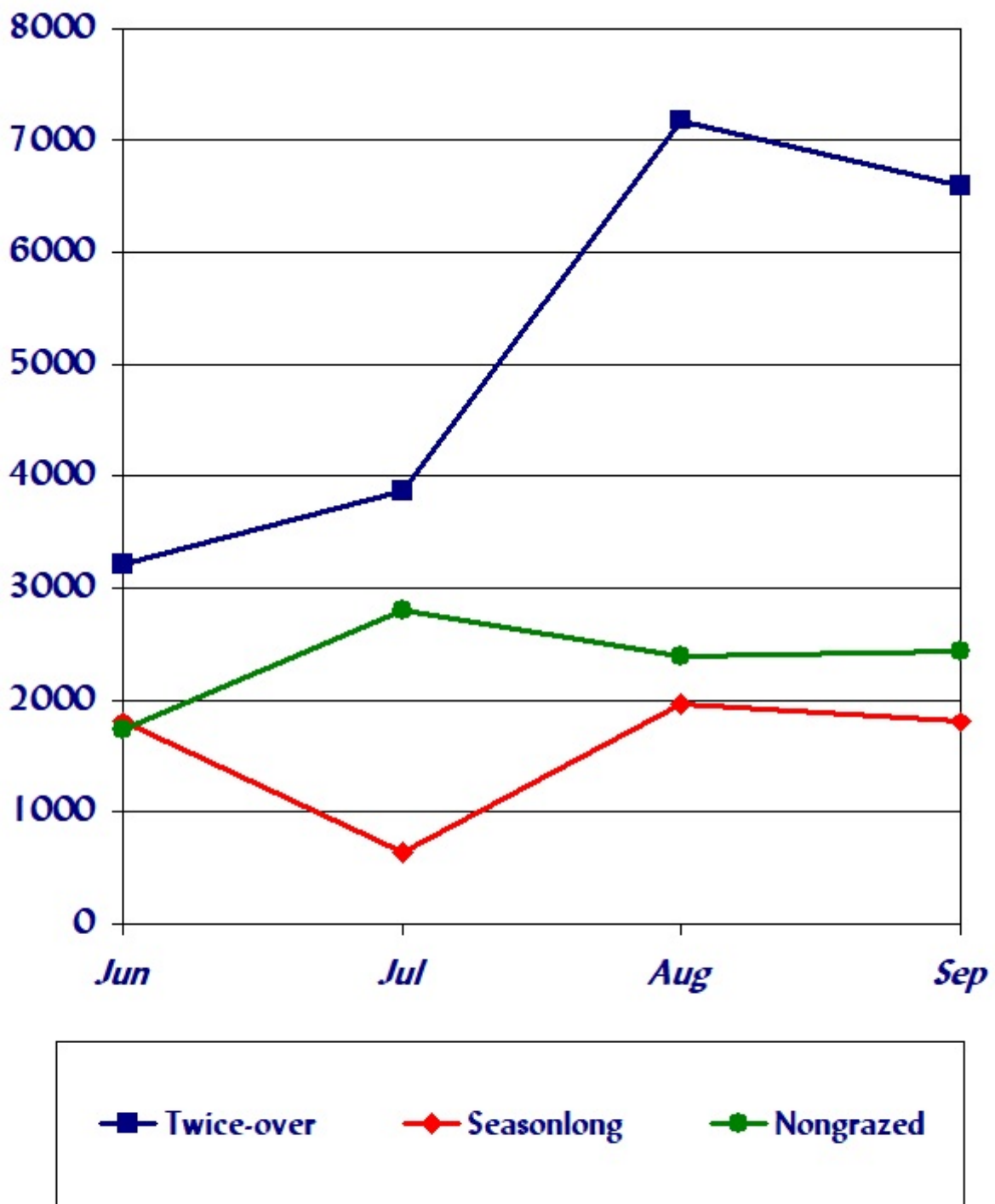
Figure 3. Rhizosphere volume (cm^3) per cubic meter of soil

Table 29. Rhizosphere volume in cubic centimeters per cubic meter of soil (cm^3/m^3), 2002.

Grazing Management	May	Jun	Jul	Aug	Sep	Oct
Nongrazed		1725.24a	2804.61a	2391.97b	2438.47b	
Seasonlong		1800.93a	642.21b	1963.02b	1802.97b	
Twice-over		3214.75a	3867.54a	7183.27a	6586.06a	

Means in the same column and followed by the same letter are not significantly different (P<0.05).
Data from Gorder, Manske, and Stroh, 2004.

Table 30. Total tiller density of western wheatgrass per square meter on silty ecological sites, 2002.

Grazing Management	May	Jun	Jul	Aug	Sep	Oct
Nongrazed		548.20y	548.20x	877.12xy	1206.04x	
Seasonlong		548.20y	657.84x	767.48y	657.84y	
Twice-over		2412.09x	1206.04x	1973.53x	1425.32x	

Means in the same column and followed by the same letter are not significantly different (P<0.05).
Data from Gorder, Manske, and Stroh, 2004.

D3. 2006 Study, Determination of the Standard Reference Rhizosphere Weight

Evaluation of the initial effects of restoration of degraded prairie ecosystems with the twice-over rotation system would require collection of quantitative data showing changes in rhizosphere size for the 2006-2011 study. During the growing season of 2002, we perfected the technique to measure rhizosphere volume which was extremely effective at delineating differences among management treatments. However, measurement of rhizosphere volume of every root within a soil core required 8 to 10 hours of tedious labor plus calculations of total rhizosphere volume per soil core and per treatment required a great deal of additional time.

Measurement of rhizosphere weight consisted of placement of each rhizosphere into a container of known weight upon being revealed from the matrix soil and determining the total weight after all rhizospheres within a soil core had been exposed. This process required 2 to 3 hours of meticulous excavation with fine hand tools per soil core.

All of the rhizosphere weight values collected from degraded ecosystems would be at some quantity less than potential, which at that time was unknown. A small study was conducted during mid June 2006 to measure the possible maximum rhizosphere weight from silty ecological sites that had been managed long-term (24 years) by the twice-over rotation strategy.

Procedure

Rhizosphere biomass was collected on three replicated grazed pastures managed by the twice-over rotation system for 24 years since 1983 by three replicated soil cores from the silty ecological sites of each pasture using a humane soil beastie catcher (Manske and Urban 2012a). The fresh rhizosphere material, which included the rhizosphere organisms, the active plant roots, and the adhered soil particles, was separated from matrix soil by meticulous excavation with fine hand tools and weighed.

Results

The resulting mean Standard Reference Rhizosphere Weight was 406.44 kg/m^3 recorded on silty ecological sites managed long-term (24 years) with a twice-over rotation grazing strategy.

D4. 1989-2018 Study, Availability of Soil Mineral Nitrogen

Nitrogen is an extremely important major essential element in grassland ecosystems. Deficiencies of available mineral nitrogen result in low herbage biomass production, deterioration of plant density and species composition, and reduced livestock weight performance. All rangeland soils managed with traditional grazing practices are deficient in mineral nitrogen (Wight and Black 1972, 1979). However, rangeland soils of the Northern Plains are not deficient in nitrogen. Most prairie soils contain 5 to 6 tons, with a range of 3 to 8 tons, of nitrogen per acre. Most of this nitrogen is immobilized in the soil as organic nitrogen and is not available for plant use. Soil organic nitrogen must be converted into mineral nitrogen through mineralization by rhizosphere microorganisms. Pastures with deficiencies in available mineral nitrogen is actually a deficiency in rhizosphere microorganism biomass which is strictly a management caused problem.

The quantity of available mineral nitrogen in grassland soils is dependant on the rate of mineralization of soil organic nitrogen (Coleman et al. 1983). The mineralization rate is determined by the rhizosphere microorganism biomass, and the microorganism biomass is limited by access to simple carbohydrate energy (Curl and Truelove 1986).

The available energy from soil organic matter is inadequate to increase soil microorganism biomass substantially. The small amount of readily accessible energy available to soil microorganisms in fresh organic material comes from short chain carbohydrates of sugars and starches composing only 1% to 5% and from water soluble proteins composing very low percentage (Brady 1974).

Grassland plants naturally leak small quantities of exudate substances that include sugars, amino acids, proteins, and numerous carbon compounds (Coyne et al. 1995). The quantity of root exudate leakage from ungrazed grassland plants is low and cannot support anything but a small microorganism biomass capable of performing biogeochemical processes only at low activity rates (Manske 2012c).

Partial defoliation that removes 25% to 33% of grass tillers biomass at vegetative phenological

growth stages between the three and a half new leaf stage and the flower stage by large grazing graminivores causes great quantities of exudates containing simple carbohydrate energy to be released from the grass tillers through roots into the rhizosphere (Hamilton and Frank 2001, Manske 2011b). This increase in availability of energy from carbon compounds in the rhizosphere, increases the biomass and activity of the microorganisms (Anderson et al. 1981, Curl and Truelove 1986, Whipps 1990). Increasing the rhizosphere organism biomass and activity results in greater rates of mineralization of soil organic nitrogen into mineral nitrogen available for plant use (Coleman et al. 1983, Klein et al. 1988, Burrows and Pfleger 2002, Rillig et al. 2002, Bird et al. 2002, Driver et al. 2005).

Differences in rhizosphere microorganism biomass and activity are directly related to the differences in the quantities of soil organic nitrogen mineralized into mineral nitrogen. The quantities of available mineral nitrogen, nitrate (NO_3) and ammonium (NH_4), can be measured from soil samples.

The quantity of available soil mineral nitrogen varies with changes in soil temperature, soil microorganism biomass, and plant phenological growth and development during the growing season (Whitman 1975) and is the net difference between the total quantity of organic nitrogen mineralized by soil microorganisms and the quantity of mineral nitrogen immobilized by plants (Brady 1974, Legg 1975). The relationships between soil microorganism activity and phenology of plant growth activity results in the observed variations of available mineral nitrogen (Goetz 1975). When soil microorganism activity is greater than plant growth activity, the quantity of available mineral nitrogen increases. When plant growth activity is greater than soil microorganism activity, the quantity of available mineral nitrogen decreases.

When the quantity of available mineral nitrogen is between 100 lbs/ac and 165 lbs/ac (112 kg/ha-185 kg/ha) during periods of active plant growth, rangeland ecosystems can sustain herbage production at biological potential levels (Wight and Black 1972). Rangeland ecosystems that have soil mineral nitrogen available at quantities less than 100 lbs/ac (112 kg/ha) have a soil mineral nitrogen deficiency and are functioning below biological potential production capacity.

Procedure

Soil mineral nitrogen, nitrate (NO_3) and ammonium (NH_4), was determined for the first time on the 1983-2018 Study grazing management practices in 1989 (during year 7) from silty ecological sites managed by four treatments: a) long-term nongrazed, b) seasonlong grazed 6.0 months, c) traditional seasonlong grazed 4.5 months, and d) twice-over rotation grazing strategy. During 1989, a mid June sample was collected using the standard technique for mixed soils of cropland sites with a bucket auger 2 inches (5.08 cm) in diameter and 6 inches (15.24 cm) long. Each treatment field sample included five soil cores to 12 inches (30.48 cm) in depth, located in a large circle, placed into a gallon bucket, and thoroughly mixed. Two replicate samples were selected from the bucket each consisting of about twenty percent of the collected soil material, placed on ice and frozen. Analysis of soil core samples for available mineral nitrogen (NO_3 and NH_4) was conducted by the North Dakota State University Soil Testing Laboratory using wet chemistry methods.

Soil mineral nitrogen, nitrate (NO_3) and ammonium (NH_4), was determined for replicated soil core samples collected in 2013, 2014, 2016, 2017, and 2018 from silty ecological sites managed by three treatments: a) long-term nongrazed, b) traditional seasonlong grazed 4.5 months, and c) twice-over rotation grazing strategy with the 1 inch Veihmeyer soil tube at incremental depths of 0-6, 6-12, and 12-24 inches. During 2013 and 2014, 6 monthly periods from May to October were collected and air dried. During 2016, 6 monthly periods from May to October were collected and frozen. During 2017, 2 monthly periods from June and August were collected and frozen. During 2018, 3 monthly periods from May, June, and July were collected and frozen. Analysis of soil core samples for available mineral nitrogen (NO_3 and NH_4) was conducted by the North Dakota State University Soil Testing Laboratory using wet chemistry methods.

Results

The 6-year period (1987-1992) was a long period with near drought conditions receiving average perennial plant growing season (April to October) precipitation of 9.97 inches (68.90% of LTM). Drought conditions did occur during the growing season of 1988 receiving only 5.30 inches (36.65% of LTM) from April to October. During 1989, June received only 1.63 inches (51.10% of LTM) and during the 6 month growing season received 10.60

inches (73.30% of LTM) with water deficiency conditions occurring during July, August, and September.

During the seventh growing season, 1989, the rhizosphere microorganism biomass on the twice-over rotation treatment had increased sufficiently to mineralize greater than 100 lbs/ac of mineral nitrogen on all three pastures (table 31). The amount of available mineral nitrogen on the two seasonlong treatments was less than the threshold quantity of 100 lbs/ac (table 31). The amount of mineral nitrogen available on the long-term nongrazed treatment was slightly more than the threshold quantity (table 31).

The 2013-2014 soil samples for available mineral nitrogen were air dried awaiting analysis. The quantities of available ammonium on all sample dates on all three management treatments were unusually low, with a loss of about 60% of the ammonium, resulting in greatly reduced total available mineral nitrogen on all three management treatments (appendix tables 1, 2, and 3). The low quantity of remaining available ammonium was still 30% greater on the twice-over strategy than that on the nongrazed and was 15% greater than that on the seasonlong strategy. Available ammonium was 13% greater on the seasonlong strategy than that on the nongrazed grassland.

