THESIS

LONG-TERM BIOSOLIDS APPLICATIONS TO OVERGRAZED RANGELANDS IMPROVE SOIL HEALTH

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ABSTRACT

LONG-TERM BIOSOLIDS APPLICATIONS TO OVERGRAZED RANGELANDS IMPROVE SOIL HEALTH

Overgrazed rangelands can lead to soil degradation, yet long-term land application of organic amendments (i.e., biosolids) may play a pivotal role in improving overgrazed rangelands in terms of soil health. However, the long-term effects on soil health properties in response to Single or Repeated, low to excessive biosolids applications, on semi-arid, overgrazed grasslands have not been quantified. Using the Soil Management Assessment Framework (SMAF), soil physical, biological, chemical, nutrient, and overall soil health indices between biosolids applications (0, 2.5, 5, 10, 21, or 30 Mg ha^{-1}) and application time (Single: 1991, Repeated: 2002) was determined. Results showed no significant changes in soil physical and nutrient health indices. However, the chemical soil health index was greater when biosolids were applied at rates < 30 Mg ha^{-1} and within the Single compared to Repeated applications. The biological soil health index was positively affected by increasing biosolids application rate, was overall greater in the Repeated as compared to the Single application, and was maximized at 30 Mg ha^{-1}. The overall soil health index was maximized at rates < 30 Mg ha^{-1}. When all indices were combined, and considering past plant community findings at this site, overall soil health appeared optimized at a biosolids application rate of ~ 10 Mg ha^{-1}. The use of soil health tools can help determine a targeted organic amendment application rate to overgrazed rangelands so the amendment provides maximum benefits to soils, plants, animals, and the environment.

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DEDICATION

This work is dedicated to my parents, Carma and Randy Berry, who raised me to be an independent thinker and to chase after what I want in life. To my brother and sister-in-law, Cory and Amanda Berry, who often challenge be to be the best version of myself regardless of the obstacles in my way. To my grandparents, Stan and Berniece Bertagnolli, who have always loved and supported me as I have walked my own path like only grandparents can. Lastly, to my husband, Matthew Buchanan who has stood by my side and provided support, stability, motivation, and love every single day.

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CHAPTER I INTRODUCTORY AND BACKGROUND INFORMATION

Introduction

Soil is an essential, non-renewable resource with potentially rapid degradation rates and extremely slow formation and regeneration processes (Diacono, et al. 2010); soil degradation and regeneration are functions of soil utilization. According to the USDA-National Agricultural Statistics Service (2020), in 2019 the total amount of land used for agricultural purposes in the United States was 897,400,000 acres of the 2.43 billion acres of total land in the U.S. (roughly 40%). This available land has slowly decreased over time due to urban expansion as well as soil degradation.

Loss of land productivity is a challenge that impacts food availability and cost, climate change, biodiversity, and ecosystem services (Diacono, et al. 2010). Thus, improving the fundamental understanding of how well soils function at their greatest level is paramount for sustaining all facets of life; this, in essence, is soil health. The USDA-NRCS defines soil health as "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (NRCS, 2017).

Soil health is the product of interdependent processes and therefore cannot be determined directly by measuring a single characteristic, although the goal of soil health assessments is to reduce the number of soil health indicators into an easily manageable subset of data. Subsets are typically a combination of physical, biological, chemical, and nutrient properties used as indicators for quantifying soil health. Subsequently, soil health indicators typically vary depending on the management goal, site, and multiple soil factors (Ippolito et al., 2017). In systems that are not fully understood in terms of their soil health, oftentimes a comprehensive set of analyses is initially required, with those analyses subsequently reduced in the future to the most important soil health indicators. The goal of soil health indicator subsets is to guide management decisions that promote or maximize overall soil functionality. Ultimately, increasing the understanding between soil functionality, crop yield, biodiversity, ecosystem services, and human benefits, will undoubtedly help address and improve upon complex global environmental quality issues (Ohno and Hettiarachchi, 2018).

Globally, soil health has been accessed utilizing a variety of indicators in multiple ecosystems. In an agroforestry ecosystem in Costa Rica, Rousseau et al. (2012) mostly relied on physical and chemical indicators, such as bulk density, pH, and the soils carbon (C) content to categorize soil health into classes of low or high soil quality. In New Zealand, Stevenson et al. (2015) utilized over 700 sites across 12 different geological locations to create soil health clusters based on soil organic status (i.e., soil organic carbon (SOC) and nitrogen (N)) and physical indicators (i.e., macroporosity). In India, Tripathi et al. (2016) tested for soil health differences between mangrove forest ecosystems and ecosystems that were originally under mangroves but currently under rice cultivation. Utilizing multiple physical, chemical, and biological indices, the authors determined that forested soils had greater soil health than cultivated rice fields, likely because rice cultivation caused soil degradation. In China, Li et al. (2020) evaluated the impact on soil health between the application of nine different organic and chemical amendments (no fertilizer, chemical fertilizer, 60% chemical fertilizer, chemical fertilizer + straw, 60% chemical fertilizer + straw, chemical fertilizer + biochar, 60% chemical fertilizer + biochar, 60% chemical fertilizer + pig manure, 60% chemical fertilizer + vermicompost). The researchers showed that nutrient and biological indices could determine overall soil health, including the available phosphorous (P), nitrogen (N), potassium (K), and gram-positive bacteria. They concluded that the application of pig manure had the greatest positive impact on overall soil health. Significant differences were noted within each of these studies. However, none of them were able to determine the overall soil health of each ecosystem as a whole.

Soil health can be difficult to quantify; therefore, it is beneficial to use an established tool that is able to present soil health information in a way that can be more easily understood. In the US, there are two major tools that are used to accomplish soil health quantification, the Cornell Soil Health Test and the Soil Management Assessment Framework (SMAF). Both tools function using a similar premise: 1) soil indicator characteristics are quantified; 2) data is entered into the soil health assessment program, and then are assigned unitless values with varying degrees of whether more, less, or somewhere in the middle is considered "better"; and 3) the scores are summed and weighted to provide a relative measure of the soil's ability to perform the functions necessary for its intended use (Andrews et al., 2004).

Specifically, the SMAF provides site-specific interpretations to produce an overall soil health index value (Andrews et al., 2004). The SMAF uses a series of decision rules that are in a database format, to generate soil health assessments. These rules use site management goals, associated soil functions, as well as other site-specific factors such as region, climatic conditions, and cropping rotations as selection criteria. These rules, in conjunction with quantified soil characteristics, serve to ascertain soil health indicators. Once selected, the indicators are grouped according to the critical soil function (physical, chemical, nutrient, or biological). After data is input, each indicator value is transformed into unitless values using nonlinear scoring curves, then scored as either physical, chemical, nutrient, or biological soil health indices, and finally all indices are combined to form a single, overall soil health score (Andrews et al., 2004).

Physical Soil Attributes

Soil physical health is the ability of a given soil to meet plant and ecosystem requirements for water, aeration, strength over time, and to resist and recover from processes that might diminish that ability (Chakraborty et al., 2010). Soil physical health typically encompasses an integrative approach, determining a range of physical properties to obtain an overall soil physical assessment. Soil properties obtained for use in the SMAF are commonly texture, aggregate stability, and bulk density. It is important to note that soil physical properties are dependent on, among other characteristics, soil organic matter content.

