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Long-Term Biosolids Applications to Overgrazed Rangelands Improve Soil Health

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Abstract: 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 degraded 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) were 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 rates, 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 material provides maximum benefits to soils, plants, animals, and the environment.

Keywords: biosolids; overgrazed rangelands; soil health; Soil Management Assessment Framework

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1. Introduction

Soil is an essential, non-renewable resource with potentially rapid degradation rates and extremely slow formation and regeneration processes [1]; 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 [1]. The negative effects of soil degradation are especially important in rangelands.

Rangelands comprise 25% of Earth's surface [2], with 30% (312 million ha) of the total U.S. land base under rangelands [3]. 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 [2,4]. 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" [5].

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, combinations 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 [6]. 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 degraded. 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 degraded 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 are 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, and microorganism activity) and encourage plant growth [7]. 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 [8,9], 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. [10] and Saviozzi et al. [11] reported decreases in soil bulk density with biosolids applied at either rates of 50 or 5 Mg ha^{-1} yr^{-1} over 12 years, respectively. Barbarick et al. [12] 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 [13] concluded that increasing biosolids application rates (up to 90 Mg ha^{-1}) in a degraded semi-arid grassland soil increased N, P, K, and soil microbial communities associated with bacteria, fungi, and ammonium oxidizers. However, Sullivan et al. [14], who studied single or repeated biosolids applications (0 to 30 Mg ha^{-1}) to degraded rangelands, reported that mineralization activities were only stimulated at the greatest repeated biosolids application rates (i.e., 30 Mg ha^{-1}). Sullivan et al. [14,15] 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 degraded rangelands. However, to the best of our knowledge, no study has quantified alterations in soil health with respect to biosolids land application to degraded 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.

2. Materials and Methods

2.1. Experimental Site Design

This 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 (1750 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 [16]). In 1991, 15 m × 15 m plots were originally established and arranged in a randomized, complete block design with four replicates with anaerobic biosolids application rates equal to 0, 2.5, 5, 10, 21, or 30 Mg ha^{-1}. In 2002, each plot was divided in half (7.5 m × 15 m)

and a second application equaling the first application was applied to the eastern $\frac{1}{2}$ of each plot. Re-application occurred in this manner due to 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. [14], while select biosolids characteristics that could directly affect soil health are presented in Table 1.

Table 1. Selected biosolids characteristics applied to the Meadow Springs Ranch shortgrass steppe rangeland site in 1991 or 2002 (adapted from Sullivan et al. [14]).

Constituent	Unit	1991 Biosolids	2002 Biosolids
pH		7.3	7.3
EC	dS m^{-1}	5.0	20
Organic N	mg kg^{-1}	41,160	41,750
NH_4-N	mg kg^{-1}	3640	5440
NO_3-N	mg kg^{-1}	98	2.9
Total P	mg kg^{-1}	16,140	11,350
Total K	mg kg^{-1}	1900	420

2.2. 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 °C for at least 24 h, 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 °C, 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 dry soil was powder ground.

2.3. 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 [6]; a detailed description of indicator scoring functions and outcomes can be found in Andrews et al. [6]. The soil indicators include:

- Soil physical health indicators: (1) bulk density and (2) water-stable aggregates (WSAs);
- 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. [6] for a detailed description). Finally, individual indicator scores are grouped into physical, biological, chemical, nutrient, and overall soil health indices (SHI).

2.4. 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 [17] 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-sized screens). A Yoder sieving machine was set to 30 strokes per minute for 5 min, 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 °C, after which the soil weight was determined.

2.5. 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 [18,19]. Soil slurries were shaken for 2 h, after which pH was directly measured with a pH electrode and meter. The tubes were then centrifuged and EC was measured in the liquid phase using a conductivity meter.

2.6. 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 min and then filtering through Whatman #2 filter paper [20]. 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).

2.7. 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 [21]. Specifically, a 10 g sample of the air-dried, 2 mm sieved soil was shaken for 1 h 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 °C) 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 [22], 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: $MBC = EC/kEC$ where *kEC* is soil specific, but is often estimated as 0.45 [23].

Beta-glucosidase activity was determined using the procedure published by Green et al. [24]. 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 samples 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 h. 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, USA; [25]. Soil inorganic C was quantified using the pressure transducer method [26]. 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.

2.8. Statistical Analysis

The Meadow Springs Ranch site utilized a split-plot design (with time) containing four replicates. Utilizing RStudio Verison 1.2.1073 [27], we performed ANOVA using the car package [28] and if significant differences were present (at an α of 0.05) within treatments or time, we determined mean separation using Tukey's adjusted pairwise comparisons from the agricolae package [29]. The interaction between treatment and time was also taken into consideration.

3. Results and Discussion

In this study, most often interactions did not exist. Therefore, the discussion below focuses primarily on the effect of increasing the 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).