The 2016, 2017, and 2018 available mineral nitrogen data for the long-term nongrazed strategy are in appendix tables 4, 5, and 6, for the traditional seasonlong strategy are in appendix tables 7, 8, and 9, and for the twice-over rotation strategy are in appendix tables 10, 11, and 12.

The mean monthly precipitation of 2016 to 2018 had a total of 6 perennial plant growing season months (table 32). There were no months with water deficiency conditions. Low precipitation less than 75% of LTM occurred during May (16.7%). Normal precipitation greater than 75% and less than 125% of LTM occurred during June, July, and August (50.0%). High precipitation greater than 125% of LTM occurred during September and October (33.3%). Total 3 year mean growing season precipitation was normal at 13.77 inches (105.84% of LTM).

The 2016-2018 soil samples for available mineral nitrogen were frozen within a few hours of collection and presumably little of the available ammonium was lost. In rangeland soils most of the available mineral nitrogen is in the ammonium (NH_4) form with lower amounts in the nitrate (NO_3) form.

From the 2016-2018 soil samples, mineral nitrogen was comprised of 90.6% NH_4 and 9.4% NO_3 on the nongrazed strategy, 89.9% NH_4 and 10.1% NO_3 on the seasonlong strategy, and 87.1% NH_4 and 12.9% NO_3 on the twice-over strategy.

The mean monthly total mineral nitrogen was 137.2 lbs/ac on the twice-over strategy (table 35) with 17.7 lbs/ac of nitrate and 119.5 lbs/ac of ammonium. Mean monthly total mineral nitrogen was 113.5 lbs/ac on the traditional seasonlong strategy (table 34) with 11.5 lbs/ac of nitrate and 102.0 lbs/ac of ammonium. Mean monthly total mineral nitrogen was 104.6 lbs/ac on the nongrazed strategy (table 33) with 9.8 lbs/ac of nitrate and 94.8 lbs/ac of ammonium. Total mineral nitrogen was 20.9% greater, ammonium was 17.1% greater, and nitrate was 55.0% greater on the twice-over strategy than that on the seasonlong strategy (tables 34 and 35). Total mineral nitrogen was 31.2% greater, ammonium was 26.1% greater, and nitrate was 80.6% greater on the twice-over strategy than that on the nongrazed strategy (tables 33 and 35). Total mineral nitrogen was 8.5% greater, ammonium was 7.7% greater, and nitrate was 16.5% greater on the seasonlong strategy than that on the nongrazed strategy (tables 33 and 34).

Available quantities of nitrate and ammonium for intact soils of rangeland sites fluctuate with changes in organic nitrogen mineralization by soil microbial activity and by immobilization of mineral nitrogen with changes in plant growth rate (Brady 1974, Goetz 1975, Legg 1975, Whitman 1975). The rates of the mineralization and immobilization processes are additionally affected by the quantity of soil water, and the biologically effectiveness of the grazing management strategy.

The quantity of available mineral nitrogen occurs in a dynamic cycle during each growing season that generally follows a typical pattern with some variations occurring on different ecological sites and at different soil depths (Goetz 1975). The variance in amplitude of the quantity of mineral nitrogen available between the peaks and low periods in the cycle oscillations usually is around 25% to 50%.

Mineralization and nitrification processes of soil microbe activity start slowly in early spring when soil temperature permits formation of liquid water around 30° F. Quantity of available mineral nitrogen increases with rising soil temperature and microorganism biomass reaching the first peak in mineral nitrogen around mid May, which is usually

the greatest peak. Soon afterward, plant growth rates rapidly increase with plant growth activity greater than soil microbe activity resulting in the first low period during June and the first two weeks of July when the rapid growth of reproductive lead tillers slows. The second peak in mineral nitrogen is reached during late July to mid August as reproductive lead tiller growth moves into senescents and the slow growth rate of vegetative tillers start to increase. A second low period in mineral nitrogen occurs from mid August to late September as grass plants store carbohydrates during the winter hardening process and as fall tillers of cool season grasses and fall buds of warm season grasses develop, later to become vegetative tillers during the subsequent growing season. The third peak in mineral nitrogen occurs around mid October if some soil water is present and as perennial plant growth slows with decreasing air temperatures. Available mineral nitrogen declines during the third low period as soil temperatures decrease at the end of the growing season and winter freeze up approaches (Goetz 1975, Whitman 1975).

This typical dynamic cycle of available mineral nitrogen was followed on the silty ecological sites of the long-term nongrazed strategy (table 33) and the twice-over rotation strategy (table 35). The available mineral nitrogen on the traditional seasonlong strategy (table 34) followed the typical dynamic cycle during the first peak and low period and into the second peak, however, the second low period usually occurring from mid August to late September during the winter hardening and fall tiller and fall bud development processes appears to have been delayed until near the end of the growing season during October which would greatly reduce the winter survival chances for a high proportion of the carry over tillers resulting in lower grass density and decreased herbage biomass during the next growing season.

The four major internal grass plant growth mechanisms of precipitation (water) use efficiency, compensatory physiological processes, vegetative reproduction by tillering, and resource uptake competitiveness all require a threshold quantity of 100 lbs/ac (112 kg/ha) mineral nitrogen to be available before they can be fully activated. Traditional management practices that focus only on the aboveground ecosystem components rarely mineralize 100 lbs/ac of mineral nitrogen. Manske (2012b) documented quantities of available mineral nitrogen (table 36) on five long-term traditional management treatments with operational histories of 20 to 75 years that ranged from a low 31.2 lbs/ac on

deferred grazing, to 39.5 lbs/ac on long-term nongrazing, to 42.4 lbs/ac and 61.6 lbs/ac on long duration seasonlong grazing, and to a high 76.7 lbs/ac on recommended duration (4.5 month) seasonlong grazing treatments. These low quantities of available mineral nitrogen that are well below the threshold level of 100 lbs/ac indicate that traditional grazing management and nongrazing practices are the direct cause of inhibitory mineral nitrogen deficiencies in rangeland ecosystems. The limiting factor on traditional practices is a low soil microbe biomass which are achlorophyllous and cannot fix carbon energy and must depend on the carbon energy fixed by grass plants during photosynthesis. However, releasing (exudation) of surplus grass plant carbon energy through the roots into the rhizosphere requires partial defoliation by large grazing graminivores when grass lead tillers are at vegetative phenological growth stages between the three and a half new leaf stage and the flower stage that occurs from early June to mid July. As a result of the increased carbon energy, the rhizosphere microbes can increase in biomass to a level that can mineralize 100 lbs/ac of nitrogen.

The twice-over rotation strategy has two grazing periods on each pasture of a three to six pasture system. The periods of partial defoliation by grazing are coordinated with the grass tillers phenological growth stages. Grazing on each of the pastures during the first period (1 June to 15 July) removes 25% to 33% of the leaf weight of grass lead tillers at vegetative growth stages between the three and a half new leaf stage and the flower stage causing adequate quantities of fixed carbon energy to be exudated through the roots into the rhizosphere permitting an increase of the microorganism biomass to levels that are capable of mineralizing available mineral nitrogen at rates of 100 lbs/ac or greater that maintains full activation of the four major internal grass plant growth mechanisms.

Full activation of the compensatory physiological processes within grass plants accelerates growth rates of replacement leaves and shoots, increases photosynthetic capacity of remaining mature leaves that increase the quantity of available fixed carbon, and increases restoration of biological and physiological processes enabling rapid and complete recovery of partially defoliated grass tillers.

Full activation of the asexual processes of vegetative production increases secondary tiller development from axillary buds, increases initiated tiller density during the grazing season, and increase

herbage biomass production and improves herbage nutritional quality.

Full activation of the nutrient resource uptake processes increases root absorption of soil water and the major and minor essential elements, improves the robustness of grass growth and development, increases competitiveness of healthy grasses, and increases suppression of undesirable grass, weedy forb, and shrub seedlings or rhizomes from encroachment and establishment within grassland communities.

Full activation of the precipitation (water) use efficiency processes increases herbage biomass production 50.4% per inch of rainfall received, and reduces the detrimental effects during water deficiency periods and from drought conditions.

Discussion

The soil microorganism biomass and biological activity on the grazingland ecosystems managed with the twice-over rotation strategy were great enough to mineralize nitrogen at 112 lbs/ac to 157 lbs/ac during the grazing period of June to October. The soil microorganisms that occupy intact soil mostly inhibit that narrow zone around active perennial grass roots; the rhizosphere. Rhizosphere microorganisms are limited in production by access to energy from simple carbon chains. Grass plants fix large quantities of surplus carbon through photosynthesis during vegetative growth stages. The grazing periods on the twice-over rotation strategy are coordinated with grass tiller phenological growth and development. Partial defoliation by grazing graminivores that removes 25% to 33% of the aboveground leaf weight on about 60% to 80% of the lead grass tillers at vegetative growth stages between the three and a half new leaf stage and the flower stage intentionally causes large quantities of grass leaf surplus simple carbohydrates to be exudated through the roots into the rhizosphere. The large increase in available simple carbon energy increases the microorganism biomass and elevates microbe activity increasing the quantity of mineralized nitrogen and other biogeochemical processes permitting the grassland ecosystem managed with the twice-over rotation strategy to function at the good - as - new biological potential production capacity.

The soil microorganism biomass and biological activity on the grazingland ecosystems managed with the traditional seasonlong strategy mineralized nitrogen at 97 lbs/ac to 122 lbs/ac during the June to October grazing period in a three year

period that received normal growing season precipitation at 106% of LTM. Traditional seasonlong practices rarely mineralized nitrogen at rates greater than 100 lbs/ac. Seasonlong treatments are managed by long time traditional concepts that consider the things that can be seen aboveground i.e. the plants and the livestock. Unfortunately, the importance of the microorganisms belowground is not even acknowledged. The grazing periods on seasonlong strategies are not coordinated with grass tiller phenological growth stages; with grazing often starting before the three and a half new leaf stage and continuing past mid October. Some partial defoliation by grazing graminivores does cause exudation of quantities of carbohydrates at rates greater than typical leakage, however, multiple defoliation events and removal of greater than 50% leaf weight does not permit large quantities of simple carbon energy to be consistently released into the rhizosphere. Any enhancement of biological activity of microbes below that soil surface is purely unintentional. Usually the traditional management concepts and the characteristic seasonlong stocking rates cause quantities greater than 50% of the leaf weight to be removed from grass tillers. The remaining leaf area is insufficient to photosynthesize adequate quantities of carbohydrates to meet the demand for average growth. Without consistent large quantities of exudated simple carbon energy, the rhizosphere microorganism biomass on seasonlong managed ecosystems usually remain at mediocre levels and sometimes increase to higher quantities during good precipitation growing seasons.

The soil microorganism biomass and biological activity on grazingland ecosystem managed with a long-term nongrazed strategy mineralized nitrogen at 93 lbs/ac to 112 lbs/ac during the June to October grazing period in a three year period that received normal growing season precipitation at 106% of LTM on plant communities with greatly increased abundance of introduced replacement Canada bluegrass plants that have labile root material which provides a new source of carbon energy for soil microbes that native grass root material does not provide. On the nongrazed strategy, native grasses provide simple carbohydrate energy to soil microbes only at the typical low leakage rate. The lack of partial defoliation by grazing graminivores prevent large quantities of simple carbon energy from being exudated from grass roots into the rhizosphere, resulting in a small biomass of microorganisms and low quantities of available mineral nitrogen. The traditional purposes of the nongrazed treatment removes grazing defoliation by graminivores with the intention of resting the grazingland ecosystem as a

restoration management practice developed from long-time concepts that do not go deeper than the soil surface. This misguided practice was based on a naive assumption that the observed vigor depletion of grassland communities was caused by livestock grazing, rather than being caused by poor management of livestock grazing, and that the lost vigor of the grass plants could be restored by resting. Ironically, removal of livestock grazing does not rest an ecosystem, the grass plants are not invigorated, and the soil microorganisms are not enhanced. The biomass of soil microorganisms on a nongrazed ecosystem can fluctuate with the soil moisture levels but cannot increase above the biomass that can be supported by the small quantities of carbon energy provided at the normal low leakage rate plus a small amount of carbon energy and water soluble crude protein remaining within recently dead plant residue. As a result, the microorganism biomass on nongrazed ecosystems remains small and the quantity of mineralized nitrogen remains low.

Rangelands of the Northern Plains are not inherently low producing ecosystems. The typical low grass herbage biomass and low calf weight gains on rangeland pastures managed with traditional grazing management practices result from deficient quantities of available soil mineral nitrogen below 100 lbs/ac. Rangelands with intact soils are not deficient in nitrogen. The biomass of rhizosphere microorganisms is too low to mineralize adequate quantities of organic nitrogen into the threshold quantity of 100 lbs/ac of mineral nitrogen required by grass plants to fully activate the four major internal growth mechanisms. The biomass of the rhizosphere microorganisms can be increased by implementation of the twice-over rotation strategy that consistently provide large quantities of surplus carbon energy from partially defoliated lead tillers to the microorganisms in the rhizosphere that in turn mineralize adequate quantities of mineral nitrogen for the plants that can then provide forage with adequate quantities of crude protein through the entire grazing season so cow and calf pairs can produce at their genetic potentials.

Table 31. Total mineral nitrogen (NO$_3$-N plus NH$_4$-N) in lbs/acre-foot collected in mid June from silty ecological sites managed by four treatments at DREC ranch, ND, 1989.

Management Treatment	Soil Depth (inches)	mid June
Long-term Nongrazed	0-12	106.05
Seasonlong grazed 6.0 m	0-12	61.61
Traditional seasonlong	0-12	76.70
Twice-over rotation	0-12	177.84
1st Pasture	0-12	199.05
2nd Pasture	0-12	163.97
3rd Pasture	0-12	170.50

Table 32. Precipitation in inches during perennial plant growing season months with mineral nitrogen soil samples collected, 2016-2018.