Soil texture is the classification of the relative primary mineral particle distribution, or sand, silt, and clay in a soil (Dexter, 2004). Soil texture influences soil characteristics that affect plant growth, including water-holding capacity, permeability, and soil workability. Typically, soils with relatively higher clay content have a greater water holding capacity due to greater pore space present. However, water infiltration rate tends to be slower than that of silt or sand dominated soil due to the small size of the pores (Dexter, 2004). Texture also plays a major role in determining soil usability, such as what plants are able to flourish due to the above characteristics influenced by texture, and by nutrient availability (discussed below).

The resistance of soil aggregate structure to physical stresses determines soil sensitivity to crusting and erosion, cultivated plant germination, rooting, plant maturation, and the ability of a soil to store carbon through physical protection of organic moieties (Abiven et al., 2009). Over time, whether in cropping season or years, aggregate stability fluctuates due to climatic conditions, varying agricultural practices (e.g., tillage, cover crops, residue management, irrigation events, etc.), and the decomposition of organic compounds. Returning fresh organic matter periodically to a soil could be the solution for maintaining healthy soils and rehabilitating overgrazed soils.

Bulk density is an indicator of soil compaction, accounted for by soil weight per unit volume. Bulk density also can be used to quantify the volume of pores per unit volume of soil. Bulk density reflects a soil's ability to function as structural support, water and solute movement, and soil aeration. Relatively high bulk density (e.g., greater than 1.6 g cm^{-1}) is an indicator of relatively high soil compaction, leading to reductions in soil water content, water and air infiltration, and root growth impediment (Bruand and Gilkes, 2002; Tesfahunegn, 2016). This may also lead to poor plant establishment, lack of vegetative cover and the inability to protect soil from erosion (Chakraborty et al., 2010).

Chemical Soil Attributes

In terms of soil health, soil chemical properties are important because they impact overall nutrient (im)balances and soil biological properties. Soil chemistry is largely determined by soil organic matter content, parent material, weathering processes, and anthropogenic manipulations. Several soil chemical factors that are measured both within and outside of the SMAF are outlined below.

Cation exchange capacity (CEC) denotes the amount of cations that can be sorbed to negatively charged soil exchange sites. These cations typically include Ca^{2+}, Mg^{2+}, K^+, Na^+, and to a lesser extent NH_4^+ , Al^{3+}, Fe^{3+}, Fe^{2+}, Mn^{2+}, and H^+. Adequate Ca^{2+} and Mg^{2+} on exchange sites leads to bridging between soil particles, leading to improved soil structure. Excessive K^+ or Na^+ on exchange sites can lead to particle dispersion and thus a reduction in soil structure. The balance between these major cations is therefore crucial for proper soil structure as both are intimately linked, as well as linked to soil biological activity (described later).

Soil pH is a measure of the free hydrogen ion (H^+) concentration present. A high H^+ concentration is represented as a relatively low pH, while a low concentration equals a relatively high pH. Soils below pH 7 are acidic, while those above pH 7 are basic; soils above 7.2 could contain free lime. Most plants thrive at pH values between 6-7.5 (Stoffella et al., 2003). The form in which macro-elements and micro-elements occur in soils is also depends on pH. Relatively low soil pH values typically lead to increased cation availability (and may lead to nutrient or elemental toxicity effects), while pH values between 6 and 6.5 favor P availability. Thus, pH exercises a significant influence over nutrient availability for plants. Soil microbial activity and consequently the mineralization of organic matter and nutrient cycling are also affected by soil pH. In general, soil fungi can thrive across a wide range of pH values, yet bacteria thrive between ~ pH 5.5 and 8 (Havlin et al., 2015).

Soil salinization, measured in part as electrical conductivity (EC), is a major obstacle to the optimal utilization of land resources. Salt-affected soils are widely distributed throughout the world, and about 20% of the world's cultivated land is salt affected (Wang et al., 2014). When soil salt concentrations are relatively high as compared to within plant roots, an osmotic gradient is created whereby moisture is drawn from the roots to soil-borne salts, causing root die-off. Over time, this impedes plant moisture and nutrient absorption, leading to reduced plant growth or death.

Nutrient Soil Attributes

Soil is the major source of nutrients needed for plant growth. Nutrients supplied by soil are divided into two groups, macro- and micronutrients. The macronutrients are then separated into two categories known as the primary and secondary macronutrients. Primary macronutrients are required by plants in relatively large proportions compared to the secondary macronutrients. Three primary macronutrients, N, P, and K, are most commonly in demand by plants globally, and thus close attention is paid to these three elements with the SMAF.

Nitrogen plays a fundamental role in living organism (e.g., soil microbes, plants) growth, production, and reproduction. Common soil N sources include chemical fertilizers, decomposing plant residues, compost, manure or biosolids applications, and exudates from living plant roots. Inorganic fertilizers can directly supply plant-available N [nitrate (NO_3^-) or ammonium (NH_4^+)], while N in organic amendments need to undergo mineralization and nitrification in order to release NH_4^+ and NO_3^-. Aspects such as soil texture, soil pH, climate conditions, irrigation methods, and fertilizing practices all influence the level of available soil N. If a N deficiency is present, the most common signs are reduced plant growth rates and older leaves become chlorotic (or if severe, necrotic) due to N being mobile within plants (Caberara et al., 2008). The SMAF utilizes potentially mineralizable N (PMN) as an input value, with the USDA defining potentially mineralizable N as "the fraction of organic N converted to plant-available (or mineral) forms under specific conditions of temperature, moisture, aeration and time (USDA-NRCS, 2014)." In Israel and the US Midwest, Osterholz et al. (2017) evaluated the PMN response between six long-term cropping experiments with treatments in each experiment consisting of different crop rotations and N fertility management. The N fertility management was classified as either organic (animal manures, green manures, compost, and sludge) or inorganic (synthetic N fertilizers or treated wastewater with relatively high inorganic N concentrations). The authors concluded that the N fertility management strategy affected PMN across these widely varying agricultural experiment sites, with treatments utilizing the organic amendments demonstrating consistently greater values than the inorganic treatments.

Phosphorus is an essential element for all living organisms, used for energy storage and transfer (e.g., ATP), growth, and reproductivity (Havlin et al., 2015). Soil P is found in either organic or inorganic forms; however, P is absorbed by plants as $H_2PO_4^-$ or HPO_4^{2-}, with the form driven by soil pH. Typically, inorganic P is absorbed relatively quickly by plants or microorganisms, or is precipitated as Al, Fe, or Ca mineral phases (i.e., all pH dependent reactions). Due to mineral precipitation and dissolution reactions, plants obtain P from soil primarily through diffusion. If a P deficiency is present, plants become stunted and purplish discoloration occurs on older leaves as P is mobile within plants (Havlin et al., 2015). In the SMAF, plant-available P is determined by either Mehlich-1, Mehlich-3, Bray, Olsen, Resin, or iron oxide strip methods. In 2019, Wade et al. studied corn-soy cropping systems from three regions of Ohio that were subjected to 11 years of P restriction to measure impacts on soil P availability and agronomic performance. They concluded that with decreased fertilization rates, both soil P availability and plant tissue P decreased, however the crops did not exhibit signs of P stress. In the plots that received no P fertilization, soil organic P concentrations increased.

