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Tracking Soil Health Changes in a Management-Intensive Grazing Agroecosystem

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Abstract: Management-intensive Grazing (MiG) has been proposed to sustainably intensify agroecosystems through careful management of livestock rotations on pastureland. However, there is little research on the soil health impacts of transitioning from irrigated cropland to irrigated MiG pasture with continuous livestock rotation. We analyzed ten soil health indicators using the Soil Management Assessment Framework (SMAF) to identify changes in nutrient status and soil physical, biological, and chemical health five to six years after converting irrigated cropland to irrigated pastureland under MiG. Significant improvements in biological soil health indicators and significant degradation in bulk density, a physical soil health indicator, were observed. Removal of tillage and increased organic matter inputs may have led to increases in β-glucosidase, microbial biomass carbon, and potentially mineralizable nitrogen, all of which are biological indicators of soil health. Conversely, trampling by grazing cattle has led to increased bulk density and, thus, a reduction in soil physical health. Nutrient status was relatively stable, with combined manure and fertilizer inputs leading to stabilized plant-available phosphorous (P) and increased potassium (K) soil concentrations. Although mixed effects on soil health were present, overall soil health did increase, and the MiG system appeared to have greater overall soil health as compared to results generated four to five years earlier. When utilizing MiG in irrigated pastures, balancing the deleterious effects of soil compaction with grazing needs to be considered to maintain long-term soil health.

Keywords: soil health; management-intensive grazing; irrigation; SMAF

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1. Introduction

Sustainable intensification of agroecosystems has become an important topic for land managers, climate scientists, and numerous stakeholders looking to meet environmental goals while maintaining highly productive farms and ranches. One increasingly popular management strategy for meeting producer on-site goals is Management-intensive Grazing (MiG), which is a management scheme that may increase stocking rates, improve forage quality, and reduce negative environmental impacts from production [1,2].

Martz et al. [3] defined MiG as a "flexible approach to rotational grazing management whereby animal nutrient demand through the grazing season is balanced with forage supply and available forage is allocated based on animal requirements". This style of management may broadly apply to a variety of more specific practices, such as rotational stocking, strip grazing, creep grazing, limit grazing, and many other methods that require intense management techniques and frequent cattle movement to maintain high productivity in a pasture throughout a grazing season [4]. As management techniques improve and increased land efficiency of these intensive operations competes with fluctuating commodity prices in conventional cropland, producers may find benefit in transitioning from cropland to MiG systems, using existing irrigation infrastructure to support high forage productivity goals. However, there is little research on how this transition may impact soil health, a primary driver of long-term productivity and sustainability in MiG agroecosystems.

Research exists comparing annual cropland to perennial grassland, but much of this research is focused on conversion to Conservation Reserve Program land or compares long-term cropland sites to long-term grassland sites [5–10]. However, there is little research on converting to grassland and much less that examines conversion in systems that are characterized by intense management and frequent livestock rotation. Studies that examine not only the transition from annual cropland to perennial grassland but, more specifically, the transition to intensively grazed rotational systems may provide insight into the soil characteristics that promote healthy, productive agroecosystems that provide filtration of water, physical stability, and resistance to erosion, and adequate nutrient cycling to maintain soil fertility [11]. However, we may look to past research on perennial grasslands and pastures to guide our investigation of Management-intensive Grazing.

Within perennial pasture systems, soil biological activity associated with the healthy cycling of key plant nutrients to support sustainable crop production may be improved as compared to crop production systems [5,6]. Soil enzymatic activity has often been used as an indicator of soil health due to the high sensitivity of exoenzyme activity to management practice change and the important role that microbial enzymes play in nutrient cycling [11–14]. Moreover, the deep rooting systems and lack of intense tillage in perennial grasslands have been shown to increase soil organic carbon [15–18], an important source of energy for microbial communities. Microbial biomass carbon (MBC) has been shown to be greater in perennial grassland or pasture systems than in cropland systems [8,19], supporting the theory that these management systems may support larger and more active microbial communities. Grazing of varying intensities has also been shown to shift microbial community structure, activity, and diversity [20,21], indicating that the interactions of livestock, plants, and soil have a profound impact on soil microbial activity.

However, the potential soil health benefits of perennial grassland management are not limited to microbial activity. Manure inputs provide a way to supplement soil fertility and limit the need for inorganic fertilizers [22,23]. Decreased inorganic fertilizer use in perennial pasture systems may decrease fertilizer acidification and salinization of soils [8], both of which are threats to long-term sustainability. However, changes in soil pH and accumulation of salts in managed perennial grasslands tend to be relatively small and likely insignificant in terms of either directly or indirectly affecting plant growth and soil fertility.