	May	Jun	Jul	Aug	Sep	Oct	Growing Season
Long-term Mean	2.65	3.19	2.36	1.96	1.50	1.35	13.01
2016-2018	1.74	2.49	2.81	2.27	2.66	1.80	13.77
% of LTM	65.66	78.06	119.07	115.82	177.33	133.33	105.84

Table 33. Mean mineral nitrogen, nitrate (NO$_3$) and ammonium (NH$_4$), in lbs/ac in incremental depths to 24 inches during growing season months on silty ecological sites of the long-term nongrazed, 2016-2018.

Soil Depth (inches)	May	Jun	Jul	Aug	Sep	Oct
NO$_3$ nitrate						
0-6	3.88	4.25	3.75	3.75	3.00	5.00
6-12	3.00	2.92	1.75	3.00	2.00	1.00
12-24	3.50	4.67	2.00	4.50	4.00	3.00
0-24	10.38	11.83	7.50	11.25	9.00	9.00
NH$_4$ ammonium						
0-6	39.73	32.50	38.10	40.63	30.67	44.70
6-12	31.21	31.22	28.15	31.25	28.88	31.98
12-24	27.13	27.03	26.03	28.31	24.80	26.27
0-24	98.06	90.75	92.27	100.18	84.35	102.95
NO$_3$ + NH$_4$						
0-6	43.61	36.75	41.85	44.38	33.67	49.70
6-12	34.21	34.14	29.90	34.25	30.88	32.98
12-24	30.63	31.70	28.03	32.81	28.80	29.27
0-24	108.44	102.58	99.77	111.43	93.35	111.95

Table 34. Mean mineral nitrogen, nitrate (NO_3) and ammonium (NH_4), in lbs/ac in incremental depths to 24 inches during growing season months on silty ecological sites of the traditional seasonlong, 2016-2018.

Soil Depth (inches)	May	Jun	Jul	Aug	Sep	Oct
NO_3 nitrate						
0-6	5.59	4.61	6.34	6.17	5.67	4.00
6-12	3.67	2.45	2.59	2.34	2.67	2.00
12-24	3.84	3.11	2.67	4.34	4.00	2.67
0-24	13.09	10.17	11.59	12.84	12.34	8.67
NH_4 ammonium						
0-6	47.18	40.61	40.60	45.88	48.40	37.09
6-12	36.25	31.02	28.37	28.69	34.59	27.19
12-24	31.90	31.43	25.21	26.98	26.32	24.36
0-24	115.33	103.06	94.17	101.54	109.31	88.64
$NO_3 + NH_4$						
0-6	52.77	45.22	46.93	52.04	54.07	41.09
6-12	39.92	33.46	30.95	31.02	37.26	29.19
12-24	35.74	34.55	27.87	31.31	30.32	27.03
0-24	128.42	113.23	105.75	114.39	121.65	97.31

Table 35. Mean mineral nitrogen, nitrate (NO_3) and ammonium (NH_4), in lbs/ac in incremental depths to 24 inches during growing season months on silty ecological sites of the twice-over rotation system, 2016-2018.

Soil Depth (inches)	May	Jun	Jul	Aug	Sep	Oct
NO_3 nitrate						
0-6	8.67	7.11	6.59	8.25	5.00	6.67
6-12	7.17	4.17	3.00	4.67	2.00	6.67
12-24	6.84	6.00	2.34	5.34	6.67	9.33
0-24	22.68	17.28	11.92	18.25	13.67	22.67
NH_4 ammonium						
0-6	58.06	46.25	38.10	52.62	40.57	50.03
6-12	43.40	42.06	37.42	39.81	31.65	36.33
12-24	33.31	35.37	29.37	45.93	25.89	30.67
0-24	134.77	123.68	104.89	138.36	98.11	117.03
$NO_3 + NH_4$						
0-6	66.73	53.36	44.69	60.87	45.57	56.70
6-12	50.57	46.23	40.42	44.48	33.65	43.00
12-24	40.15	41.37	31.70	51.26	32.56	40.00
0-24	157.44	140.96	116.81	156.61	111.78	139.70

Table 36. Mineral nitrogen (lbs/ac) available on mixed grass prairie ecosystems effected by traditional management treatments.

Traditional Management Treatment	Operational Duration Years	Available Mineral Nitrogen lbs/ac
4.5 m Seasonlong	20 yr	76.7
6.0 m Seasonlong	20 yr	61.6
7.0 m Seasonlong	75 yr	42.4
Nongrazed	75 yr	39.5
Deferred Grazing	50 yr	31.2

Data from Manske 2012b.

D5. 2013-2018 Study, Soil Organic Matter (SOM) Affects Soil Quality

The quantity of soil organic matter (SOM) is a direct indication of soil primary productivity and is a sensitive measure of changes in soil quality and ecosystem functionality (Burke et al. 1989, Gregorich et al. 1994).

Increased quantities of microbe, plant, and animal biomass results in greater amounts of residue added annually to the soil organic matter. The quantity of organic matter in a soil can increase at annual increments until the steady state is reached where the inputs and the outputs are at equilibrium levels. The equilibrium level of soil organic matter is variable and affected by difference in soil texture, mean temperature, growing season precipitation, and type of management practice. Soil organic matter equilibrium level increases with reduction in the texture of the soils. Lower quantities of soil organic matter occur with coarse textured sandy soils and greater quantities occur in finer textured silty and clayey soils. The quantity of soil organic matter increases as mean temperatures cool and as growing season precipitation increases (Weaver et al. 1935, Brady 1974, Parton et al. 1987). The equilibrium level of soil organic matter for any specific soil type in a region can be decreased or increased depending on whether the effects from the type of management practice implemented causes a decrease or an increase in the soil microorganism biomass (Dormaar et al. 1977, Campbell and Sonster 1982, Naeth et al. 1991, McLaunchlan et al. 2006).

Soil organic matter is the primary nutrient reservoir of grassland ecosystems and contains the organic forms of the essential elements, carbon (C), hydrogen (H), nitrogen (N), oxygen (O), phosphorus (P), and sulfur (S); the ionic forms of the macronutrients, calcium (Ca), magnesium (Mg), and potassium (K); and the ionic forms of the micronutrients, boron (B), copper (Cu), molybdenum (Mo), and zinc (Zn) (Brady 1974, Van Veen and Paul 1981, Burke et al. 1989). The other nine minor essential elements are present in grassland ecosystem soils in the mineral form at the same level as each element was present in the parent material.

Essential elements stored in the soil organic matter as unavailable organic forms or as ions adsorbed by colloidal complexes are biologically and chemically immobilized, respectively, and these stable forms are not subjected to potential losses through volatilization or leaching movement (Legg 1975, Gibson 2009). Availability of the immobilized essential elements is conducted through the ecosystem biogeochemical cycles performed by soil microorganisms (McGill and Cole 1981, Cheng and Johnson 1998, Manske 2012b, 2014c).

The quantity of available essential elements is determined by the rates of soil organic matter decomposition and mineralization that are directly regulated by the biomass of active soil microorganisms and are not affected by the quantity or rate of residue accession (Van Veen and Paul 1981). Increases in the organic matter content of a soil improves the stability of soil aggregates, improves the physical and chemical properties, improves air and water infiltration and water holding capacity, improves soil fertility, and increases cation exchange capacity (Schimel, Coleman, and Horton 1985, Six et al. 1998, 2004).

Procedure

The percent soil organic matter (SOM) was determined by the loss on ignition (% LOI) analysis conducted by the NDSU Soil Testing Laboratory from replicated soil core samples collected during June of 2013, 2014, 2016, 2017, and 2018 at silty ecological sites with the 1 inch Veihmeyer soil tube at incremental depths of 0-6, 6-12, and 12-24 inches on three management treatments a) long-term nongrazed, b) traditional seasonlong, and c) twice-over rotation strategy. During 2013 and 2014 the soil samples were air dried. During 2016 to 2018 the soil samples were frozen. The quantity of organic carbon and nitrogen in soil was determined from the weight of soil and the percent soil organic matter. The weight of silty soil in southwestern North Dakota was determined from average silty soil bulk density from analysis of comparable soils (Anonymous circa early 1980's) at incremental depths of 0-6, 6-12, and 12-24 inches (table 37). Weight of soil organic carbon (SOC) was determined from the weight of silty soil and percent of soil organic matter multiplied by 0.58 (58% organic carbon content of soil organic matter) (Anonymous nd, NRCS Staff 2009, Pluske et al. 2015) of soil core samples. Weight of soil organic nitrogen (SON) was determined from the weight of silty soil and percent soil organic matter multiplied by 0.058 (estimated 5.8% organic nitrogen content of soil organic matter) of soil core samples (table 35).

Table 37. Generalized soil bulk density and soil weight at incremental depths on silty ecological sites of rangeland in southwestern North Dakota.

Soil Depth	Soil Bulk Density	Soil Weight	
(inches)	(g/cm^3)	(lbs/ac)	(tons/ac)
0-6	1.15	1,563,667.08	781.83
6-12	1.30	1,767,623.65	883.81
12-24	1.30	3,535,247.31	1,767.62
0-24		6,866,538.04	3,433.27

Average silty soil bulk density from Anonymous. circa early 1980's.
NDSU Soils Department.

Table 38. Weight of soil, soil organic matter (SOM), soil organic carbon (SOC), and soil organic nitrogen (SON) in pounds per acre per incremental depth.

Mathematical Formula

Soil weight per increment of soil depth per acre

Soil bulk density in g/cm^3 X depth of soil in cm X 100,000,000 cm^2/1 hectare

X 1 ha/2.471 ac X 1 lb/453.5924 g = soil weight in lbs/ac

Weight of soil organic matter (SOM)

Weight of soil in lbs/ac X % SOM/100 = weight of SOM in lbs/ac

Weight of soil organic carbon (SOC)

Weight of soil in lbs/ac X (% SOM/100 X 0.58) = weight of SOC in lbs/ac

Weight of soil organic nitrogen (SON)

Weight of soil in lbs/ac X (% SOM/100 X 0.058) = weight of SON in lbs/ac

Results

The percent soil organic matter (SOM) on the long-term nongrazed strategy was 3.58% (0-6), 2.55% (6-12), and 2.43% (12-24). The percent soil organic matter (SOM) on the traditional seasonlong strategy was 6.30% (0-6), 3.20% (6-12), and 2.40% (12-24). The percent soil organic matter (SOM) on the twice-over rotation strategy was 7.15% (0-6), 4.50% (6-12), and 3.15% (12-24) during 2016-2018 (Figure 4). The percent SOM at 0-6 inch depth on the twice-over strategy was 13.5% greater than that on the seasonlong strategy and was 99.7% greater than that on the nongrazed strategy, and percent SOM on the seasonlong strategy was 76.0% greater than that on the nongrazed strategy. The percent SOM at 6-12 inch depth on the twice-over strategy was 40.6% greater than that on the seasonlong strategy and was 76.5% greater than that on the nongrazed strategy, and percent SOM on the seasonlong strategy was 25.5% greater than that on the nongrazed strategy. The percent SOM at 12-24 inch depth on the twice-over strategy was 31.3% greater than that on the seasonlong strategy and was 29.6% greater than that on the nongrazed strategy, and percent SOM on the seasonlong strategy was 1.2% lower than that on the nongrazed strategy (Figure 4).

The quantity of soil organic matter (SOM) on the silty ecological sites to the 24 inch soil depth on the long-term nongrazed strategy was 66.3 tons/ac during 2013-2014 and was 93.5 tons/ac during 2016-2018 with an increase of 40.9% (tables 39 and 40). One of the replicate nongrazed exclosures was lost to oil exploration during this study. The elimination of data from this lost site caused most of the change in the mean quantity of soil organic matter, organic carbon, and organic nitrogen. The quantity of soil organic matter (SOM) to the 24 inch soil depth on the traditional seasonlong strategy was 122.3 tons/ac during 2013-2014 and was 120.0 tons/ac during 2016-2018 with a decrease of 1.9% (tables 41 and 42). The quantity of soil organic matter (SOM) to the 24 inch soil depth on the twice-over rotation strategy was 143.4 tons/ac during 2013-2014 and was 151.4 tons/ac during 2016-2018 with a increase of 5.6% (tables 43 and 44). During 2013-2014, the quantity of SOM on the seasonlong strategy was 84.3% greater than that on the nongrazed strategy (tables 39 and 41). The quantity of SOM on the twice-over strategy was 116.2% greater than that on the nongrazed (tables 39 and 43) and was 17.3% greater than that on the seasonlong strategy (tables 41 and 43). During 2016-2018, the quantity of SOM on the seasonlong strategy was 28.3% greater than that on the nongrazed strategy (tables 40 and 42). The

quantity of SOM on the twice-over strategy was 61.9% greater than that on the nongrazed (tables 40 and 44) and was 26.2% greater than that on the seasonlong strategy (tables 42 and 44).

Soil organic matter (SOM) has been accumulating in the top 24 inches of soil at mean rates of 5193 lbs/ac/yr, 6664 lbs/ac/yr, and 8408 lbs/ac/yr on the nongrazed, seasonlong, and twice-over strategies, respectively, with rates of 2807 lbs/ac/yr, 4307 lbs/ac/yr, and 5315 lbs/ac/yr in the top 12 inches of soil and at rates of 2386 lbs/ac/yr, 2357 lbs/ac/yr, and 3093 lbs/ac/yr in the second 12 inches of soil, respectively. The rate of soil organic matter (SOM) accumulation on the seasonlong strategy was 28.6% greater than that on the nongrazed strategy. The rate of soil organic matter (SOM) accumulation on the twice-over strategy was 62.2% greater than that on the nongrazed and was 26.1% greater than that on the seasonlong strategy.