3.1. Soil Physical Indicators, Indicator Scores, and Physical Soil Health

Bulk density and WSAs 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 WSAs were nearly maximized in this system, with indicator scores near 1.00 (Table 3). Within the SMAF, both Bd and WSAs 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 degraded 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}) †	WSAs (%)	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	6.15 a	0.10	23.7 d	607	22.2	99.1	24.2	1.79 ab
2.5	0.72	76.2	6.20 a	0.11	33.3 d	577	25.3	106	23.5	1.54 b
5	0.74	72.3	6.07 ab	0.12	55.3 cd	600	29.2	95.8	19.7	1.89 ab
10	0.77	70.0	5.95 ab	0.13	77.7 bc	661	35.4	88.6	21.7	1.90 ab
21	0.76	67.7	5.90 ab	0.12	108 b	547	31.2	111	15.1	1.96 ab
30	0.72	81.2	5.65 b	0.12	147 a	575	25.5	116	12.8	2.11 a
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	6.52 A	0.15	21.3 E	570	23.3	97.6	24.2	1.46 C
2.5	0.70	76.9	6.48 AB	0.21	37.7 DE	589	28.6	100	26.2	1.85 BC
5	0.72	80.9	6.28 ABC	0.17	64.9 CD	649	33.9	95.5	19.5	1.83 BC
10	0.73	72.5	5.85B CD	0.14	99.9 BC	597	28.8	98.4	22.7	2.26 B
21	0.78	70.5	5.68 CD	0.18	126 B	611	29.9	116	14.6	2.17 B
30	0.69	76.0	5.50 D	0.31	175 A	653	38.8	128	26.0	2.88 A
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, WSAs = 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 degraded 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 †	WSAs	pH	EC	P	K	PMN	MBC	BG	SOC
1991	Physical		Chemical		Nutrient			Biological		
0	0.99	1.00	0.98 a	1.00	1.00 a	1.00	0.99	0.14	0.03	0.31 ab
2.5	0.99	1.00	0.99 a	1.00	1.00 a	1.00	1.00	0.16	0.03	0.23 b
5	0.99	1.00	0.97 a	1.00	1.00 a	1.00	1.00	0.12	0.03	0.36 ab
10	0.99	1.00	0.88 ab	1.00	0.96 a	1.00	1.00	0.13	0.03	0.36 ab
21	0.99	1.00	0.86 ab	1.00	0.76 a	1.00	1.00	0.16	0.03	0.38 ab
30	0.99	1.00	0.71 b	1.00	0.16 b	1.00	0.99	0.16	0.03	0.45 a
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	1.00 A	1.00	1.00	0.12	0.03	0.21 C
2.5	0.99	1.00	0.80	1.00	1.00 A	1.00	1.00	0.13	0.03	0.34 BC
5	0.99	1.00	0.92	1.00	1.00 A	1.00	1.00	0.12	0.03	0.33 BC
10	0.99	1.00	0.85	1.00	0.82 AB	1.00	1.00	0.13	0.03	0.50 B
21	0.99	1.00	0.74	1.00	0.59 B	1.00	1.00	0.16	0.02	0.47 B
30	0.99	1.00	0.60	1.00	0.06 C	1.00	1.00	0.21	0.03	0.72 A
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, WSAs = 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, * = $p < 0.05$, and ** = $p < 0.01$.

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 degraded 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	0.75 a
2.5	1.00	0.99 a	1.00	0.35	0.74 a
5	1.00	0.98 a	1.00	0.38	0.75 a
10	1.00	0.94 ab	0.98	0.38	0.74 a
21	1.00	0.93 ab	0.88	0.39	0.72 a
30	1.00	0.86 b	0.83	0.41	0.65 b
1991 AVG:	1.00	0.95	0.95	0.38	0.73
2002					
0	1.00	0.93	0.99	0.34 C	0.72 A
2.5	1.00	0.90	1.00	0.37 BC	0.73 A
5	1.00	0.96	1.00	0.37 BC	0.74 A
10	1.00	0.93	0.91	0.42 B	0.73 A
21	1.00	0.87	0.79	0.41 BC	0.70 AB
30	1.00	0.81	0.91	0.49 A	0.66 B
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 ** = $p < 0.01$.

3.2. 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. [30]. 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 rates. 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 [6].

3.3. Soil Nutrient Indicators and Nutrient Soil Health

Olsen-extractable P significantly increased with increasing biosolids application rates 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 [6,31]. Extractable K concentrations ranged from 547 to 661 mg kg^{-1}, yet there was no significant difference in soil indicator characteristics or scores (Tables 2 and 3). This result was not surprising given that plant-available K is much greater than the recommended 120 mg kg^{-1} for these ecosystems [32]. 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).

3.4. 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 rates, similar to an Ippolito et al. [30] 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 (Tables 2 and 3). However, similar to PMN, there was a positive trend with increasing biosolids application rates 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 [12,33,34] have found increases in MBC shortly after biosolids were applied (as compared to controls). Hargreaves et al. [35] 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 rates. 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 [36,37]. 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 [34]. 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 (Tables 2 and 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 [35–37]. 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. [30] 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 [38].

3.5. 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 and 21 and 0 and 10 Mg ha^{-1} biosolids treatments, respectively. The biological SHI was the only index that continued to increase with increasing biosolids application rates, and was maximized at 30 Mg ha^{-1} biosolids; this finding was identical to those of Sullivan et al. [14] at this research location. Table 4 also shows that the overall SHI was similar between 0 and 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 considered 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 degraded, 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. [14,15], 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.

4. 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 the 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. [14,15]), suggests that biosolids land application to this degraded 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 degraded rangeland soils.

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