Similarly, soil physical properties may be altered by the conversion from cropland to MiG systems. While perennial root systems may reduce bulk density over annual crop systems, the lack of regular tillage and the repeated hoof action of cattle and other livestock in MiG systems may increase bulk density (Bd) and disrupt soil aggregates in grazed soils [24], potentially limiting root growth, reducing available water content, and decreasing infiltration rate [25–27]. Thus, MiG agroecosystems need to be focused upon to further understand the tradeoffs between improvements versus degradation of soil biological, chemical, and physical properties, and, ultimately, the creation of resilient and sustainable agroecosystems.

The current study builds upon the work by Shawver et al. [1], which used SMAF (see Andrews et al. [11] for full framework details) to monitor soil health changes during the early transition from irrigated cropland to an irrigated MiG perennial pasture. While the Shawver et al. [1] study examined years 1 and 2 following the transition, we present the soil health measurements of years 5 and 6 to reflect upon the continued changes to soil health following cropland to MiG perennial pasture transition.

Based on current literature, we hypothesized that as this MiG pasture matures, the soil will experience (a) negative changes in physical soil health from continued trampling via hoof pressure, (b) positive changes in biological soil health from manure inputs with low soil disturbance, (c) no change in chemical soil health, as high $CaCO_3$ content present is likely to buffer pH change and EC is already low, with irrigation likely to push salts deeper into the soil profile, (d) a positive change in nutrient status from manure inputs of P and K, and an improvement in overall soil health from biological soil health and nutrient content improvements outweighing physical soil health impairments.

2. Materials and Methods

2.1. Site Description

This study was conducted under an 82 ha center pivot at the Colorado State University Agricultural Research, Development, and Education Center northeast of Fort Collins, CO USA (40°39′30.16″ N, 104°59′09.00″ W) at an altitude of 1554 m. Monthly mean temperatures tend to peak in July with an average high of 30 °C and reach a low in December with a minimum average temperature of −10 °C. Total average precipitation is approximately 340 mm, with much of this precipitation occurring between April and August [28]. The region is classified under the Köppen–Geiger classification system as a semi-arid, cold steppe [1,29]. Across this portion of Colorado, many producers graze cattle on unirrigated land, while cropland is dominated by irrigated commodity crops—namely corn and wheat. Maintaining the high productivity of intensively managed pasture consistent with this study requires additional irrigation inputs above typical climactic conditions. For example, the 2021 center pivot irrigation totals ranged from 460 to 590 mm, depending on in-field location and irrigation demands by the perennial grasses. Soil fertility requirements were supplemented by inorganic fertilizer inputs. For all years, fertilizer needs were determined by commercial soil testing and are as follows:

- 2017: No fertilizer added;
- 2018: ~14 kg N ha^{-1} and ~67 kg P ha^{-1} as monoammonium phosphate;
- 2019: no fertilizer added;
- 2020: no fertilizer added;
- 2021: ~90 kg N ha^{-1}, 22 kg P ha^{-1}, and 13 kg S ha^{-1} as a mix of monoammonium phosphate, urea, and ammonium sulfate;
- 2022: ~56 kg N ha^{-1}, 22 kg P ha^{-1}, and 13 kg S ha^{-1} as a mix of monoammonium phosphate, urea, and ammonium sulfate.

Before 2016, the 82 ha pivot field was managed as a sprinkler-irrigated, fully tilled crop rotation of grain corn, silage corn, dry beans, and alfalfa. Between the 2016 and 2017 growing seasons, the field was converted to a cool-season grass-forage mix of multiple bromes, clover species, and other common forages, such as alfalfa. The full details of the grass mix and livestock grazing pattern are available in Shawver et al. [1]. In the spring of 2017, the field was split into ~2.6 ha paddocks. Cattle were then introduced, and approximately 230 animal units consisting of cow-calf pairs, yearling heifers, and yearling steers were rotated through the paddocks depending on forage availability and dietary needs. Broadly, the system was comprised of intense grazing in small paddocks for 1–4 days to remove approximately 50% of forage biomass, with cattle subsequently being moved into adjacent paddocks delineated with mobile electric fences; GPS tools were used to precisely manage the entire area. Decisions to move cattle through paddocks were based on maintenance of stand health and to avoid over-grazing.

2.2. Soil Sampling and Processing

Soils were sampled in the same location as in the Shawver et al. [1] study, focusing sampling on the primary soil series in the pivot: Nunn clay loam (fine, montmorillonitic, mesic Aridic Argiustolls, 26 ha; [30]), Kim loam (fine-loamy, mixed, calcareous, mesic Ustic Torriorthents, 10 ha), and Garrett loam (fine-loamy, mixed, mesic Pachic Argiustolls, 7.5 ha; [30]). In order to maintain continuity between samples from 2017 to 2022, soil was not collected in the northwest quarter of the pivot due to poor initial forage establishment in the spring of 2017. Thus, while the pivot encompasses 82 ha, sampled soil only represented 62 ha. The sample locations were randomly chosen within the paddocks using ArcMap (Version 10.5.1, ArcMap GIS) in 2017, but the paddocks were chosen to represent both the primary soil textures in the field and the forage mixtures. For the current study, soil sampling occurred in late June of 2021 and late May of 2022.