The quantity of soil organic carbon (SOC) on the silty ecological sites to the 24 inch soil depth on the long-term nongrazed strategy was 38.5 tons/ac during 2013-2014 and was 54.2 tons/ac during 2016-2018 with an increase of 40.7% (tables 39 and 40). The quantity of soil organic carbon (SOC) to the 24 inch soil depth on the traditional seasonlong strategy was 70.9 tons/ac during 2013-2014 and was 69.8 tons/ac during 2016-2018 with a decrease of 1.6% (tables 41 and 42). The quantity of soil organic carbon (SOC) to the 24 inch soil depth on the twice-over rotation strategy was 83.2 tons/ac during 2013-2014 and was 87.9 tons/ac during 2016-2018 with a increase of 5.7% (tables 43 and 44). During 2013-2014, the quantity of SOC on the seasonlong strategy was 84.4% greater than that on the nongrazed strategy (tables 39 and 41). The quantity of SOC on the twice-over strategy was 116.0% greater than that on the nongrazed (tables 39 and 43) and was 17.3% greater than that on the seasonlong strategy (tables 41 and 43). During 2016-2018, the quantity of SOC on the seasonlong strategy was 28.8% greater than that on the nongrazed strategy (tables 40 and 42). The quantity of SOC on the twice-over strategy was 62.2% greater than that on the nongrazed (tables 40 and 44) and was 25.9% greater than that on the seasonlong strategy (tables 42 and 44).

Soil organic carbon (SOC) has been accumulating in the top 24 inches of soil at mean rates of 3010 lbs/ac/yr, 3878 lbs/ac/yr, and 4881 lbs/ac/yr on the nongrazed, seasonlong, and twice-over strategies, respectively. The rate of soil organic carbon (SOC) accumulation on the seasonlong strategy was 27.8% greater than that on the nongrazed

strategy. The rate of soil organic carbon (SOC) accumulation on the twice-over strategy was 61.6% greater than that on the nongrazed and was 26.4% greater than that on the seasonlong strategy.

The quantity of soil organic nitrogen (SON) on the silty ecological sites to the 24 inch soil depth on the long-term nongrazed strategy was 3.9 tons/ac during 2013-2014 and was 5.4 tons/ac during 2016-2018 with an increase of 41.3% (tables 39 and 40). The quantity of soil organic nitrogen (SON) to the 24 inch soil depth on the traditional seasonlong strategy was 7.1 tons/ac during 2013-2014 and was 7.1 tons/ac during 2016-2018 with a decrease of 0.6% (tables 41 and 42). The quantity of soil organic nitrogen (SON) to the 24 inch soil depth on the twice-over rotation strategy was 8.3 tons/ac during 2013-2014 and was 9.0 tons/ac during 2016-2018 with a increase of 8.5% (tables 43 and 44). During 2013-2014, the quantity of SON on the seasonlong strategy was 84.2% greater than that on the nongrazed strategy (tables 39 and 41). The quantity of SON on the twice-over strategy was 116.1% greater than that on the nongrazed (tables 39 and 43) and was 17.4% greater than that on the seasonlong strategy (tables 41 and 43). During 2016-2018, the quantity of SON on the seasonlong strategy was 29.6% greater than that on the nongrazed strategy (tables 40 and 42). The quantity of SON on the twice-over strategy was 66.0% greater than that on the nongrazed (tables 40 and 44) and was 28.1% greater than that on the seasonlong strategy (tables 42 and 44).

Soil organic nitrogen (SON) has been accumulating in the top 24 inches of soil at mean rates of 304.9 lbs/ac/yr, 381.1 lbs/ac/yr, and 495.5 lbs/ac/yr on the nongrazed, seasonlong, and twice-over strategies, respectively. The rate of soil organic nitrogen (SON) accumulation on the seasonlong strategy was 25.0% greater than that on the nongrazed strategy. The rate of soil organic nitrogen (SON) accumulation on the twice-over strategy was 62.5% greater than that on the nongrazed and was 30.0% greater than that on the seasonlong strategy.

Discussion

The major essential elements of carbon, hydrogen, nitrogen, and oxygen have separate but closely linked biogeochemical cycles that transform the elements between organic forms and inorganic forms. Large quantities of the major essential elements are retained in the soil organic matter of grassland ecosystems as immobilized organic compounds. Soil microorganisms cycle a portion of the major essential elements from organic detritus

into inorganic forms each growing season. The quantity of essential elements mineralized by soil microorganisms is a major factor that determines the quantity of annual biomass production.

Soil organisms and grassland plants use the essential elements in the inorganic form to synthesize vital organic compounds of carbohydrates, proteins, and nucleic acids. Grass plants produce double the quantity of leaf biomass than needed for normal plant growth (Crider 1955, Coyne et al. 1995). All of the aboveground herbage biomass produced by perennial grasses in a growing season represents about 33% of the total biomass production. About 67% of the annual perennial grass biomass is produced belowground. About 50% of the aboveground biomass is expendable by the plant. About half of the expendable leaf material is removed as senescent leaves that are broken from the plant and fall to the ground, or as leaf material consumed by insects and wildlife. About half of the expendable leaf material, or 25% of the aboveground biomass can be consumed by grazing livestock.

Perennial grass leaf material consists of digestible nutrients and nondigestible structural components. About 15% of the nutrients contained in the consumed leaf material is extracted by stocker heifers and steers and retained for growth. About 30% of the nutrients contained in the consumed leaf material is extracted by lactating cows, with a portion retained by the cow for production, and the remainder of the extracted nutrients passed on to her calf for growth (Russelle 1992, Gibson 2009).

All of the nondigestible dry matter and most of the nutrients consumed by grazing livestock are deposited on the ground as manure in a couple of days. Most of the nutrients consumed and used by livestock for maintenance are returned to the ecosystem in the feces and urine. None of the herbage biomass dry matter produced during a growing season is removed by livestock from the grazingland ecosystem. All of the essential elements contained in the belowground biomass and those contained in the nonconsumed aboveground biomass stay in the ecosystem. Nearly all of the essential elements use in the annual production of herbage biomass and soil organism biomass are retained and recycled in the ecosystem.

Some major essential elements are lost or removed from the ecosystem as output. The metabolic process of respiration in soil organisms, plants, and animals results in a loss of some essential elements as carbon dioxide, water vapor, and heat

energy. Some essential elements are removed from the ecosystem as weight biomass produced by insects and wildlife. The essential elements transferred from grass plants to grazing animals and used for animal growth are removed from the ecosystem (Gibson 2009). If the grassland ecosystem is burned, almost all of the essential elements in the aboveground herbage are volatilized, and if the soil is dry, some of the belowground essential elements are also lost (Russelle 1992).

The small proportion of the ecosystem major essential elements that are lost or removed annually need to be replenished by capturing major essential elements as input through ecosystem processes. Atmosphere carbon dioxide is the ecosystem input for carbon. Precipitation of water is the ecosystem input for hydrogen. Wet deposition of nitrogen oxides following lightning discharges is the ecosystem input for nitrogen. Carbon dioxide, water, and nitrogen oxides are the ecosystem input for oxygen. Radiant light from the sun is the ecosystem input for energy. The input source of major essential elements are not part of the ecosystem resources until the ecosystem processes capture the input essential elements.

The grassland ecosystems managed with the twice-over strategy annually captured 1003 lbs/ac greater organic carbon than that captured on the ecosystems managed with the seasonlong strategy, and annually captured 1870 lbs/ac greater organic carbon that that captured on the ecosystems managed with the nongrazed strategy, and the annually captured organic carbon on the ecosystem managed with the seasonlong strategy were 867 lbs/ac greater than that captured on the ecosystems managed with the nongrazed strategy.

The grassland ecosystems managed with the twice-over strategy annually captured 114 lbs/ac greater organic nitrogen than that captured on the ecosystems managed with the seasonlong strategy and annually captured 191 lbs/ac greater organic nitrogen than that captured on the ecosystems managed with the nongrazed strategy, and the annually captured organic nitrogen on the ecosystems managed with the seasonlong strategy were 76 lbs/ac greater than that captured on the ecosystems managed with the nongrazed strategy.

The grassland ecosystems managed with the twice-over strategy annually captured 1744 lbs/ac greater organic matter than that captured on the ecosystems managed with the seasonlong strategy and annually captured 3215 lbs/ac greater organic matter than that captured on the ecosystems managed with

the nongrazed strategy, and the annually captured organic matter on the ecosystems managed with the seasonlong strategy were 1471 lbs/ac greater than that captured on the ecosystems managed with the nongrazed strategy.

The quantities of annually captured major essential elements were greater on the ecosystems managed with the twice-over strategy, while the quantities of annually captured major essential elements were lowest on the ecosystems managed with the nongrazed strategy, and the quantities of annually captured major essential elements on the ecosystems managed with the seasonlong strategy were in between.

The twice-over rotation grazing system is the biologically effective management strategy that was intentionally designed to increase the soil rhizosphere microorganism biomass enough to mineralize between 100 lbs/ac and 165 lbs/ac of mineral nitrogen and inorder to fully activate the four major internal grass plant growth mechanisms and to perform the other ecosystem biogeochemical processes at biologically potential levels in order to capture great enough quantities of major essential elements to completely replace the quantities that are annually lost or removed from the ecosystem.
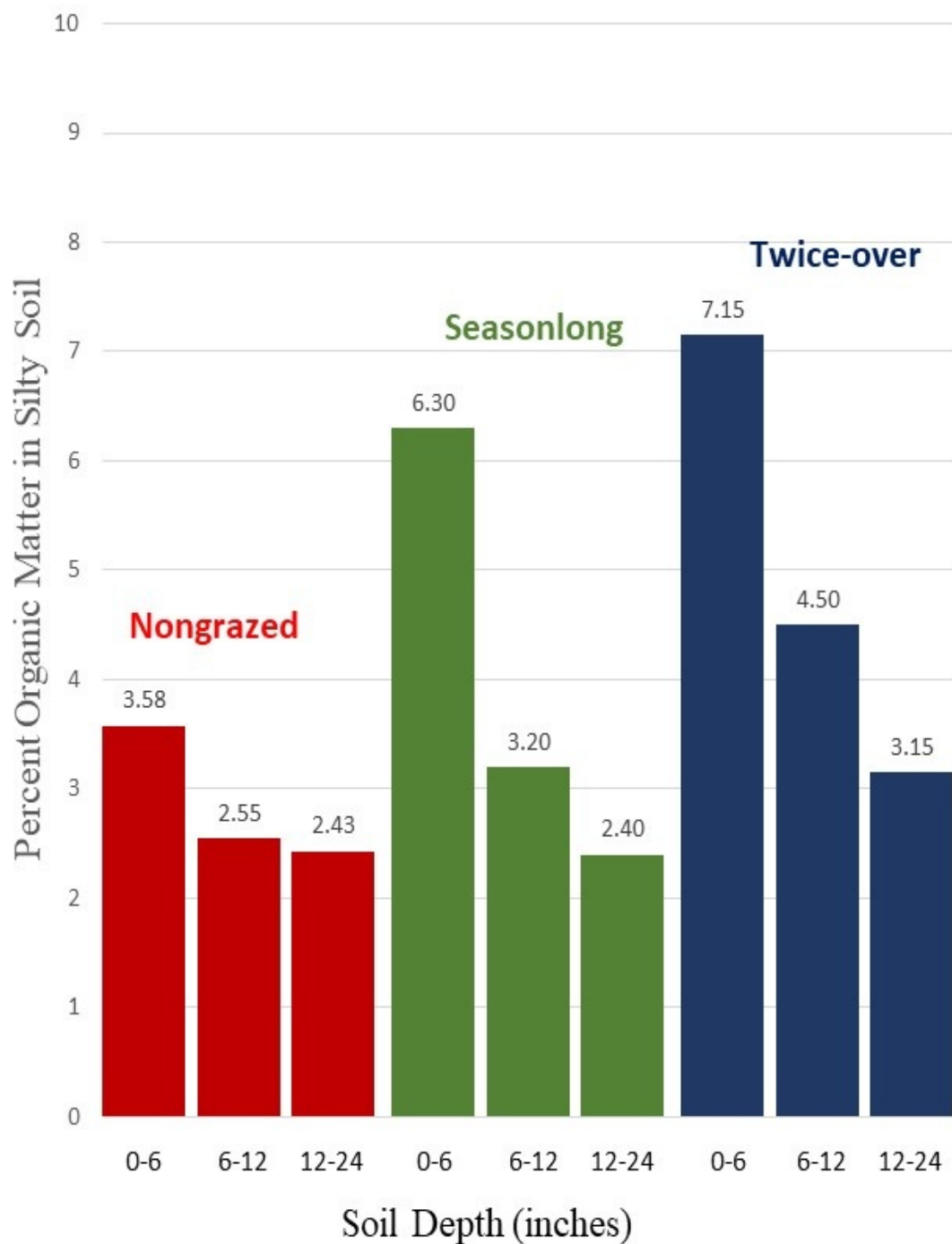
Figure 4. Distribution of organic matter (SOM) at incremental depths to 24 inches during June on silty ecological sites, 2016-2018

Table 39. Soil organic components, soil organic matter (SOM), carbon (SOC), and nitrogen (SON), at incremental depths to 24 inches during June on the silty ecological sites of the long-term nongrazed, 2013-2014.

Soil Organic Components	Soil Depth (inches)			
	0-6	6-12	12-24	0-24
SOM				
%	3.08	1.89	1.45	1.93
lbs/ac	48,053.99	33,333.89	51,281.84	132,669.72
tons/ac	24.03	16.67	25.64	66.33
SOC				
%	1.79	1.10	0.84	1.12
lbs/ac	27,927.48	19,400.68	29,708.10	77,036.26
tons/ac	13.96	9.70	14.85	38.52
SON				
%	0.18	0.11	0.08	0.11
lbs/ac	2,792.74	1,940.07	2,970.81	7,703.62
tons/ac	1.40	0.97	1.49	3.85

Table 40. Soil organic components, soil organic matter (SOM), carbon (SOC), and nitrogen (SON), at incremental depths to 24 inches during June on the silty ecological sites of the long-term nongrazed, 2016-2018.