Potassium is an essential element for plant growth, playing a role in photosynthesis, enzymatic activation, and in particular, related to ionic strength and osmotic gradients of solutions within plant cells (Havlin et al., 2015). Within soils, the vast majority of K is unavailable to plants as it is found as a structural component of soil minerals, leaving relatively small amounts available for plant growth. In soil, K occurs in water-soluble (solution K; a pool with the least amount of K present), exchangeable, non-exchangeable, and structural or mineral forms (a pool with the most amount of K present). Water-soluble and exchangeable pools directly contribute to available K for plant uptake. Several factors such as soil moisture, aeration, temperature, and tillage practices impact K uptake by plants. If there is a K deficiency is present, chlorosis occurs along the leaf margins of older leaves (i.e., K is mobile within plants), plants may be stunted, and lodging may occur. Within the SMAF, plant-available K is determined by the Mehlich-3 extraction method. However, the Olsen method is often used in soils with pH values greater than 7.2, with a correlation/conversion made between Olsen and Mehlich-3 K values for input purposes using the following equation: Mehlich-3 K (mg/kg) = [Olsen K (mg/kg) – 21.887]/0.7209 (Robert Miller, North American Proficiency Testing; personal communication). In China, Zhang et al. (2011) assessed the changes in soil K pools in rice cropping systems as affected by three different fertilizer treatments (NP, NK, and NPK), over eight growing seasons at the Agricultural Research Institute of Jinhua (ARI) where soil is calcareous, and over 5 growing seasons at the Shimen Research Farm (SM) where soil is acidic. The authors concluded that the treatments containing fertilizer-K application significantly increased grain yields over 5-8 consecutive crops. When fertilizer-K was only applied at a rate of 100 kg ha^{-1} per rice crop, the soil did not maintain the readily available K concentrations. In the treatments where no K addition was made, inherent soil K concentrations could only maintain rice growth for 1-2 growing seasons without significant yield loss.

Biological Soil Attributes

Soil biological attributes can also greatly influence overall health. A plethora of soil microorganisms drive soil functionality and health, including organic matter decomposition and nutrient turnover and cycling. When soil microbial populations increase or are functioning at optimal rates, enzymatic activities concomitantly increase. These attributes are commonly determined and entered into soil health programs, such as the SMAF. Specifically, the SMAF utilizes four biological health indicators: potentially mineralizable N; microbial biomass C; beta-glucosidase activity; and SOC.

The potentially mineralizable soil N (PMN) is the fraction that can be transformed into inorganic forms under ideal conditions and utilized by plants (Mahal et al., 2019). Potentially mineralizable N is related to nutrient availability and represents the relationship between soil microbial activity and plant productivity. It is considered an indirect measure of N availability during the growing season. Similar to soil organic C and microbial biomass C, PMN is influenced by the amount of organic matter available in the soil system. Ippolito et al. (2021) studied soil health alterations with increasing biosolids versus increasing inorganic N fertilizer applications to a dryland wheat-fallow rotation. The authors showed that inorganic N fertilizers did not alter PMN. However, increasing biosolids application rates (0 to 11.2 Mg ha^{-1}) significantly increased PMN and, overall, biosolids increased PMN over N fertilizers.

Microbial biomass C is the readily available carbon contained within the living, microbial component of soil. Soil microbial biomass C generally makes up 1-5% of the total soil organic C in the system, acts as an active component in the process of soil organic C turnover, and responds more rapidly to soil disturbance than bulk soil organic carbon (Xiaojun et al., 2013). Soil properties such as pH, texture, and availability of total C in the system all impact the amount of microbial biomass C present in soils. In 2011, Stott et al. quantified several soil health indicators, such as microbial biomass C, to determine if the SMAF could distinguish the soil health between three well-developed canopy sites and one poor corn canopy site within the Iowa River's South Fort Watershed. The authors determined that, among several other indicators (e.g., SOC, bulk density, EC), the microbial biomass C indicator score was significantly lower in poor canopy areas. The mean microbial biomass C scores were relatively high in the well-developed as compared to the poorly developed canopy areas (0.94 versus 0.87, respectively). Furthermore, almost 80% of the well-developed canopy sites scored 0.9 or greater, while only about 56% of the poorly developed canopy sites scoring in the same range.

No single enzyme activity accounts for all of the soil metabolic functions. However, beta-glucosidase activity is sensitive to changes in soil and residue management, and its activity is the rate-limiting, enzymatic step in the microbial degradation of cellulose to glucose. Beta-glucosidase activity has been suggested as an indicator of management effects as well as predictor for potential increases in soil organic carbon before the changes are even reflected in total or organic C analysis (Bandick and Dick, 1999; Paudel et al. 2011). Beta-glucosidase activity is generally greater in conservation or no till practices compared to typical conventional cropping systems, with increases in its enzymatic activity noticeable within 1 to 3 years after altering management practices (Stott et al., 2010).

Soil represents the most important organic C sink in terrestrial ecosystems. Compared to the vegetation that the soil system supports, the soil contains up to three times more C in the first meter of depth (Andriamananjara et al., 2017). Soil organic carbon (SOC) acts as a food source for soil fauna and flora. It also plays an important role in the soil food web by controlling the number and types of soil inhabitants which serve important functions such as nutrient cycling and availability, assisting root growth and plant nutrient uptake, and suppressing crop diseases. Soil organic carbon decreases when there is an overall decrease in soil organic matter inputs, such as fallowing, cultivation, stubble burning or removal, and overgrazing. Soil organic carbon is also often positively and significantly correlated with aggregation indices, especially in the macroaggregate range (4- to 8-mm size aggregates) (Chakraborty et al., 2010).

Changes in Soil Health as Affected by Organic Amendment Applications

Anthropogenic manipulation of land over time leads to soil degradation and typically, overall reduced system functionality (Lal, 1998). This leads to agroecosystems that are not highly sustainable or resilient in the face of changing climatic variables (Lal, 1998); properly managing agroecosystems is desperately required to improve overall soil health while continuing to produce enough food, feed, fuel, and fiber to sustain life. Soil degradation can most easily be recognized by quantifying soil organic C losses over time. Thus, increasing organic C in agroecosystems via organic amendment additions may be a means by which system resiliency, sustainability, and overall soil health, is improved.

Organic amendments can have a profound influence on almost all soil properties—such as structure (and hence on water infiltration and storage, susceptibility to surface runoff and erosion), cation exchange capacity, nutrient availability, buffering capacity in terms of pH and nutrient availability, and color. Some of the most useful and readily available organic amendments are animal manures and biosolids (stabilized sewage sludge resulting from municipal wastewater treatment processes). Both materials contain appreciable amounts of N, P, K (e.g., factors of nutrient soil health), and organic material, all of which have the potential to improve agroecosystem soil physical, chemical, and biological health, as described below.

Physical

Chakraborty et al. (2010) performed an experiment focusing on how the soil physical environment was affected by long-term application of fertilizers and manure. Five different treatments (100% recommended dose of nitrogen, phosphorus, and potassium (NPK) fertilizer, 150% recommended dose NPK fertilizer, 100% NPK fertilizer + farmyard manure, 100% NPK fertilizer + sulfur, and a control) were applied to sandy loam soils under a maize-wheat cropping system. The 100% NPK + farmyard manure treatment had the greatest impact on soil physical health, as aggregation increased and macroaggregates were > 50% of the total soil mass; bulk density was also significantly lower. The effects of all treatments were better when compared to the 100% NPK fertilizer treatment, indicating that the recommended dose of NPK was suboptimal to maintain the desired soil physical health. Soil texture, however, remained similar across all treatments. Ozores-Hampton et al. (2011) found similar results in their study where they applied composted and non-composted organic amendments to sandy soils in vegetable production over a ten-year period. Organic amendment applications decreased the bulk density of the soil from 1.6 g cm^{-3} to a 1.4 g cm^{-3}. A study by Ozlu et al. (2018) included three manure application rates - low (LM), medium (MM), and high (HM), and two different fertilizer application rates - medium (MF; only nitrogen addition), high (HF; double the amount of MF), that were applied to soils in South Dakota under corn-soybean rotation. Their results showed that the manure treatments significantly increased water-stable soil aggregates by 5.6-7.2% compared to that of the fertilizer treatments.