Soil samples were collected using a 2.5 cm diameter soil probe to a depth of 15 cm, with each core split into 0-to-5 and 5-to-15 cm depths. Approximately 25–30 cores were sampled within a 3 m radius centered around the GPS-located sampling point, composited per sample, and mixed in a plastic bucket before being transferred to a plastic bag, sealed, and placed in dry coolers. Within this sampling radius, an additional intact core at both depth increments was preserved in a metal can for gravimetric soil moisture and bulk density (Bd) determination.

Soils were returned to the laboratory the same day and stored at 4 °C until processing. Bulk density and moisture content were determined by immediately weighing moist cores stored in metal cans, drying at 105 °C for 24 h, followed by weighing. The composited soil samples were passed through an 8 mm sieve to remove rocks and large plant debris. Approximately 150 g of field-moist, 8 mm sieved soil was then stored at 4 °C prior to MBC analysis. An additional ~150 g of soil was passed through a 2 mm sieve and air-dried, while the remainder of the 8 mm sieved soil was also air-dried, both for further analysis. Both air-dried subsamples were returned to plastic bags and stored at room temperature.

2.3. Soil Health Analyses

The SMAF [11] is a Microsoft Excel-based tool used to score and provide relative interpretations of soil health measurements within the context of climactic conditions, cropping system, soil taxonomy, and texture. Selection of soil indicators may be based on an intended research goal, but are broadly split into four categories: soil physical indicators (Bd and water-stable aggregates (WSA)), soil biological indicators (soil organic carbon (SOC), MBC, potentially mineralizable nitrogen (PMN), and β-glucosidase activity (BG)), soil chemical indicators (pH and electrical conductivity (EC)), and soil nutritional indicators (plant-available P and K). Indicators are selected to represent key soil ecosystem services, agronomic needs, and sensitivity to changes in management [11]. The SMAF translates the raw measurements of these soil indicators into unitless scores from 0 to 1 (0 being "worst" and 1 being "best") based on algorithms accounting for soil texture, climate, and cropping system. The SMAF has been used previously to study cropland, pastures, cropland-to-pasture conversions, and various other management schemes [1,10,31,32].

2.3.1. Soil Physical Health Indicators

Soil moisture content and bulk density were determined using an intact soil core of known volume. The weight of the soil core was measured at field moisture and after 24 h at 105 °C until dried mass was consistent. Water-stable aggregates were determined using the method described in Kemper and Rosenau [33] using 100 g of 8 mm air-dried soil. The soil was placed on top of a stack of 23 cm diameter sieves (2.0, 1.0, 0.5, and 0.25 mm sized screens), which were attached to a Yoder sieving machine and submerged at 30 strokes per minute for 5 min. The soil remaining on all of the sieves was rinsed into an aluminum pan, and the water from the pan was evaporated until completely dry at 105 °C, at which point soil weight in the pan was determined.

2.3.2. Soil Biological Health Indicators

β-glucosidase activity was determined using the methodology published by Green et al. [34]. In triplicate, 1.0 g of air-dried 2 mm sieved soil was weighed into 50 mL Erlenmeyer flasks to create three sets of each sample. One set was treated as the control and contained an additional blank empty Erlenmeyer flask. The other two sets were treated as a sample set and a duplicate set. Following this, 4 mL of modified universal buffer (MUB) at pH 6.0 and 0.25 mL of toluene were added to all flasks, and 1 mL of 0.05 M ρ-nitrophenyl-β-glucopyranoside (PNG) was added to the sample flasks and duplicate flasks, but PNG is not yet added to the control flasks. All samples were swirled and incubated at 37 °C for 1 h, at which point 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M TRIS (hydroxymethyl) aminomethane (THAM) buffer at pH 12 is added to all flasks and 1 mL of 0.05 M PNG is added to the control flasks. These soil suspensions were filtered through Whatman #2 filter paper, and the filtrate was diluted by adding 4 mL of 0.1 M THAM to 1 mL of sample. B-glucosidase activity was measured using a Genesys 10S UV-VIS spectrophotometer at 410 nm, using a standard curve of *p*-nitrophenol at 0, 10, 20, 30, 40, and 50 $\mu g\ L^{-1}$ in 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.1 M THAM. Microbial biomass carbon was determined using the chloroform fumigation/non-fumigation method [35], which estimates MBC by measuring dissolved C analyzed on a TIC/TOC analyzer (Shimadzu TOC-L; Shimadzu Scientific Instruments, Inc., Kyoto, Japan), subtracting dissolved C extracted from unfumigated samples from dissolved C extracted from fumigated samples and using a ratio of chloroform-labile C to microbial biomass C (0.45).