Soil Organic Components	Soil Depth (inches)			
	0-6	6-12	12-24	0-24
SOM				
%	3.58	2.55	2.43	2.72
lbs/ac	55,979.28	45,074.40	85,906.51	186,960.19
tons/ac	27.99	22.54	42.95	93.48
SOC				
%	2.07	1.48	1.41	1.58
lbs/ac	32,367.91	26,160.83	49,846.99	108,375.75
tons/ac	16.18	13.08	24.92	54.18
SON				
%	0.21	0.15	0.14	0.16
lbs/ac	3,283.70	2,651.44	4,949.35	10,884.49
tons/ac	1.64	1.33	2.47	5.44

Table 41. Soil organic components, soil organic matter (SOM), carbon (SOC), and nitrogen (SON), at incremental depths to 24 inches during June on the silty ecological sites of the traditional seasonlong, 2013-2014.

Soil Organic Components	Soil Depth (inches)			
	0-6	6-12	12-24	0-24
SOM				
%	6.07	3.38	2.55	3.56
lbs/ac	94,703.80	59,612.99	90,185.30	244,502.09
tons/ac	47.35	29.81	45.09	122.25
SOC				
%	3.52	1.96	1.48	2.07
lbs/ac	54,918.84	34,568.48	52,342.84	141,830.16
tons/ac	27.46	17.28	26.17	70.90
SON				
%	0.35	0.20	0.15	0.21
lbs/ac	5,491.88	3,456.85	5,234.28	14,183.01
tons/ac	2.75	1.73	2.62	7.09

Table 42. Soil organic components, soil organic matter (SOM), carbon (SOC), and nitrogen (SON), at incremental depths to 24 inches during June on the silty ecological sites of the traditional seasonlong, 2016-2018.

Soil Organic Components	Soil Depth (inches)			
	0-6	6-12	12-24	0-24
SOM				
%	6.30	3.20	2.40	3.49
lbs/ac	98,511.03	56,563.96	84,845.94	239,920.93
tons/ac	49.26	28.28	42.42	119.96
SOC				
%	3.66	1.86	1.40	2.03
lbs/ac	57,230.22	32,877.80	49,493.46	139,601.48
tons/ac	28.12	16.44	24.75	69.80
SON				
%	0.37	0.19	0.14	0.21
lbs/ac	5,785.57	3,358.48	4,949.35	14,043.40
tons/ac	2.89	1.68	2.47	7.05

Table 43. Soil organic components, soil organic matter (SOM), carbon (SOC), and nitrogen (SON), at incremental depths to 24 inches during June on the silty ecological sites of the twice-over rotation system, 2013-2014.

Soil Organic Components	Soil Depth (inches)			
	0-6	6-12	12-24	0-24
SOM				
%	5.98	4.19	3.38	4.18
lbs/ac	93,299.62	73,898.95	119,539.74	286,738.31
tons/ac	46.65	36.95	59.77	143.37
SOC				
%	3.47	2.43	1.96	2.42
lbs/ac	54,138.74	42,857.86	69,318.90	166,315.50
tons/ac	27.07	21.43	34.66	83.16
SON				
%	0.35	0.24	0.20	0.24
lbs/ac	5,413.87	4,285.79	6,931.89	16,631.55
tons/ac	2.71	2.14	3.47	8.32

Table 44. Soil organic components, soil organic matter (SOM), carbon (SOC), and nitrogen (SON), at incremental depths to 24 inches during June on the silty ecological sites of the twice-over rotation system, 2016-2018.

Soil Organic Components	Soil Depth (inches)			
	0-6	6-12	12-24	0-24
SOM				
%	7.15	4.50	3.15	4.41
lbs/ac	111,802.20	79,543.06	111,360.29	302,705.55
tons/ac	55.90	39.77	55.68	151.35
SOC				
%	4.15	2.61	1.83	2.56
lbs/ac	64,892.18	46,134.98	64,695.03	175,722.19
tons/ac	32.45	23.07	32.35	87.86
SON				
%	0.42	0.27	0.19	0.26
lbs/ac	6,567.40	4,772.58	6,716.97	18,056.95
tons/ac	3.28	2.39	3.36	9.03

D6. 2016-2018 Study, Evaluation of Soil Microbe Biomass and Activity

Soil functions are directly or indirectly related to the soil microorganisms. The soil biogeochemical processes are driven by the biomass and activity of soil microbes. Soil microorganisms recycle the essential elements required for life on earth. Measuring soil microbe biomass and activity is fundamental to understanding and managing the processes performed by these critical organisms. However, measuring the biomass and activity of soil microbes is a perplexing difficulty.

These critters are tiny, microzoan, less than 0.5 microns in diameter, with one thousand microns equal to one millimeter.   Most of these soil organisms cannot be cultured on agar plates. Fortunately, microbial cell membranes contain phospholipid fatty acids (PLFA's). The PLFA's can be extracted from soil samples with the amount of total PLFA's indicating the viable microbial biomass and the various different lipid classes serving as unique signatures (biomarkers) for specific functional groups of microorganisms.

The microbes in cropland mixed soils in which annual plants grow are all free-living in the soil edaphosphere (earth sector or zone). Some of these microbes do not associate with plants. Two types of free-living microbes associate with plants, some of these microbes are plant-detrimental (pathogenic) and some are plant-beneficial. The free-living plant-beneficial microbes live in a zone of unaltered soil surrounding active annual roots. This volume of loose soil is called a rhizosphere which exists only as long as the annual root remains active.

The microbes in intact soils of grasslands in which perennial plants grow are symbiotic with the plants and inhabit a cylinder of soil particles bonded by fungal secreted adhesive polysaccharides that surrounds perennial roots are also called a rhizosphere (root sector or zone). The rhizosphere microorganisms perform grassland ecosystem biogeochemical processes that renew nutrient flow activities in the intact soil. Biogeochemical processes transform stored essential elements from organic forms into plant-usable inorganic forms. These biogeochemical processes also capture replacement quantities of lost or removed major essential elements of carbon, hydrogen, nitrogen, and oxygen, with assistance from active live plants, and transform them into storage as organic forms for later use. And they also decompose complex unusable organic material into compounds and then into reusable essential elements (McNaughton 1979, 1983; Coleman et al.

1983; Ingham et al. 1985; Mueller and Richards 1986; Richards et al. 1988; Briske 1991; Murphy and Briske 1992; Briske and Richards 1994, 1995). The quantity of biogeochemical processes taking place in grassland ecosystems is dependent on the rhizosphere volume and microorganism biomass (Coleman et al. 1983). Both these factors are limited by access to simply carbohydrate energy (Curl and Truelove 1986). Healthy grass plants produce double the quantity of leaf biomass (Crider 1955; Coyne et al. 1995), capture and fix large amounts of carbon during photosynthesis, and produce carbohydrates in quantities greater than the amount required for normal growth and maintenance (Coyne et al. 1995). Partial defoliation of grass tillers at the vegetative phenological growth stages by large grazing graminivores cause significant quantities of exudates containing simple carbohydrates to be released from the grass tillers through the roots into the rhizosphere (Hamilton and Frank 2001). As a consequence, the biomass and activity of microorganisms also increase (Anderson et al. 1981; Curl and Truelove 1986; Whipps 1990), resulting in greater biogeochemical cycling of essential elements (Coleman et al. 1983; Biondini et al. 1988; Klein et al. 1988; Burrows and Pfeger 2002; Rillig et al. 2002; Bird et al. 2002; Driver et al. 2005).

The rhizosphere is comprised of bacteria, protozoa, nematodes, springtails, mites, endomycorrhizal fungi, and ectomycorrhizal fungi. Bacteria are microscopic single-celled saprophitic organisms that collectively consume large quantities of soil organic matter and are one of the primary producers of the rhizosphere. Protozoa are single-celled microorganisms that are mainly small amoeba and feed primarily on bacteria. Nematodes are a diverse group of small nonsegmented worms. Most nematodes feed on bacteria or fungi, some feed on protozoa, and some eat other nematodes. Springtails are the most abundant insect in grassland soils and they ingest considerable quantities of soil organic matter in order to eat fungi and bacteria. Mites are small eight-legged arachnids that feed on fungi, nematodes, small insects, and other mites. The nematodes, springtails, and mites travel among rhizosphere structures. Fungi are achlorophyllous saprophytes that live on dead organic matter. Endomycorrhizal fungi develop arbuscules, vesicles, and hyphae within root tissue of the host plant and secrete adhesive polysaccharides that bond soil particles around grass roots forming the structural environment for rhizosphere organisms. Ectomycorrhizal fungi develop a sheath around the

root with hyphae and do not enter the tissue of the host plant (Harley and Smith 1983). The bacteria and fungi are the microflora saprotrophic organisms at the bottom of the food chain and makeup the greatest biomass of the rhizosphere.

Most European Union (EU) countries established programs to monitor soil quality and the state of biodiversity following the adoption of the Treaty on Biological Diversity of Rio de Janeiro in 1992. As a result, refined microbiological methods for assessing soil quality required development with contributions from many countries over a period from 1992 until 2006. This research activity in EU countries stimulated the development of specific methods for Soil Health Analysis for North American soils conducted over a period from 1996 to 2011. Development of these new sophisticated methods are important because soil quality and ecosystem biogeochemical processes depend on the biomass and activity levels of microorganisms which perform the key functions in decomposition and nutrient cycling.

Soil microbe biomass and activity in grassland ecosystems of the Northern Mixed Grass Prairie were evaluated using the recently developed methods for the Haney-Soil Health Analysis conducted at the Ward Laboratories, Kearney, NE.

Procedure

Biomass of functional groups of soil microbes, including Total Microbes, Total Bacteria, Total Fungi, Protozoa, and Unknown, were determined by phospholipid fatty acid (PLFA) analysis. Microbial activity in soil was measured with the Solvita 1-day CO_2-C procedure that determined soil biological CO_2 respiration from the quantity of CO_2-C in ppm released in 24 hr from soil microbes after the soil had been dried and rewetted. Percent organic matter (% LOI), soluble salts, and soil pH were determined by standard methods. Total organic carbon and total nitrogen were determined by the water extraction method. Inorganic nitrogen (both nitrate and ammonium), and total phosphorus (both organic and inorganic) were determined by the plant organic acids (H3A) extraction method. Plant essential elements of Potassium, Calcium, Magnesium, Sulfur, Iron, Manganese, Zinc, and Copper, plus Sodium, and Aluminum were analyzed by the plant organic acids (H3A) extraction method. These analysis were conducted by the Ward Laboratory in Kearney, NE, on replicated soil core samples collected with the 1 inch Veihmeyer soil tube at incremental depths of 0-6, 6-12, and 12-24 inches on silty ecological sites of three management strategies: a) long-term nongrazed, b) traditional seasonlong, and c) twice-over rotation system during June and July monthly periods 2016 and 2018 and during June 2017.

Results

During the 3 year period of 2016 to 2018, the mean June and July precipitation was normal at 4.60 inches (82.88% of LTM). During 2016, June and July received 5.57 inches (100.36% of LTM). During 2017, June and July received 1.99 inches (35.86% of LTM). And during 2018, June and July received 6.24 inches (112.43% of LTM).

During 2016 to 2018, relatively new soil biological methods were used to analyze soil microbe activity. Annual data analyzed by the Salvita CO_2 and PLFA (phospholipid fatty acid) analysis are in appendix tables 22 to 24. Annual data analyzed by standard methods and water extraction are in appendix tables 25 to 27. Annual data analyzed by H3A (plant organic acids) extraction are in appendix tables 28 to 35.

Aerobic respiration by soil microbes uses oxygen to metabolize carbohydrates in order to gain chemical energy; complete oxidation during this process releases carbon dioxide and water. The quantity of carbon dioxide (CO_2) released in 24 hours indicates the level of microorganism activity. The weight of microbial carbon dioxide released in 24 hours on the twice-over strategy was 1265.37 lbs/ac CO_2 with a microbe biomass of 8014.30 ng/g, on the seasonlong strategy microbial activity released 1091.20 lbs/ac CO_2 with a microbe biomass of 8882.92 ng/g, and on the nongrazed strategy microbial activity released 752.53 lbs/ac CO_2 with a microbe biomass of 7024.04 ng/g (table 45). The quantity of CO_2 released on the seasonlong was 45.0% greater with a 26.5% greater microbe biomass than that on the nongrazed strategy. The quantity of CO_2 released on the twice-over strategy was 68.2% greater with a 14.1% greater microbe biomass than that on the nongrazed strategy, and the quantity of CO_2 released was 16.0% greater with a 9.8% smaller microbe biomass than that on the seasonlong strategy.

The biomass of fungi was 814.69 ng/g (11.6%) on the nongrazed strategy, 1040.55 ng/g (11.7%) on the seasonlong strategy, and 1146.40 ng/g (14.3%) on the twice-over strategy (table 45). Fungi biomass on the twice-over strategy was 40.7% greater than that on the nongrazed strategy and was 10.2% greater than that on the seasonlong strategy. Fungi

biomass on the seasonlong strategy was 27.7% greater than that on the nongrazed strategy.

The biomass of protozoa was 32.16 ng/g (0.5%) on the nongrazed strategy, 41.47 ng/g (0.5%) on the seasonlong strategy, and 42.07 ng/g (0.5%) on the twice-over strategy (table 45). Protozoa biomass on the twice-over strategy was 30.8% greater than that on the nongrazed strategy and was 1.5% greater than that on the seasonlong strategy. Protozoa biomass on the seasonlong strategy was 29.0% greater than that on the nongrazed strategy.