Chemical

The effect that organic amendments (e.g., manures, biosolids) have on soil chemical properties is often dependent upon the chemical properties of the applied amendment. In the Ozlu et al. (2018) experiment described above, manure application maintained soil pH at the 0 to 10 cm depth, whereas the inorganic fertilizer treatments decreased pH compared to the control. However, manure application also increased the EC (1.56 dS m^{-1}) by 2.2 times compared to that of fertilizer (0.71 dS m^{-1}) for 0- to 10-cm depth. Fortunately, EC values were not great enough to affect corn or soybeans. Acidic soils are commonly corrected by liming; however, Whalen et al. (2000) provide evidence that animal manure amendments can increase the pH in acidic soils. The authors applied fresh cattle manure to soils with pH ranging from 4.8-5.5. The effect was immediate and persisted during their eight-week incubation. The greatest application rate (40 g manure kg^{-1}, dry weight basis) increased the soil pH to 6.0-6.3.

Biological

Biological soil properties are typically the most reactive to small changes occurring in management practices. In 2014, Maillard et al. looked at 42 research articles totaling 49 sites and 130 observations across the globe. The authors determined that a dominant effect of cumulative manure-C input on SOC response occurred. At least 53% of the variability in SOC stock differences could be attributable to manure input as compared to mineral fertilized or unfertilized reference treatments. Ozlu et al. (2019) compared soil β-Glucosidase activity after various rates of manure or inorganic fertilizer applications under a corn-soybean rotation. The authors found that β-Glucosidase activity was 6-14% greater with manure than with inorganic fertilizers.

Based on the information presented above, our research objectives were to ascertain the influences of long-term biosolids land application effects on overall long-term soil health of a short-grass steppe, overgrazed rangeland ecosystem. Collecting data related to soil physical, chemical, nutrient, and biological properties, and concomitantly utilizing the SMAF, our goal was to suggest a biosolids application rate that improve soil health to the greatest degree.

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CHAPTER II LONG TERM BIOSOLIDS APPLICATIONS TO OVERGRAZED RANGELANDS

Introduction

Soil is an essential, non-renewable resource with potentially rapid degradation rates and extremely slow formation and regeneration processes (Diacono et al., 2010); soil degradation and regeneration are functions of soil utilization. Utilizing soils erroneously leads to lost land productivity, ultimately impacting food availability and cost, climate change, biodiversity, and ecosystem services (Diacono et al., 2010). The negative effects of soil degradation is especially important in rangelands.

Rangelands comprise 25% of Earth's surface (Zegra, 2015), with 30% (312 million ha) of the total U.S. land base under rangelands (USDA-NRCS, 2020). Rangeland degradation occurs as a result of vegetation removal or limited/lack of grazing management, causing shifts in species composition, loss of biodiversity, biomass, animal productivity, and soil erosion (Zegra, 2015 and Distel, 2016). Thus, improving the fundamental understanding of how rangeland soils may function at their greatest level is paramount for sustaining all facets of life. This concept essentially references soil health, or "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans" (USDA-NRCS, 2017).

Soil health, in the context of range management, is a product of many related and independent processes and therefore cannot be determined directly by measuring only several soil characteristics. Most often, a combination of soil physical, biological, chemical, and nutrient properties are used as indicators to quantify soil health. It has also been emphasized that soil health indicators will vary depending on management goals, site, and soil factors (Andrews et al., 2004). Therefore, a comprehensive analysis is required to guide management decisions that promote soil functions to improve overall rangeland soil health, especially after these ecosystems have been overgrazed. Built within these comprehensive analyses may be the use of various organic amendments to improve soil C dynamics, ultimately leading to improvements in soil health; biosolids may play a pivotal role in improving overgrazed rangeland soil health.

Decades of scientific research have showed the positive impact of biosolids applications on disturbed lands, which may (in)directly affect soil health. Biosolids are nutrient-rich organic material that is a byproduct of municipal wastewater treatment. Once treated and processed, these residuals are often recycled onto agricultural lands as an amendment to improve various soil properties (e.g., bulk density, plant-available nutrients, microorganism activity) and encourage plant growth (U.S. EPA, 2000). The controlled land application of biosolids completes a natural cycle in the environment, recycling plant nutrients and maintaining plant yields in an environmentally sound manner (Barbarick et al., 2007 and Barbarick et al., 2012), and is preferable over taking up space in a landfill or other disposal facilities.

Several studies have reported on the short- and long-term changes in soil physical, chemical, nutrient, or biological properties in response to biosolids land application. For example, both Tsadilas et al. (2005) and Saviozzi et al. (1999) reported decreases in soil bulk density with biosolids applied at either 50 or 5 Mg ha^{-1} yr-1 over 12 years, respectively. Barbarick et al. (2004) reported greater soil microbial biomass activity in overgrazed rangeland plots six years after amended with biosolids land application (30 Mg ha^{-1}) as compared to a control. Dennis and Fresquez (1989) concluded that increasing biosolids application rates (up to 90 Mg ha^{-1}) in a overgrazed semi-arid grassland soil increased N, P, K, and soil microbial communities associated with bacteria, fungi, and ammonium oxidizers. However, Sullivan et al. (2006b), who studied Single or Repeated biosolids applications (0 to 30 Mg ha^{-1}) to overgrazed rangelands, reported that mineralization activities were only stimulated at the greatest Repeated biosolids application rates (i.e., 30 Mg ha^{-1}). Sullivan et al. (2006a and 2006b) further showed that significant long-term effects on chemical and nutrient soil health were evident, even at relatively low rates (i.e., up to 5 Mg ha^{-1}).

In most of the above studies, biosolids application rates were used to target physical, chemical, or biological soil alterations in rangeland settings, yet none of these studies grouped together all facets of soil alterations due to biosolids land application. Because of the positive effects of biosolids in enhancing overall soil functionality, there is a need to expand our understanding on how organic amendments can be utilized to simultaneously improve soil physical, chemical, nutrient, and biological attributes, or the overall soil health, of overgrazed rangelands. However, to the best of our knowledge no study has quantified alterations in soil health with respect to biosolids land application to overgrazed rangeland soils. Thus, the study objective was to quantify increasing Single or Repeated biosolids application (0 to 30 Mg ha^{-1}, applied in either 1991 or again in 2002) effects on rangeland soil health, with a goal to suggest a targeted biosolids application rate that would not cause detrimental effects but would enhance these systems to the greatest extent.

Materials and Methods

Experimental Site Design

The study was conducted on long-term experimental research plots within the Meadow Springs Ranch, Larimer County, Colorado, USA (40 53’46”N, 104 52’28”W). The ranch (1,750 m elevation) is owned by and located north of the City of Fort Collins, and is used for the city’s land-based biosolids recycling program. The study site was a semi-arid, shortgrass steppe rangeland ecosystem dominated by perennial grasses. The surface soil was classified as an

Altvan loam (Altvan series; fine-loamy over sandy or sandy-skeletal, mixed, superactive, Mesic Aridic Argistoll with 0 to 3% slopes; California Soil Resource Lab, (2008). In 1991, 15m x 15m plots were originally established and arranged in a randomized, complete block design with four replicates with anaerobically biosolids application rates equal to 0, 2.5, 5, 10, 21, or 30 Mg ha^{-1}. In 2002, each plot was divided in half (7.5x15 m) and a second application equaling the first application was applied to the eastern ½ of each plot. Re-application occurred in this manner due wind direction and attempting to prevent biosolids drift to other plots. A detailed description of the 1991 and 2002 biosolids chemical properties can be found in Sullivan et al. (2006b), while select biosolids characteristics that could directly affect soil health are presented in Table 1. (adapted from Sullivan et al. (2006)).