The biomass of bacteria was 3288.40 ng/g (46.8%) on the nongrazed strategy, 4206.22 ng/g (47.4%) on the seasonlong strategy, and 3794.91 ng/g (47.4%) on the twice-over strategy (table 45). Bacteria biomass on the seasonlong strategy was 27.9% greater than that on the nongrazed strategy, and was 10.8% greater than that on the twice-over strategy. Bacteria biomass on the twice-over strategy was 15.4% greater than that on the nongrazed strategy.

The biomass of unknown microbes was 2888.26 ng/g (41.1%) on the nongrazed strategy, 3607.42 ng/g (40.6%) on the seasonlong strategy, and 3032.37 ng/g (37.8%) on the twice-over strategy (table 45). Unknown microbe biomass on the seasonlong strategy was 24.9% greater than that on the nongrazed strategy, and was 19.0% greater than that on the twice-over strategy. Unknown microbe biomass on the twice-over strategy was 5.0% greater than that on the nongrazed strategy.

The quantity of unknown microbes was about 40% of the total microbe biomass which was a large portion, most likely related to being from grassland soils rather than from cropland soils. Only 0.5% of the microbe biomass was identified as protozoan which was extremely low and assumed that most grassland protozoa are unknown in cropland soils.

Soil pH is the relationship of the activity of the hydrogen ions related to the activity of the hydroxyl ions. When the quantity of hydrogen ions is increasingly greater than the quantity of hydroxyl ions, the soil acidity becomes greater. When the quantity of hydroxyl ions is increasingly greater than the quantity of hydrogen ions, the soil alkalinity becomes greater. When the quantity of hydrogen ions and the quantity of hydroxyl ions are balanced, the soil is neutral. The soil pH at incremental depths to 24 inches during June and July on the silty ecological sites of all management strategies at all soil depths

were neutral, except the 12-24 inch soil depth on the twice-over strategy was moderately alkaline (table 46).

The quantity of soluble salts are measured by electrical conductivity (EC in mmhos/cm) to indicate relative degree of growth problems from salts for plants; at low concentrations of soluble salts, plant growth is unimpeded and at high concentrations of soluble salts, the salts become toxic and hinder plant growth. The quantity of soluble salts at incremental depths to 24 inches during June and July on the silty ecological sites of all management strategies at all soil depths were low causing no problems for plant growth (table 46).

Soil organic matter (SOM) is a portion of a soil comprised of organic plant and animal residue at various stages of decomposition and is the primary nutrient reservoir for essential elements. The quantity of soil organic matter (SOM) analyzed by the new methods were 22.8% lower on the nongrazed, 10.6% lower on the seasonlong, and 24.0% lower on the twice-over strategies than that analyzed by the traditional methods from replicate soil samples. The soil organic matter on the twice-over strategy was 59.5% greater than that on the nongrazed strategy, and was 7.4% greater than that on the seasonlong strategy. The soil organic matter on the seasonlong strategy was 48.6% greater than that on the nongrazed strategy (table 46).

Carbon and nitogen are extremely important major essential elements. The water extractable organic carbon (WEOC) is a very small portion of carbon energy from the soil organic matter (SOM) that is readily available to soil microbes. The WEOC was 1.6% of SOM on the nongrazed strategy, the WEOC was 1.2% of SOM on the seasonlong strategy, and the WEOC was 1.5% of SOM on the twice-over strategy (table 46). The WEOC on the nongrazed strategy was 61.7 times smaller than the SOM, on the seasonlong strategy the WEOC was 83.9 times smaller than the SOM, and on the twice-over strategy the WEOC was 66.6 times smaller than the SOM. The quantity of WEOC on the twice-over strategy was 35.0% greater than that on the seasonlong strategy and was 47.6% greater than that on the nongrazed strategy, and WEOC on the seasonlong strategy was 9.3% greater than that on the nongrazed strategy.

The water extractable organic nitrogen (WEON) is an extremely small portion of the total soil nitrogen from the soil organic matter (SOM) and is the minute quantity available to soil microbes that

is easily broke down into inorganic nitrogen forms. The WEON on the nongrazed strategy was 0.11% of SOM, the WEON was 0.08% of SOM on the seasonlong strategy, and the WEON was 0.10% of SOM on the twice-over strategy (table 46). The quantity of WEON on the twice-over strategy was 28.2% greater than that on the seasonlong strategy and was 35.8% greater than that on the nongrazed strategy, and WEON on the seasonlong strategy was 6.0% greater than that on the nongrazed strategy.

H3A is a produced mixture of organic acids used in soil test laboratories as a soil extractant that mimics the plant roots' natural process for acquiring nutrients unassisted by symbiotic soil microbes. Inorganic nitrogen analyzed by H3A extraction had much lower results than traditional wet chemistry. Nitrate determined by H3A extraction was about 2.1% lower than nitrate determined by wet chemistry and ammonium determined by H3A extraction was about 68.6% lower than ammonium determined by wet chemistry. Mineral nitrogen analyzed by H3A plant organic acid extract was 59.08 lbs/ac with 14.73 lbs/ac of nitrate and 44.35 lbs/ac of ammonium of the twice-over strategy. Mineral nitrogen was 40.54 lbs/ac with 11.38 lbs/ac of nitrate and 29.16 lbs/ac of ammonium on the seasonlong strategy. Mineral nitrogen was 32.12 lbs/ac with 8.54 lbs/ac of nitrate and 23.58 lbs/ac of ammonium on the nongrazed strategy (table 47). Mineral nitrogen was 45.7% greater, ammonium was 52.1% greater, and nitrate was 29.4% greater on the twice-over strategy than that on the seasonlong strategy. Mineral nitrogen was 83.9% greater, ammonium was 88.1% greater, and nitrate was 72.5% greater on the twice-over strategy than that on the nongrazed strategy. Mineral nitrogen was 26.2% greater, ammonium was 23.7% greater, and nitrate was 33.3% greater on the seasonlong strategy than that on the nongrazed strategy (table 47).

Phosphorus, potassium, calcium, magnesium, and sulfur are macronutrients required by both plants and animals. Sodium is a macronutrient required by animals and used by warm season plants and cacti. Iron, manganese, zinc, and copper are micronutrients required by both plants and animals. Carbon, nitrogen, phosphorus, and sulfur have both organic and inorganic forms that require soil microbe activity for transformation to be plant available. Calcium, magnesium, potassium, zinc, and copper have ionic forms that are chemically immobilized as ionic-colloidal complexes that require soil microbe activity to be plant available. These macro- and micronutrients extracted from soil by H3A plant organic acids mimic the natural process used by plant roots unaided by soil microbes and are thus only a small portion of the macro- and micronutrients present in the soil, albeit, not as readily available as the portion extracted by H3A and require soil microbe action to be plant available.

Phosphorus analyzed by H3A plant organic acid extract was 91.85 lbs/ac with 38.50 lbs/ac organic and 53.35 lbs/ac inorganic on the twice-over strategy. Total phosphorus was 77.27 lbs/ac with 42.05 lbs/ac organic and 35.22 lbs/ac inorganic on the seasonlong strategy. Total phosphorus was 84.33 lbs/ac with 39.65 lbs/ac organic and 44.68 lbs/ac inorganic on the nongrazed strategy (table 47). Total phosphorus was 18.9% greater, organic was 8.4% lower, and inorganic was 51.5% greater on the twice-over strategy than that on the seasonlong strategy. Total phosphorus was 8.9% greater, organic was 2.9% lower, and inorganic was 19.4% greater on the twice-over strategy than that on the nongrazed strategy. Total phosphorus was 8.4% lower, organic was 6.1% greater, and inorganic was 21.2% lower on the seasonlong strategy than that on the nongrazed strategy (table 47).

The macronutrient potassium (K) analyzed by H3A plant organic acid extract was 1414.50 lbs/ac on the twice-over strategy, 1179.34 lbs/ac on the seasonlong strategy, and 1033.05 lbs/ac on the nongrazed strategy (table 48). Potassium on the twice-over strategy was 36.9% greater than that on the nongrazed strategy, and was 19.9% greater than that on the seasonlong strategy. Potassium on the seasonlong strategy was 14.2% greater than that on the nongrazed strategy.

The macronutrient calcium (Ca) analyzed by H3A plant organic acid extract was 11,683.55 lbs/ac on the twice-over strategy, 6824.79 lbs/ac on the seasonlong strategy, and 6464.49 lbs/ac on the nongrazed strategy (table 48). Calcium on the twice-over strategy was 80.7% greater than that on the nongrazed strategy, and was 71.2% greater than that on the seasonlong strategy. Calcium on the seasonlong strategy was 5.6% greater than that on the nongrazed strategy.

The macronutrient magnesium (Mg) analyzed by H3A plant organic acid extract was 2971.88 lbs/ac on the twice-over strategy, 1686.62 lbs/ac on the seasonlong strategy, and 1491.25 lbs/ac on the nongrazed strategy (table 48). Magnesium on the twice-over strategy was 99.3% greater than that on the nongrazed strategy, and was 76.2% greater than that on the seasonlong strategy. Magnesium on

the seasonlong strategy was 13.1% greater than that on the nongrazed strategy.

The macronutrient sulfur (S) analyzed by H3A plant organic acid extract was 68.94 lbs/ac on the twice-over strategy, 34.68 lbs/ac on the seasonlong strategy, and 32.24 lbs/ac on the nongrazed strategy (table 48). Sulfur on the twice-over strategy was 113.8% greater than that on the nongrazed strategy, and was 98.8% greater than that on the seasonlong strategy. Sulfur on the seasonlong strategy was 7.6% greater than that on the nongrazed strategy.

The plant available macronutrients on the twice-over strategy were 82.7% greater than those on the nongrazed strategy, and was 66.5% greater than those on the seasonlong strategy. The plant available macronutrients on the seasonlong strategy were only 10.1% greater than those on the nongrazed strategy.

Sodium (Na) is not an essential element for plants and has the potential to result in sodic affected soils. Sodium analyzed by H3A plant organic acid extract was 220.26 lbs/ac on the twice-over strategy, 150.52 lbs/ac on the seasonlong strategy, and 171.73 lbs/ac on the nongrazed strategy (table 48). Sodium on the twice-over strategy was 28.3% greater than that on the nongrazed strategy, and was 46.3% greater than that on the seasonlong strategy. The twice-over strategy had 108.09 lbs/ac of increased sodium at the 12-24 inch soil depth which also had an elevated pH of 8.15, moderately alkaline, and an electrical conductivity of 0.3 mmhos/cm, low, indicating that at these levels, the sodium is not much of a problem. Sodium on the seasonlong strategy was 12.4% lower than that on the nongrazed strategy.

The micronutrient manganese (Mn) analyzed by H3A plant organic acid extract was 49.01 lbs/ac on the twice-over strategy, 24.60 lbs/ac on the seasonlong strategy, and 16.74 lbs/ac on the nongrazed strategy (table 49). Manganese on the twice-over strategy was 192.8% greater than that on the nongrazed strategy, and was 99.2% greater than that on the seasonlong strategy. Manganese on the seasonlong strategy was 47.0% greater than that on the nongrazed strategy.

The micronutrient copper (Cu) analyzed by H3A plant organic acid extract was 1.18 lbs/ac on the twice-over strategy, 0.81 lbs/ac on the seasonlong strategy, and 0.64 lbs/ac on the nongrazed strategy (table 49). Copper on the twice-over strategy was 84.4% greater than that on the nongrazed strategy, and was 45.7% greater than that on the seasonlong

strategy. Copper on the seasonlong strategy was 26.6% greater than that on the nongrazed strategy.

The micronutrient iron (Fe) analyzed by H3A plant organic acid extract was 923.92 lbs/ac on the nongrazed strategy, 730.31 lbs/ac on the seasonlong strategy, and 581.80 lbs/ac on the twice-over strategy (table 49). Iron on the nongrazed strategy was 58.8% greater than that on the twice-over strategy, and was 26.5% greater than that on the seasonlong strategy. Iron on the seasonlong strategy was 25.5% greater than that on the twice-over strategy.

The micronutrient zinc (Zn) analyzed by H3A plant organic acid extract was 3.07 lbs/ac on the seasonlong strategy, 2.31 lbs/ac on the nongrazed strategy, and 1.75 lbs/ac on the twice-over strategy (table 49). Zinc on the seasonlong strategy was 75.4% greater than that on the twice-over strategy, and was 32.9% greater than that on the nongrazed strategy. Zinc on the nongrazed strategy was 32.0% greater than that on the twice-over strategy.

Manganese and Copper had the greatest plant availability on the twice-over strategy and the lowest plant availability on the nongrazed strategy. Iron had the greatest plant availability on the nongrazed strategy, zinc had the greatest plant availability on the seasonlong strategy and both iron and zinc had the lowest plant availability on the twice-over strategy.

Aluminum is not an essential element for either plants or animals. Aluminum ions at high concentrations can bond with enough hydroxyl ions removing the hydroxyl ions from solution moving the soil pH towards acidity. Aluminum (Al) analyzed by H3A plant organic acid extract was 1969.17 lbs/ac on the nongrazed strategy, 1656.79 lbs/ac on the seasonlong strategy, and 1488.81 lbs/ac on the twice-over strategy (table 49). Aluminum on the nongrazed strategy was 32.3% greater than that on the twice-over strategy, and was 18.9% greater than that on the seasonlong strategy. Aluminum on the seasonlong strategy was 11.3% greater than that on the twice-over strategy. The pH level on all of the management strategies at all of the soil depths were neutral or moderately alkaline and no pH level was acid (table 46).

The quantities of macronutrients and micronutrients analyzed by H3A plant organic acid extract consist of the small portion that is readily available to plants from cropland soils by secreted organic acids unassisted by soil microbes. In

grassland soils where symbiotic soil microbes are surrounding native grass plant active roots, the quantities of readily available macronutrients and micronutrients would be expected to be much greater than the amounts analyzed by H3A plant organic acid extract techniques.