Table 1: Chemical and nutrient properties on a dry-weight basis of biosolids applied to Meadow Springs Ranch experimental plots in 1991 and 2002.

Constituent	Unit	1991 Biosolids	2002 Biosolids	Biosolids Range
pH		7.3	7.3	ND[a]
EC	dS m^{-1}	5.0	20.2	ND
Organic N	mg kg^{-1}	41,161	41,750	<1,000-176,000
NH_4–N	mg kg^{-1}	3,640	5,440	ND
NO_3-N	mg kg^{-1}	98	2.9	ND
Total P	mg kg^{-1}	16,141	11,350	<1,000-143,000
Total K	mg kg^{-1}	1,896	420	200-26,000

[a] *ND Not determined*

Sample Collection and Processing

In September 2018, a hydraulic Giddings probe was used to collect four soil cores (0-15 cm depth; 5 cm diameter) from each plot. Three cores were composited, placed in Ziploc bags and into coolers, while the fourth core was used for bulk density (Bd) and moisture content determination. Once returned to the lab, cores for moisture content and Bd were weighed, immediately dried at 105 ºC for at least 24 hours, and then weighed again. Composite soils were immediately passed through an 8-mm sieve; a sub-sample (~150 g) of the 8-mm sieved, field-moist soil was stored in a Ziploc bag at 4 ºC, another sub-sample (~300 g) of the 8-mm sieved soil was passed through a 2-mm sieve and allowed to air dry, and the remaining 8-mm sieved soil was also allowed to air dry. Once dry, a small sub-sample (~5 g) of the 2-mm sieved air-dried soil was powder ground.

Soil Health and Laboratory Soil Analysis – The Soil Management Assessment Framework

The Soil Management Assessment Framework (SMAF) is an assessment tool that provides a foundation for quantifying soil health by utilizing 11 soil indicators, in conjunction with soil management practices, climatic conditions, and taxonomy (Andrews et al., 2004); a detailed description of indicator scoring functions and outcomes can be found in Andrews et al. (2004). The soil indicators include:

- soil physical health indicators: 1) bulk density (ρ_b) and 2) water stable aggregates (WSA);
- soil chemical health indicators: 3) pH and 4) electrical conductivity (EC);
- soil nutrient health indicators: plant-available 5) phosphorus (P) and 6) potassium (K); and
- soil biological health indicators: 7) potentially mineralizable nitrogen (PMN), 8) microbial biomass carbon (MBC), 9) beta-glucosidase activity (BG), and 10) soil organic carbon (SOC).

The SMAF also utilizes 11) clay content, determined via soil textural analysis, as a dependent variable due to the influence clay content has on most other indicators involved in soil health quantification. Once all indicators have been entered into the SMAF, individual indicators are given unitless scores from 0 to 1 based on a) more is better, b) less is better, or c) somewhere in the middle is better (see Andrews et al. (2004) for a detailed description). Finally, individual indicator scores are grouped into physical, biological, chemical, nutrient, and overall soil health indices (SHI).

Soil Physical Health Indicators

Soil moisture content and bulk density were determined using an intact soil core as mentioned above. Water stable aggregates were determined based on the method of Kemper and Rosenau (1986) using 100 g of the 8-mm, air-dried soil place on top of a series of 23-cm diameter sieves (2.0, 1.0, 0.5, and 0.25-mm size screens). A Yoder sieving machine was set to 30 strokes per minute for 5 minutes, and then the soil was removed from each sieve with water and collected in a previously weighed aluminum pan. All water within the pan was evaporated at 105 ºC, after which the soil weight was determined.

Soil Chemical Health Indicators

Soil pH and electrical conductivity (EC) were determined on the 2-mm sieved, air-dried soil using a 1:1 soil:solution (20 g soil:20 mL DI in a 50 mL centrifuge tube) ratio (Thomas, 1996 and Rhoades, 1996). Soil slurries were shaken for 2 hours, after which pH was directly measured with a pH electrode and meter. The tubes were then centrifuged and EC was measured on the liquid phase using a conductivity meter.

Soil Nutrient Health Indicators

Olsen extractable P and K were determined by shaking 2 g of air-dried, 2-mm sieved soil with 40 ml of 0.5 M sodium bicarbonate solution for 30 minutes and then filtering through Whatman #2 filter paper (Olsen et al., 1954). Filtrates were gently covered with parafilm and left out over night to allow for loss of CO_2 gas. The filtrates were then diluted ten-fold using DI water and analyzed for P and K using inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Soil Biological Health Indicators

Potentially mineralizable nitrogen was calculated by subtracting baseline mineral N (i.e., NO_3-N and NH_4-N) from 28-day aerobically mineralized N concentrations (Curtin et al., 2004). Specifically, a 10 g sample of the air-dry, 2-mm sieved soil was shaken for 1 hour with 50 mL of 2 M KCl and then filtered through Whatman #2 filter paper. Baseline mineral N ((NO_2+NO_3) + NH_4) was determined colorimetrically using a Lachat Flow Injection Autoanalyzer. Another 30 g sub-sample of air-dried soil was placed into a 50 mL beaker, the soil in the beaker was gently tapped to a uniform bulk density of 1.0 g cm^{-3}, and then adjusted to 60% water-filled pore space with deionized water (DI). The beaker was placed in a quart Mason jar to which a small amount of water has been added at the bottom to maintain 100% relative humidity. The jars were incubated in the dark at room temperature (~ 22 ºC) for 28 days, with jars opened every 7 days to allow for air exchange. After the 28-day incubation period, approximately 10 g of wet sub-sample was removed from the beaker, weighed, and extracted for mineral N as described above. A separate soil sample was removed from the beaker to determine gravimetric soil moisture content to correct from a wet- to a dry-weight basis.

Microbial biomass carbon was determined on the 8-mm field-moist soil using a modified version of the chloroform fumigation/non-fumigation method (Hobbie, 1998), with total dissolved C analyzed on a TIC/TOC analyzer (Shimadzu TOC-L; Shimadzu Scientific Instruments, Inc., Kyoto, Japan). The difference between C in the fumigated and non-fumigated samples was considered the chloroform-labile C pool (*EC*), and is proportional to MBC as: MB*C=EC/kEC* where *kEC* is soil-specific, but is often estimated as 0.45 (Beck et al., 1997).

Beta-glucosidase activity was determined using the procedure published by Green et al. (2007). Triplicate, 1.0 g of air-dried, 2-mm sieved soil were placed into 50 mL Erlenmeyer flasks; two sets were treated the same (i.e., the original samples set and a duplicate sample set); the remaining third set was treated as controls for each sample, and also included a single blank. Next, 4 mL of modified universal buffer (MUB) adjusted to pH 6.0, 0.25 mL toluene, and 1 mL 0.05 *M* ρ-nitrophenyl-ß-D-glucopyranoside (PNG) solution was added to the duplicate sample sets. The PNG was not added to the set of controls until after incubation. All samples were swirled and then incubated at 37 °C for 1 hour. The reaction was stopped by adding 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M Tris (hydroxymethyl) aminomethane (THAM) buffer solution (pH ~12), then swirling the flask. The soil suspension was filtered through Whatman # 2 filter paper and the filtrate was diluted using 1 mL of sample and 4 mL of 0.1 M THAM. Beta-glucosidase enzyme activity was then measured on a Genesys 10S UV-VIS spectrophotometer set at 410 nm with a standard curve created using *p*-nitrophenol (1 g p-nitrophenol L^{-1}) at 0, 10, 20, 30, 40, and 50 ug L^{-1}; all standards also contained 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M THAM.