Summary of Results

The Haney Soil Health Test analyzes numerous chemical and biological components of soil to quantitatively evaluate soil health. Most of these components were greater in the soils managed with the twice-over strategy, while most of these components were lower in the soils managed with the long-term nongrazed strategy, and the components in soils managed with the traditional seasonlong strategy were usually in between.

The nongrazed strategy had the lowest microbial activity and the lowest microbe biomass. The twice-over strategy had the greatest microbial activity at 16.0% greater than that on the seasonlong strategy, and had similar but 9.8% lower microbial biomass than that on the seasonlong strategy. The soil microorganisms on the twice-over strategy appear to have transformed through some selection process resulting in fewer microorganisms with a lower microbial biomass that can maintain a greater activity level and perform greater quantities of biogeochemical processes.

The nongrazed strategy had the lowest quantity of soil organic matter (SOM) that was 32.7% lower than that on the seasonlong strategy and was 37.2% lower than that on the twice-over strategy. The twice-over strategy had the greatest SOM that was 7.4% greater than that on the seasonlong strategy. The nongrazed strategy had the lowest water extractable organic carbon and organic nitrogen that was 8.5% lower and 5.6% lower than the WEOC and WEON on the seasonlong strategy, respectively. The twice-over strategy had the greatest WEOC and WEON that was 35.0% greater and 28.2% greater than the WEOC and WEON on the seasonlong strategy, respectively.

The nongrazed strategy had the lowest quantity of total inorganic nitrogen that was 20.8% lower than that on the seasonlong strategy and had the lowest quantities of nitrate and ammonium that were 25.0% lower and 19.1% lower than those on the seasonlong strategy, respectively. The twice-over strategy had the greatest quantity of total inorganic nitrogen that was 45.7% greater than that on the seasonlong strategy and had the greatest quantities of nitrate and ammonium that were 29.4% greater and 52.1% greater than those on the seasonlong strategy, respectively.

The seasonlong strategy had the lowest quantities of total phosphorus and inorganic phosphorus that were 8.4% lower and 21.2% lower than those on the nongrazed strategy, respectively, and the twice-over strategy had the lowest quantities of organic phosphorus that was 2.9% lower than that on the nongrazed strategy. The twice-over strategy had the greatest quantities of total phosphorus and inorganic phosphorus that were 8.9% greater and 19.4% greater than those on the nongrazed strategy, respectively, and the seasonlong strategy had the greatest quantity of organic phosphorus that was 6.1% greater than that on the nongrazed strategy.

The nongrazed strategy had the lowest quantities of the macronutrients, potassium, calcium, magnesium, and sulfur, that were 12.4% lower, 5.3% lower, 11.6% lower, and 7.0% lower, than those on the seasonlong strategy, respectively. The twice-over strategy had the greatest quantities of the macronutrients, potassium, calcium, magnesium, and sulfur, that were 19.9% greater, 71.2% greater, 76.2% greater, and 98.8% greater, than those on the seasonlong strategy, respectively.

The nongrazed strategy had the lowest quantities of the micronutrients, manganese and copper, that were 32.0% lower and 21.0% lower, than those on the seasonlong strategy, respectively. The twice-over strategy had the greatest quantities of the micronutrients, manganese and copper, that were 99.2% greater and 45.7% greater, than those on the seasonlong strategy, respectively.

The twice-over strategy had the lowest quantities of the micronutrient, iron, that was 20.3% lower than that on the seasonlong strategy, and had the lowest quantity of the micronutrient, zinc, that was 24.2% lower than that on the nongrazed strategy. The nongrazed strategy had the greatest quantity of the micronutrient, iron, that was 26.5% greater than that on the seasonlong strategy. The seasonlong strategy had the greatest quantity of the micronutrient, zinc, that was 32.9% greater than that on the nongrazed strategy,

Discussion

The Haney Soil Health Test was designed to evaluate soil health of mixed cropland soils. The Haney Soil Health data sorted the intact grassland soils of the three management strategies into the same

order as the wet chemistry data. The soil health number for the twice-over strategy was 18% greater than that for the seasonlong strategy and 48% greater than that for the nongrazed strategy. The soil health number for the seasonlong strategy was 25% greater than that for the nongrazed strategy. The pounds per acre of available nitrogen, phosphorus, and potassium on the twice-over strategy was 35%, 28%, and 22% greater than those on the seasonlong strategy, and was 71%, 12%, and 58% greater than those on the nongrazed strategy, respectively. The pounds per acre of available nitrogen, and potassium on the seasonlong strategy was 26% and 29% greater than those on the nongrazed strategy, respectively, and the pounds per acre of available phosphorus on the nongrazed strategy was 14% greater than that on the seasonlong strategy.

The soil carbon dioxide respiration test measures soil microbe activity braking down the available carbohydrates in the soil organic matter. The microbe activity on the twice-over strategy was 16% greater than that on the seasonlong strategy and was 68% greater than that on the nongrazed strategy. The microbe activity on the seasonlong strategy was 45% greater than that on the nongrazed strategy. The solvita test does not measure microbe activity in the rhizosphere resulting for exudation of surplus grass plant fixed carbon energy.

The Phospholipid Fatty Acid (PLFA) analysis measures the quantity of the different biomarkers of phospholipid fatty acids representing the various functional groups of soil microbes. Except about 40% of the microbes in grassland soils are unknown. The twice-over strategy had greater biomass of fungi and protozoa than the other two management strategies but had lower bacteria biomass and total microbe biomass than those on the seasonlong strategy.

The quantities of available essential elements were measured by water extraction or by H3A plant organic acid extraction which evaluate the amount of each essential element available in the soil that could be obtained by plant processes alone without assistance from soil microbes. The quantities of available essential elements were greatest on the twice-over strategy, second on the seasonlong strategy, and lowest on the nongrazed strategy for all essential elements except phosphorus, iron, and zinc.

The mixed cropland soils contain only free living edaphosphere microbes. The plant beneficial microbes congregate near annual crop plant roots that leak substance that microbes can use for energy. The plant beneficial microbes may assist the annual plants by procuring some essential elements, but probably only a small portion of their needs.

The intact grassland soils with perennial grasses contain symbiotic rhizosphere microbes that vary in biomass depending on the quantity of plant surplus carbon energy exudated into the rhizosphere. Perennial grasses on the nongrazed strategy would contain the lowest microbe biomass that receive carbon energy at the plant leakage rate. The microbe biomass would be a little greater on the seasonlong strategy than that on the nongrazed strategy because the microbes would receive the regular leakage amount plus some exudated plant carbon energy unintentionally released through cattle grazing. The microbe biomass would be greatest on the twice-over strategy as a result of coordinated partially defoliated lead tillers at vegetative growth stages between the three and a half new leaf stage and the flower stage causing large quantities of surplus carbon energy to be exudated through the grass roots into the rhizosphere available for microbe biomass to increase to levels that can mineralize greater than 100 lbs/ac of mineral nitrogen and that can reestablish the full functionality of all ecosystem biogeochemical processes.

Table 45. Soil microbe biomass (ng/g) by phospholipid fatty acid (PLFA) analysis at soil depths to 24 in deep, June and July, 2016-2018.

Soil Microbes	Soil Depth inches	Management Treatments		
		Nongrazed 34 -36 yrs.	Seasonlong 30-32 yrs	Twice-over 34-36 yrs.
Microbial Activity CO$_2$-C released/24 hr				
lbs/ac	0-6	434.13	666.41	729.49
	6-12	216.15	258.94	357.35
	12-24	102.25	165.68	178.53
	0-24	752.53	1091.02	1265.37
Total Microbes				
ng/g	0-6	4240.39	5192.99	4354.85
	6-12	1554.22	1852.45	2120.24
	12-24	1229.44	1837.49	1539.22
	0-24	7024.04	8882.92	8014.30
Total Bacteria				
ng/g	0-6	2149.59	2607.37	2166.11
	6-12	641.89	825.49	1002.58
	12-24	496.93	773.37	626.22
	0-24	3288.40	4206.22	3794.91
Total Fungi				
ng/g	0-6	575.21	670.01	583.00
	6-12	128.52	170.16	315.15
	12-24	110.96	200.39	248.26
	0-24	814.69	1040.55	1146.40
Protozoa				
ng/g	0-6	27.38	31.73	29.30
	6-12	4.78	4.73	10.79
	12-24	0.00	5.02	1.98
	0-24	32.16	41.47	42.07
Unknown				
ng/g	0-6	1488.41	1896.41	1577.88
	6-12	779.08	852.29	791.72
	12-24	620.78	858.73	662.77
	0-24	2888.26	3607.42	3032.37

Table 46. Essential element weight (lbs/ac) by water extract at soil depths to 24 in deep, June and July, 2016-2018.

Essential Elements		Soil Depth inches	Management Treatments		
			Nongrazed 34 -36 yrs.	Seasonlong 30-32 yrs	Twice-over 34-36 yrs.
Soil pH					
		0-6	7.00	6.81	6.83
		6-12	6.94	6.79	7.14
		12-24	7.22	7.34	8.15
Soluble Salts					
	mmho/cm	0-6	0.15	0.18	0.23
		6-12	0.12	0.11	0.20
		12-24	0.14	0.19	0.30
Organic Matter					
	%	0-6	3.38	6.00	5.52
		6-12	2.24	2.85	3.54
		12-24	1.47	1.99	2.29
Total Organic Carbon					
	lbs/ac	0-6	951.20	1148.55	1511.90
		6-12	792.93	829.10	1258.47
		12-24	595.52	579.75	683.35
		0-24	2339.65	2557.40	3453.72
Total Nitrogen					
	lbs/ac	0-6	81.66	98.66	136.56
		6-12	59.85	62.61	95.75
		12-24	43.54	37.57	37.70
		0-24	185.05	198.84	270.00
Organic Nitrogen					
	lbs/ac	0-6	69.64	81.48	104.44
		6-12	51.70	55.78	79.60
		12-24	39.16	32.79	33.91
		0-24	160.50	170.05	217.95

Table 47. Essential element weight (lbs/ac) by H3A (plant organic acids) extract at soil depths to 24 in deep, June and July, 2016-2018.

Essential Elements		Soil Depth inches	Nongrazed 34 -36 yrs.	Seasonlong 30-32 yrs	Twice-over 34-36 yrs.
			Management Treatments		
Inorganic Nitrogen					
	lbs/ac	0-6	15.93	23.37	31.63
		6-12	10.42	10.24	22.03
		12-24	5.68	6.85	6.14
		0-24	32.04	40.46	59.79
Nitrate					
	lbs/ac	0-6	5.52	8.01	10.02
		6-12	2.18	2.12	2.96
		12-24	0.84	1.25	1.75
		0-24	8.54	11.38	14.73
Ammonium					
	lbs/ac	0-6	10.50	15.40	21.60
		6-12	8.24	8.14	18.41
		12-24	4.84	5.62	4.34
		0-24	23.58	29.16	44.35
Total Phosphorus					
	lbs/ac	0-6	26.10	32.90	40.52
		6-12	25.02	27.19	47.05
		12-24	34.53	17.13	3.27
		0-24	85.65	77.23	90.84
Organic Phosphorus					
	lbs/ac	0-6	15.45	19.00	20.25
		6-12	13.25	14.43	15.91
		12-24	10.96	8.62	2.34
		0-24	39.65	42.05	38.50
Inorganic Phosphorus					
	lbs/ac	0-6	10.28	14.01	20.32
		6-12	11.20	12.62	31.54
		12-24	23.20	8.58	1.49
		0-24	44.68	35.22	53.35

Table 48. Essential element weight (lbs/ac) by H3A (plant organic acids) extract at soil depths to 24 in deep, June and July, 2016-2018.

Essential Elements		Soil Depth inches	Management Treatments		
			Nongrazed 34 -36 yrs.	Seasonlong 30-32 yrs	Twice-over 34-36 yrs.
Potassium (K)					
	lbs/ac	0-6	485.93	518.02	673.50
		6-12	352.96	387.22	486.02
		12-24	194.16	274.10	254.98
		0-24	1033.05	1179.34	1414.50
Calcium (Ca)					
	lbs/ac	0-6	1588.32	1775.95	1892.87
		6-12	1415.65	1496.95	1965.21
		12-24	3460.52	3551.89	7825.47
		0-24	6464.49	6824.79	11683.55
Magnesium (Mg)					
	lbs/ac	0-6	473.15	547.39	738.68
		6-12	441.75	505.78	739.10
		12-24	576.35	633.45	1494.11
		0-24	1491.25	1686.62	2971.88
Sodium (Na)					
	lbs/ac	0-6	41.61	48.13	52.62
		6-12	47.73	46.91	59.56
		12-24	82.40	55.48	108.09
		0-24	171.73	150.52	220.26
Sulfur (S)					
	lbs/ac	0-6	12.65	13.87	20.81
		6-12	11.02	11.42	22.03
		12-24	8.57	9.39	26.11
		0-24	32.24	34.68	68.94

Table 49. Essential element weight (lbs/ac) by H3A (plant organic acids) extract at soil depths to 24 in deep, June and July, 2016- 2018.