Total C and total N were determined using a dry combustion LECO Tru-SPEC Elemental Analyzer (Leco Corp., St. Joseph, MI; Nelson et al., 1996). Soil inorganic C was quantified using the pressure transducer method (Sherrod et al., 2002). Soil organic carbon (SOC) was calculated as the difference between total C and soil inorganic carbon.

Ten percent duplicates and blanks were utilized for the above analyses. In addition, it is noted that no standard soil was concurrently utilized in the above analyses; there is no such material available on the market that specifically targets soil health protocols.

Statistical Analysis

The Meadow Springs Ranch site utilized a split-plot design (with time) containing four replicates. Utilizing RStudio Verison 1.2.1073 (2020), we performed ANOVA using the car package (Fox et al., 2019) and if significant differences were present (at an α of 0.05) within treatments or time, we determined mean separation using Tukey adjusted pairwise comparisons from the agricolae package (Mendiburu, 2020). The interaction between treatment and time was also taken into consideration.

Results and Discussion

In this study, most often interactions did not exist. Therefore, the discussion below focuses primarily on the effect of increasing biosolids application rate within the Single application or Repeated application, or on the overall effect of applying biosolids once (in 1991) or twice (in 1991 and 2002).

Soil Physical Indicators, Indicator Scores, and Physical Soil Health

Bulk density and WSA did not change significantly between treatments, application times, or within the interaction of treatment and time (Table 2). Bulk densities ranged from 0.69 to 0.78 g cm^{-3}, and although well within the range of ideal Bd for plant growth, were low due to the gravel component within the soil. Bulk density and WSA were nearly maximized in this system, with indicator scores near 1.00 (Table 3). Within the SMAF, both ρ_b and WSA are key components used to quantify physical soil health. Since both indicator scores were near 1.00, their combined contribution to the soil physical health index (SHI) led to a 1.00 in this soil health score (Table 4). This outcome suggests that this soil has the ability to meet rangeland plant and ecosystem requirements for water, aeration, and soil strength over time.

Table 2: Mean soil indicator characteristics (0-15-cm depth; collected in 2018) as affected by increasing Single (applied in 1991) or Repeated (applied in 2002) biosolids applications to a overgrazed rangeland at the Fort Collins, Colorado, USA Meadow Springs Ranch. When present, different letters between treatments within a given application year represent significant differences at the $p<0.05$ level.

Year trt (Mg ha^{-1})	ρ_b (g cm^{-3})†	WSA (%)	pH	EC (dS m^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	PMN (mg kg^{-1})	MBC (mg g^{-1})	BG (mg pnp kg^{-1} soil h^{-1})	SOC (%)
1991	Physical		Chemical		Nutrient		Biological			
0	0.72	71.1	6.15a	0.10	23.7d	607	22.2	99.1	24.2	1.79ab
2.5	0.72	76.2	6.20a	0.11	33.3d	577	25.3	106	23.5	1.54b
5	0.74	72.3	6.07ab	0.12	55.3cd	600	29.2	95.8	19.7	1.89ab
10	0.77	70.0	5.95ab	0.13	77.7bc	661	35.4	88.6	21.7	1.90ab
21	0.76	67.7	5.90ab	0.12	108b	547	31.2	111	15.1	1.96ab
30	0.72	81.2	5.65b	0.12	147a	575	25.5	116	12.8	2.11a
1991 AVG:	0.74	73.1	5.99	0.12	74.2	595	28.1	102.8	19.5	1.87
2002										
0	0.70	77.2	6.52A	0.15	21.3E	570	23.3	97.6	24.2	1.46C
2.5	0.70	76.9	6.48AB	0.21	37.7DE	589	28.6	100	26.2	1.85BC
5	0.72	80.9	6.28ABC	0.17	64.9CD	649	33.9	95.5	19.5	1.83BC
10	0.73	72.5	5.85BCD	0.14	99.9BC	597	28.8	98.4	22.7	2.26B
21	0.78	70.5	5.68CD	0.18	126B	611	29.9	116	14.6	2.17B
30	0.69	76.0	5.50D	0.31	175A	653	38.8	128	26.0	2.88A
2002 AVG:	0.72	75.7	6.05	0.19	87.5	612	30.6	105.9	22.2	2.08
ANOVA (between trts)	NS	NS	**	NS	**	NS	NS	NS	NS	**
ANOVA (between years)	NS	NS	NS	**	**	NS	NS	NS	NS	**
ANOVA (trt x yr interaction)	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

† ρ_b = bulk density, WSA = water-stable aggregates, EC = electrical conductivity, P = extractable phosphorus, K = extractable K, SOC = soil organic C, PMN = potentially mineralizable N, MBC = microbial biomass C, and BG = β-glucosidase activity (pnp = *p*-nitrophenol). NS=non-significant, *=p<0.05, and **=p<0.01.

Table 3. Mean soil indicator scores (0.00 to 1.00; greater is “better”; determined by the SMAF) from soil collected from the 0-15-cm depth (collected in 2018), as affected by increasing Single (applied in 1991) or Repeated (applied in 2002) biosolids applications to a overgrazed rangeland at the Fort Collins, Colorado, USA Meadow Springs Ranch. When present, different letters between treatments within a given application year represent significant differences at the *p*<0.05 level.

Year trt (Mg ha^{-1})	ρ_b†	WSA	pH	EC	P	K	PMN	MBC	BG	SOC
1991	Physical		Chemical		Nutrient		Biological			
0	0.99	1.00	0.98a	1.00	1.00a	1.00	0.99	0.14	0.03	0.31ab
2.5	0.99	1.00	0.99a	1.00	1.00a	1.00	1.00	0.16	0.03	0.23b
5	0.99	1.00	0.97a	1.00	1.00a	1.00	1.00	0.12	0.03	0.36ab
10	0.99	1.00	0.88ab	1.00	0.96a	1.00	1.00	0.13	0.03	0.36ab
21	0.99	1.00	0.86ab	1.00	0.76a	1.00	1.00	0.16	0.03	0.38ab
30	0.99	1.00	0.71b	1.00	0.16b	1.00	0.99	0.16	0.03	0.45a
1991 AVG:	0.99	1.00	0.90	1.00	0.81	1.00	1.00	0.15	0.03	0.35
2002										
0	0.99	1.00	0.87	1.00	1.00A	1.00	1.00	0.12	0.03	0.21C
2.5	0.99	1.00	0.80	1.00	1.00A	1.00	1.00	0.13	0.03	0.34BC
5	0.99	1.00	0.92	1.00	1.00A	1.00	1.00	0.12	0.03	0.33BC
10	0.99	1.00	0.85	1.00	0.82AB	1.00	1.00	0.13	0.03	0.50B
21	0.99	1.00	0.74	1.00	0.59B	1.00	1.00	0.16	0.02	0.47B
30	0.99	1.00	0.60	1.00	0.06C	1.00	1.00	0.21	0.03	0.72A
2002 AVG:	0.99	1.00	0.80	1.00	0.75	1.00	1.00	0.15	0.03	0.43
ANOVA (between trts)	NS	NS	**	NS	**	NS	NS	NS	NS	**
ANOVA (between years)	NS	NS	*	NS	NS	NS	NS	NS	NS	**
ANOVA (trt x yr interaction)	NS	NS	NS	NS	NS	NS	NS	NS	NS	**

† ρ_b = bulk density, WSA = water-stable aggregates, EC = electrical conductivity, P = extractable phosphorus, K = extractable K, SOC = soil organic C, PMN = potentially mineralizable N, MBC = microbial biomass C, and BG = β-glucosidase activity. NS=non-significant, *=p<0.05, **=p<0.01.

Table 4. Mean soil physical, chemical, nutrient, biological, and overall soil health index (SHI) scores (0.00 to 1.00; greater is "better") from soil collected from the 0-15-cm depth (collected in 2018), as affected by increasing Single (applied in 1991) or Repeated (applied in 2002) biosolids applications to a overgrazed rangeland at the Fort Collins, Colorado, USA Meadow Springs Ranch. When present, different letters between treatments within a given application year represent significant differences at the p<0.05 level.

Year trt (Mg ha^{-1})	Physical SHI	Chemical SHI	Nutrient SHI	Biological SHI	Overall SHI
1991					
0	1.00	0.99a	1.00	0.37	0.75a
2.5	1.00	0.99a	1.00	0.35	0.74a
5	1.00	0.98a	1.00	0.38	0.75a
10	1.00	0.94ab	0.98	0.38	0.74a
21	1.00	0.93ab	0.88	0.39	0.72a
30	1.00	0.86b	0.83	0.41	0.65b
1991 AVG:	1.00	0.95	0.95	0.38	0.73
2002					
0	1.00	0.93	0.99	0.34C	0.72A
2.5	1.00	0.90	1.00	0.37BC	0.73A
5	1.00	0.96	1.00	0.37BC	0.74A
10	1.00	0.93	0.91	0.42B	0.73A
21	1.00	0.87	0.79	0.41BC	0.70AB
30	1.00	0.81	0.91	0.49A	0.66B
2002 AVG:	1.00	0.90	0.93	0.40	0.71
ANOVA (between trts)	NS	*	*	**	**
ANOVA (between years)	NS	*	NS	*	NS
ANOVA (trt x yr interaction)	NS	NS	NS	*	NS

NS=non-significant, *=p<0.05, and **=p<0.01

Soil Chemical Indicators and Chemical Soil Health

Soil pH significantly decreased with increasing biosolids application rates, but was not affected by application time (Table 2). There was also a significant change in soil pH indicator values between treatments and application times (Table 3). Soil pH decreased following increasing biosolids applications because as this material is decomposed, hydrogen ions are released. A similar pH response with increasing biosolids application rates in a dryland agroecosystems was observed by Ippolito et al. (2021). Electrical conductivity significantly increased in the Repeated as compared to the Single biosolids application (Table 2). However, EC values were all relatively low (< 0.31 dS m^{-1}), and therefore there was no significant change in EC soil indicator score between application times or treatments (Table 3). Based on pH and EC findings, alterations in soil pH must have solely influenced the chemical soil health index (Table 4). The soil health index decreased with increasing Single biosolids application rates; the Repeated biosolids application rates contained too much variability to observe significant differences, although a trend of decreasing pH existed with increasing biosolids application rate. It is important to discuss trends as these individual indicators are combined to provide an overall soil health index that may show significance (discussed below). The chemical soil health index decreased with increasing Single biosolids application rates (and followed a similar trend with Repeated biosolids applications), because within the SMAF the scoring function drop below 1.00 at pH 6.5 and lower. The decrease in the SMAF scoring function from pH 6.5 and lower is related to optimizing plant-available P at pH 6.5 (Andrews et al., 2004).

Soil Nutrient Indicators and Nutrient Soil Health

Olsen-extractable P significantly increased with increasing biosolids application rate in both the Single and Repeated applications, and greater Olsen-extractable P was present in the

Repeated as compared to the Single application (Table 2). Increases in Olsen-extractable P led to decreases in the P index value between treatments (Table 3). The decrease in the extractable P indicator score was due to excessive plant-available P in the soil, which can potentially lead to greater environmental risk via P runoff to water (Andrews et al., 2004 and Pierzynski et al., 1994). Extractable K concentrations ranged from 547 mg kg^{-1} to 661 mg kg^{-1}, yet there was no significant difference in soil indicator characteristics or scores (Table 2 and Table 3). This result was not surprising given that plant-available K is much greater than the recommended 120 mg kg^{-1} for these ecosystems (Brummer et al., 2011). Based on plant-available P and K findings, alterations in available soil P would have solely influenced the nutrient soil health index, yet no significant differences existed (Table 4). However, a trend did exist with nutrient soil health decreasing with increasing Single or Repeated biosolids applications. Again, it is important to discuss trends because when these indicator scores are combined to provide an overall soil health index, the combination may lead to significance (discussed below).

Soil Biological Indicators and Biological Soil Health

There was no significant change in PMN between treatments or application times (Table 2). There was a positive trend with increasing biosolids application rate, similar to an Ippolito et al. (2021) study that found increasing biosolids application rates (0 to 11.2 Mg ha^{-1}; applied every other year over 22 years to a dryland agroecosystem) significantly increased PMN. Discussing trends, and not significance, is important in the context of these indicators, as when indicators are combined into a final soil health score, significance may be realized. Regardless, the PMN score was maximized in nearly every plot, suggesting that the relationship between soil microbial activity responsible for N mineralization and plant productivity is positive (Table 3).

Microbial biomass carbon characteristics and indicator scores showed no significant changes between treatments and application times (Table 2 and Table 3). However, similar to PMN, there was a positive trend with increasing biosolids application rate for both the characteristics and indicator scores. Microbial biomass C is the readily available carbon contained within the living, microbial component of soil. No new organic material has been deliberately added to the system since 2002, so the relatively constant MBC content and indicator scores suggest that the readily available carbon has been utilized over time. Some studies (Barbarick et al., 2004, Sciubba et al., 2011, Stott et al., 2011) have found increases in MBC shortly after biosolids were applied (as compared to controls). Hargreaves et al. (2003) suggested that MBC is a sensitive environmental indicator that increases for a short time after organic amendments are applied, and stabilize over longer periods of time without additional applications. This contention supports observations in the current study.

Beta-glucosidase activity did not significantly change between treatments or application times (Table 2). Unlike PMN and MBC, there was somewhat of a negative trend present with increasing biosolids application rate. Because beta-glucosidase activity was relatively low, the beta-glucosidase indicator score remained low and unchanged across treatments (Table 3). Beta-glucosidase activity has been suggested as an indicator of management effects as well as a predictor for potential increases in soil organic carbon before the changes are reflected in organic C accumulation (Bandick et al., 1999 and Paudel et al., 2011). Beta-glucosidase activity is generally greater in conservation or no-till practices compared to typical conventional cropping systems, with increases in its enzymatic activity noticeable within 1 to 3 years after altering management practices (Stott et al., 2011). In the current study, these plots have been sitting unaltered since 2002, and thus likely why beta-glucosidase activity was low.

Soil organic carbon characteristics and indicator scores were all significantly different between treatments and application times (Table 2 and Table 3). The Single biosolids treatments (applied in 1991) were still showing an SOC response, although not as pronounced as the Repeated treatments (applied in 2002). Increasing biosolids application rates increased SOC, and SOC increased to a greater extent with Repeated as compared to Single biosolids application rates. Beta-glucosidase and MBC are mentioned above as early indicators of long-term C accumulation (Hargreaves et al., 2003, Bandick et al., 1999, Paudel et al., 2011). However, in the current study any differences in beta-glucosidase and MBC have likely been reduced over time as the ecosystem has come to a new steady state with respect to SOC. The positive changes that occurred in SOC with Single or Repeated biosolids applications, in conjunction with positive trends in PMN and MBC, likely led to significant biological soil health changes between treatments in the Repeated biosolids plots, as well as in application times (Table 4). In a long-term biosolids agroecosystem study, Ippolito et al. (2021) showed similar biological soil health findings. The biological soil health findings are not surprising given that the biological aspect of any soil system has been known to be more sensitive to system alterations than physical, chemical, or nutrient indices (Bastida et al., 2008).