Essential Elements Microminerals		Soil Depth inches	Management Treatments		
			Nongrazed 34 -36 yrs.	Seasonlong 30-32 yrs	Twice-over 34-36 yrs.
Iron (Fe)					
	lbs/ac	0-6	329.92	261.84	297.73
		6-12	375.02	302.08	228.45
		12-24	218.98	166.39	55.63
		0-24	923.92	730.31	581.80
Manganese (Mn)					
	lbs/ac	0-6	8.61	13.99	18.48
		6-12	4.29	6.24	29.62
		12-24	3.84	4.37	0.91
		0-24	16.74	24.60	49.01
Zinc (Zn)					
	lbs/ac	0-6	1.33	2.20	1.26
		6-12	0.71	0.65	0.47
		12-24	0.27	0.23	0.03
		0-24	2.31	3.07	1.75
Copper (Cu)					
	lbs/ac	0-6	0.19	0.20	0.48
		6-12	0.21	0.34	0.64
		12-24	0.25	0.28	0.06
		0-24	0.64	0.81	1.18
Aluminum (Al)					
	lbs/ac	0-6	601.31	505.54	615.90
		6-12	801.01	674.06	668.69
		12-24	566.85	477.19	204.22
		0-24	1969.17	1656.79	1488.81

E. Summary of the Indispensable Rhizosphere Organisms

Grassland ecosystems of the Northern Mixed Grass Prairie degrade with the removal of large grazing graminivores. The traditional purpose of the nongrazed treatment that removes grazing defoliation by graminivores with the intention of "resting" the grazingland ecosystem as a restoration management practice developed from long-time concepts that do not go deeper than the soil surface. This misguided practice was based on a naive assumption that the observed vigor depletion of grassland communities was caused by livestock grazing, rather than being caused by poor management of livestock grazing, and that the lost vigor of the grass plants could be restored by resting. Ironically, removal of livestock grazing does not rest an ecosystem like the rest experienced by a hard working human swinging in a hammock; the grass plants are debilitated and the soil microorganisms are devitalized like the enfeeblement of an elderly person sitting alone in a rocking chair. Nongrazed native grasses provide simple carbohydrate energy to soil microbes only at the typical low leakage rate resulting in a small biomass of microorganisms. The ecosystem biogeochemical processes function at increasingly regressive degrees below potential levels each growing season without grazing. Soon after the first ecosystem process fails to function properly, the other belowground processes and mechanisms begin to deteriorate. The native grass live root biomass decreases (Whitman 1974), the physiological mechanisms within grass plants diminish, the ecosystem biogeochemical processes decline rapidly, and the competitiveness of grass plant resource uptake deteriorate (Manske 2011b).

The reduction of live root surface area causes a decrease in active root length for interaction with symbiotic rhizosphere organisms and causes a decrease in absorption of water and essential nutrients from the soil. Reduction of active root biomass and diminishment of grass plant health vigor results in a loss of resource uptake efficiency and a suppression of the competitiveness of grass plants to take up mineral nitrogen, essential elements, and soil water (Li and Wilson 1998, Kochy and Wilson 2000, Peltzer and Kochy 2001). The loss of active root length is a contributing factor in the reduction of rhizosphere biomass. The primary cause for the reduction in rhizosphere biomass was, however, the great reduction in the quantity of carbohydrates exuded from the grass roots into the rhizosphere zone. The lack of partial defoliation by grazing graminivores directly prevented the exudation of large quantities of carbon energy from grass plants into the rhizosphere. The resulting small biomass of rhizosphere organisms only can mineralize small quantities of nitrogen and other essential elements (Coleman et al. 1983, Klein et al. 1988).

The decreased amounts of available mineral nitrogen below 100 lbs/ac in the ecosystem causes reductions in native grass herbage biomass production (Wight and Black 1972, 1979) and causes decreases in native grass density (basal cover). As degradation continues, numerous bare spaces between native grass plants are created in the plant communities. The open spaces are ideal habitat for growth of opportunistic introduced domesticated grass species, weedy forbs, and shrubs. The composition of grass species changes with decreases in the desirable native species and increases in the less desirable and undesirable species.

Standing dead leaves accumulate (Brand and Goetz 1986) as ecosystem deterioration progresses. Grass plants produce double the quantity of leaf biomass that is needed for normal plant growth and maintenance (Crider 1955, Coyne et al. 1995). When the extra leaf biomass is not removed by grazing annually, that extra biomass becomes detrimental. The accumulation of live and standing dead leaves reduce light penetration greatly. This reduction of sunlight to native grasses causes reduced rates of photosynthesis, decreased rates of herbage production, and increased rates of leaf senescence (Langer 1972, Briske and Richards 1995) decreasing native grass composition further. Great quantities of standing dead material do not make contact with soil preventing decomposition through microbial activity and causing litter to build up into a thick mulch layer. The thick mulch modifies soil temperature, inhibits water infiltration, and ties up carbon and nitrogen (Wright and Bailey 1982, Manske 2000, 2011b). Native grasses are further inhibited by deficiencies of soil water, cool soil temperatures during spring, and reduced ecosystem nutrients caused by thick mulch.

The change in plant composition from desirable native grasses to less desirable introduced grasses, weedy forbs, and woody shrubs is the visible symptom of ecosystem degradation; the fundamental degradation of the ecosystem is the reduction of rhizosphere biomass, the reduction of biogeochemical processes, the reduction of available mineral nitrogen below 100 lbs/ac, the reduction in availability of all the other essential elements. The degree of the aboveground plant species deterioration lags behind the degree of degradation of the belowground ecosystem biogeochemical processes and mechanisms

(Manske 2011b). Removal of cattle grazing does not promote development of stable climax plant communities and does not preserve prairie grasslands in perpetuity. Grassland communities deprived of large grazing graminivores decline steadily into unhealthy dysfunctional ecosystems with severe reductions of native grasses, considerable decreases of desirable forbs, enormous increases of introduced domesticated grasses, remarkable increases of woody shrubs and trees, and excessive increases of standing dead and litter.

Intrusive seedlings can only become established in a grassland after the ecosystem has been degraded by poor management practices. Seedlings of trees, shrubs, weedy forbs, and introduced grasses cannot become established in grassland ecosystems containing healthy grass with full nutrient resource uptake competitiveness (Peltzer and Kochy 2001). The existence of woody plant components in grasslands is not an ecologically beneficial relationship as woody plants and grasses are adversarial inhibitive competitors. Grasses and woody plants compete for sunlight, mineral nitrogen, other essential elements, and soil water. Fire in grasslands cannot prevent the invasion of, or cause the removal of, shrubs and trees that are able to reproduce by vegetative secondary suckers (Wright and Bailey 1982, Manske 2006a, b). Almost all deciduous woody plants can reproduce vegetatively, except big sagebrush (Artemisia tridentata) (Manske 2014d).

Burning cannot restore degraded grassland ecosystems and fire does not improve grassland ecosystems biologically or ecologically. Burning treatments do not increase water infiltration or soil water holding capacity. Burning treatments do not increase endomycorrhizal fungal colonization. Burning treatments do not increase rhizosphere microbe biomass and activity levels. Burning treatments do not increase mineralization of organic nitrogen into inorganic nitrogen. Burning treatments do not increase total herbage biomass production. Burning treatments do not restore functionality to degraded grassland ecosystems. The fundamental problems of weak nutrient resource uptake, reduced water use efficiency, nonfunctional compensatory physiological mechanisms and vegetative reproduction by tillering remain within the plants and diminished biogeochemical processes remain in the degraded ecosystems following repeated burning treatments. None of the biological, physiological, or asexual mechanisms within grass plants and none of the rhizosphere microbes or the biogeochemical

processes they perform are activated by burning treatments (Manske 2007a, 2011a, 2014d, 2018).

Burning grasslands exacerbates ecosystem degradation. When the losses of essential elements are greater than the quantity of captured major essential elements, the result is expanded degradation of the ecosystem (McGill and Cole 1981). Almost all of the essential elements in the aboveground herbage are volatilized when grasslands burn, and there are no active processes in burned grasslands to recapture the lost major essential elements. When burning occurs during dry soil periods, some of the belowground essential elements are also lost (Russelle 1992). Burning grasslands does not restore degraded ecosystems. Degraded grasslands continue to have the same basic problems following the repeated prescribed burning regime, and when the burning sequence stops, the undesirable replacement plants return to dominate the communities (Manske 2018). The presence of periodic fire does not prove that grassland ecosystems need or are caused by fire (Heady 1975).

All of the biological and ecological problems found on rangeland ecosystems are the result of deficiencies in the amount of available mineral nitrogen below 100 lbs/ac in the soil. The primary objective to correct any and all of these problems is to elevate the rhizosphere microorganism biomass to a level that can mineralize adequate quantities of organic nitrogen so that the quantity of available mineral nitrogen occurs at or above the threshold rate of 100 lbs/ac (112 kg/ha).

The rhizosphere microorganism biomass and biogeochemical activity are limited by the access to energy from simple carbon chains. The small quantities of energy available from short chain carbohydrates in root leakage or in recent deposition of fresh organic plant material are inadequate to support a large rhizosphere biomass. The only source of large quantities of energy from simple carbon chains is the surplus carbon fixed through photosynthesis during grass plant vegetative growth stages. This surplus carbon energy is not automatically released to soil microbes. The grass plant carbon-nitrogen ratio must be disrupted by removing greater amounts of nitrogen than carbon. The grazing periods of the twice-over rotation strategy are coordinated with grass tiller phenological growth and development. Partial defoliation by grazing graminivores that removes 25% to 33% of the aboveground leaf weight on about 60% to 80% of the lead grass tillers at vegetative growth stages between the three and a half new leaf stage and the flower

stage removes greater quantities of nitrogen as crude protein than the quantities of removed carbon which intentionally causes large quantities of grass leaf surplus simple carbohydrates to be exudated through the roots into the rhizosphere. This large increase in available simple carbon energy increases the microorganism biomass in the rhizosphere and elevates microbe biogeochemical activity resulting in great quantities of mineral nitrogen and other essential elements to become available to native grass species.

Rhizosphere microbes with low biomass from 104 kg/m^3 to 171 kg/m^3 can mineralize only 26.3 kg/ha to 65.2 kg/ha (23.5 lbs/ac to 58.2 lbs/ac) of mineral nitrogen. Rhizosphere microbes with high biomass from 214 kg/m^3 to 406 kg/m^3 can mineralize 111.3 kg/ha to 176.3 kg/ha (99.4 lbs/ac to 157.4 lbs/ac) of mineral nitrogen.

Grass plants have four major internal growth mechanisms: compensatory physiological mechanisms, asexual mechanisms for vegetative reproduction by tillering, competitive nutrient resource uptake mechanisms, and precipitation (water) use efficiency mechanisms. A threshold quantity of 112 kg/ha (100 lbs/ac) of mineral nitrogen must be available before these important growth mechanisms can be fully activated.

Full activation of the compensatory physiological mechanisms within grass plants accelerates growth rates of replacement leaves and shoots, increase photosynthetic capacity of remaining mature leaves that increase the quantity of available fixed carbon, and increases restoration of biological and physiological processes enabling rapid and complete recovery of partially defoliated grass tillers. Replacement grass biomass is about 140% of the grass biomass removed by grazing.

Full activation of the asexual mechanisms for vegetative reproduction by tillering increases secondary tiller development from axillary buds, increases initiated tiller density during the grazing season, and increases herbage biomass production and improves herbage nutritional quality through the grazing period.

Full activation of the nutrient resource uptake mechanisms increases root absorption of soil water and the major and minor essential element, improves the robustness of grass growth and development, increases competitiveness of healthy grasses, and increases suppression of undesirable grass, weedy forb, and shrub seedlings or rhizomes

from encroachment and establishment within grassland communities.

Full activation of the precipitation (water) use efficiency mechanisms increases herbage biomass production 50.4% per inch of rainfall received, and greatly reduces the detrimental effects to grass herbage production during water deficiency periods and from drought conditions.

Grass plants require 17 major and minor essential elements to execute the physiological functions needed for life of which 13 essential elements, the 4 major essential elements, the 5 macrominerals, and 4 of the 8 microminerals, require biogeochemical processes performed by soil microbes to transform these essential elements into forms that can be used by grass plants. The twice-over rotation strategy has been shown to contain the largest quantity of soil organic matter (SOM) with the highest annual mean accumulation rate at 8408 lbs/ac/yr, which is probably the closest quantitative value measurable for net primary productivity (NPP) of an ecosystem. The soils managed with the twice-over strategy had the greatest quantities of organic carbon, organic nitrogen, and total phosphorus, the soils contained the greatest quantities of available inorganic nitrogen (both nitrate and ammonium), inorganic phosphorus, and the greatest quantities of available forms of potassium, calcium, magnesium, sulfur, manganese, and copper, with adequate quantities of available forms of iron and zinc.

A large biomass of rhizosphere microorganisms can perform all of the grassland ecosystem biogeochemical processes that renew nutrient flow activities in the intact soil. Biogeochemical processes transform stored essential elements from organic forms into plant-usable inorganic forms. Biogeochemical processes also capture replacement quantities of lost or removed major essential elements of carbon, hydrogen, nitrogen, and oxygen, with assistance from active live plants, and transform the captured major essential elements into storage as organic forms for later use. And the biogeochemical processes also decompose complex unusable organic material into compounds and then into reusable essential elements (Manske 2018).

The quantity of herbage biomass production and the nutritional quality of the herbage and the quantity of cow and calf weight gains on rangeland pastures are directly related to the biomass of the rhizosphere microorganisms. Positive and negative oscillations in plant species composition and herbage

production of the aboveground plant communities follow the changes in belowground rhizosphere microbe biomass and their biogeochemical activity. When the rhizosphere microorganism biomass and activity is large enough to mineralize 100 lbs/ac or greater of available mineral nitrogen, the desirable native grass species increase, with increases in density and herbage biomass, and the less desirable and undesirable plant species decrease. The plant species composition moves in the opposite direction when the rhizosphere microorganism biomass and activity mineralize less than 100 lbs/ac of mineral nitrogen. The typical low grass herbage biomass, the low nutritional quality of forage after July, and the low calf weight gains on rangeland pastures managed with traditional management practices results from deficient quantities of available mineral nitrogen because the biomass of the rhizosphere microorganisms is too low to mineralize adequate quantities of mineral nitrogen to fulfill the required threshold level of 100 lbs/ac.

The rhizosphere microorganisms are the renewable part of grassland natural resources. These microorganisms are the hugely underrated indispensable biotic component that are the impelling force driving grassland ecosystem functions.

Acknowledgment

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