Combined Effects on Physical, Chemical, Nutrient, and Biological Soil Health on Overall Soil Health

A significant change existed in the overall soil health index between treatments within the Single or the Repeated biosolids application rates (Table 4). Overall soil health changes directly reflect significant differences and trends between treatments in three of the four soil health index categories discussed above. In Table 4, we can see that in both application years the chemical SHI and nutrient SHI was maximized between 0-21 and 0-10 Mg ha^{-1} biosolids treatments, respectively. The biological SHI was the only index that continued to increase with increasing biosolids application rate, and was maximized at 30 Mg ha^{-1} biosolids; this finding was identical to those of Sullivan et al. (2006b) at this research location. Table 4 also shows that the overall SHI was similar between 0-21 Mg ha^{-1}, then was significantly reduced at the 30 Mg ha^{-1} as compared to all (i.e., Single) or most (i.e., Repeated) other biosolids application rates.

The above soil health indices, when consider in unison, suggest that a 30 Mg ha^{-1} application rate is excessive for this location. Furthermore, results suggest that no biosolids application in this overgrazed, semi-arid rangeland site is better in terms of soil health as compared to the 30 Mg ha^{-1} application rate. This observation was due to decreases in soil chemical and nutrient health indices associated with the excessive 30 Mg ha^{-1} application rate. Previous research from this site by Sullivan et al. (2006a and 2006b), several years following the Repeated biosolids application, showed that above-ground plant community richness decreased when >10 Mg ha^{-1} of biosolids were applied, while above-ground biomass increased when >10 Mg ha^{-1} biosolids were applied. Averaging the current application ranges above for the various SHI, in combination with past findings, suggests that a 'sweet spot' exists in terms of biosolids application at this location, at approximately 10 Mg of biosolids ha^{-1}. This 'sweet spot' maximizes the positive impacts of biosolids on soil health without excessively compromising major alterations in plant community richness or above-ground biomass.

Conclusions

This study quantified the effects of increasing Single or Repeated biosolids applications (0, 2.5, 5, 10, 21, or 30 Mg ha^{-1}, applied in either 1991 or again in 2002) on rangeland soil health, utilizing the SMAF and previous findings, to suggest reasonable targeted biosolids application rate(s) that would not cause detrimental effects and instead enhance these systems to the greatest extent over time. Twenty-seven years following a one-time application of increasing biosolids rates still showed the effects of decreasing soil pH and increasing available soil P and SOC. Even after 16 years following a second application of increasing biosolids rates, soil characteristic alterations followed similar significant differences and trends. Considering the Single and Repeated biosolids applications combined, the physical SHI was maximized regardless of biosolids application rate or time, while the chemical, nutrient, biological, and overall SHI were maximized at 0-21, 0-10, 30, and 0-21 Mg ha^{-1}, respectively. This, in combination with previous plant community and biomass findings at this location (e.g., Sullivan et al. 2006a and 2006b), suggest that biosolids land application to this overgrazed rangeland would provide the most benefit at ~ 10 Mg ha^{-1}. This holistic approach of combining and interpreting soil physical, chemical, nutrient, biological, and overall soil health indices can help identify a targeted organic amendment application rate for maximum improvements in overgrazed rangeland soils.

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CHAPTER III SUMMARY AND CONCLUSIONS

Conclusions and Key Findings

This thesis explored the impact of various long-term biosolids applications on the soil physical, chemical, nutrient, and biological attributes in both general agricultural and specific rangeland contexts. Through the use of the Soil Management Assessment Framework (SMAF), this study assessed how biosolids applications affected overgrazed rangelands, providing insights that could guide more effective land management practices.

Soil Physical Health

The study demonstrated that soil physical health, measured through bulk density and water-stable aggregates, showed minimal response to biosolids application rates. The physical health indicators were already maximized in this soil system, suggesting that this soil has the ability to meet rangeland plant and ecosystem requirements for water, aeration, and soil strength over time even without biosolids application. This consistency indicates that in the semi-arid rangeland context, biosolid additions primarily influenced other aspects of soil health beyond the physical domain.

Chemical and Nutrient Soil Health

Soil pH and nutrient availability (particularly phosphorus) were significantly affected by biosolid applications. Greater biosolids application rates led to a decrease in soil pH, indicating increased soil acidity. However, nutrient health, especially phosphorus levels, showed significant increases with moderate biosolid applications, benefiting soil fertility in the short-term. Excessive biosolid applications, on the other hand, resulted in diminished nutrient soil health scores, suggesting a threshold beyond which the positive impacts are reduced. The nutrient soil health reduction was due to an increased risk of off-site phosphorus movement (i.e., in runoff) associated with excessive biosolids application rates.

Biological Soil Health

The biological indices, including microbial biomass carbon, potentially mineralizable nitrogen, and soil organic carbon, showed either strong positive responses or significant differences to biosolids applications. Although soil organic carbon was the only indicator with statistical differences among the application rates, both microbial biomass carbon and potentially mineralizable nitrogen showed positive trends with increasing biosolids application rates. This is important to note in the context of these indicators, as when indicators were combined into a final biological soil health score, the overall significance was realized. This finding underscores the critical role that organic amendments play in supporting microbial communities and enhancing soil organic matter, which are vital for nutrient cycling and overall ecosystem resilience.

Overall Soil Health

Integrating these indices, the study found that a biosolids application rate of approximately 10 Mg ha^{-1} maximized overall soil health in overgrazed rangelands. Rates above 30 Mg ha^{-1} were found to be less beneficial, with certain soil health indicators declining at these higher application rates. This suggests an optimal application threshold for achieving balanced improvements across soil health parameters without risking potential degradation from over-application.

Implications for Soil and Ecosystem Management

The results emphasize the importance of carefully calibrated biosolids application rates to optimize soil health. While biosolids provide a valuable source of organic matter and nutrients that support soil recovery and resilience, their excessive use can lead to unintended consequences, including soil acidity and nutrient imbalances. Hence, these findings have practical applications in soil conservation and sustainable land management, particularly in overgrazed rangelands where soil health restoration is essential for ecosystem sustainability.

This research also highlights the effectiveness of using a comprehensive soil health assessment tool like SMAF. By quantifying multiple soil health parameters and evaluating their combined effects, SMAF offers a holistic approach that can inform targeted and sustainable land management strategies.

Recommendations for Future Research

Building upon these findings, future research should focus on several key areas:

- *Long-term Impacts:* Extended monitoring beyond the current timeframe could reveal more about the enduring impacts of biosolids applications on soil health, particularly in semi-arid, overgrazed rangeland ecosystems.
- *Comparative Studies:* Additional studies comparing different types of organic amendments, such as compost and manure, could provide a broader understanding of their respective impacts on soil health.
- *Soil Health under Diverse Conditions:* Expanding similar research to other ecosystem types, including forested and urban soils, could further validate the adaptability of biosolids applications and soil health assessment tools across varied environments.

In conclusion, this thesis underscores the crucial role of soil health in ecosystem sustainability and demonstrates that organic amendments like biosolids, when applied thoughtfully, can significantly contribute to the restoration and maintenance of soil functionality in overgrazed landscapes. Through strategic applications informed by soil health assessment frameworks, land managers can foster healthier soils that support biodiversity, productivity, and ecosystem resilience.