Soil organic C was determined as the difference between total C and inorganic C. Total C was measured using a dry combustion VELP Dumas Elemental Analyzer (VELP Scientifica, Usmate Velate, Italy; [36]), while inorganic C was determined using the pressure transducer method [37].

Potentially mineralizable N was determined by subtracting NO_3-N and NH_4-N concentrations from non-incubated soils from those same soils that were allowed to incubate aerobically for 28 days [38]. Approximately 30 g of air-dried, 2 mm sieved soil was weighed into a 50 mL beaker, and the beaker was tapped gently to bring the soil to approximately 1.0 g cm^{-3} bulk density. The soil was then brought to 60% water-filled pore space using deionized water, and the flask was placed in a Mason jar with ~1 cm of water in the bottom of the jar to maintain soil moisture. This Mason jar was sealed and placed in a cool, dark cabinet for 28 days; every 7 days, the jars were opened briefly to allow air exchange. After 28 days, a 10 g subsample of the soil was removed and placed into a 125 mL plastic bottle. Concurrently, a 10 g sample of air-dried 2 mm sieved soil that was not incubated was weighed into a 125 mL plastic bottle to serve as the control. Both controls and samples were shaken for 30 min with 50 mL of 2M KCl and filtered through Whatman #1 filter paper. Following this, the filtrate was analyzed for NO_3-N and NH_4-N. NO_3-N was determined by a combination of 15 µL sample filtrate with 250 µL of Vanadium (III) Chloride reagent and 35 µL 2M KCl to force a Griess reaction and measure NO_2-N concentration colorimetrically on a Genesys 10S UV-VIS spectrophotometer at 540 nm. Similarly, NH_4-N was determined by combining 15 µL of sample with 25 µL citrate reagent, 50 µL salicylate-nitroprusside reagent, 25 µL hypochlorite reagent, and 160 µL 2M KCl and determining concentration colorimetrically on a Genesys 10S UV-VIS spectrophotometer at 610 nm. Both NO_3-N and NH_4-N concentrations were calculated by use of a standard curve containing 0, 0.1, 0.5, 1, 2, 5, 10, 20, and 40 mg L^{-1} of the respective analyte.

2.3.3. Soil Chemical Health Indicators

Soil pH and EC were both determined using a 1:1 soil solution (20 g air-dried 2 mm sieved soil:20 mL DI) ratio [39,40]. Soil-water slurries were shaken on low for 2 h in a 50 mL centrifuge tube. pH was read directly with a pH electrode, and EC was determined by centrifuging the samples and measuring the liquid phase in a conductivity meter.

2.3.4. Soil Nutrient Content

P and K concentrations were determined by the Olsen extraction method due to the high pH observed in all samples [41]. Briefly, 2 g of air-dried 2 mm sieved soil was shaken on low with 40 mL of 0.5 M sodium bicarbonate and filtered through the Whatman #2 filter paper. These filtrates were covered in parafilm and left out overnight to allow for release of CO_2 gas. The filtered solution was diluted at a 10:1 ratio in DI water and analyzed for P and K in a high throughput inductively coupled plasma-optical emission spectrophotometer (ICP-OES).

2.4. Statistical Analysis

Each composite soil sample was considered an individual replicate for statistical analysis. Analysis of variance (ANOVA) was performed using a linear fixed effects model with year and depth as interacting predictor variables. If the interaction term was significant at the $\alpha \leq 0.05$ level, a pairwise comparison of means was performed for the significance of depth in each year and of year at each depth. This perspective was used to acknowledge and account for the potential impact of manure deposition and trampling action occurring primarily in shallow soil depths. Analysis was performed using R (Version 4.2.2) in RStudio (Build 446) using the stats package (Version 4.2.2), emmeans package (Version 1.7.4-1), and ARTool package (Version 0.11.1). Raw measurements of soil health indicators and soil indicator scores were used as outcome variables in two separate tests to determine the effect of MiG transition on project years 5 and 6 within both depths. Significance was evaluated at $\alpha \leq 0.05$. If data was not normally distributed or otherwise violated model assumptions for ANOVA analysis, the outcome variable was log-transformed, and assumptions were rechecked. If model assumptions still failed to be met, data was examined using the Aligned-Rank Transformation in ANOVA test for non-parametric distributions [42]. The Aligned-Rank Transformation in ANOVA test was frequently required when analyzing index scores, as the curve-fitting algorithm often resulted in scores concentrated at one far end of the spectrum (e.g., for EC, where the distribution of actual measurements was normal, but the unitless soil health scores are curved to represent risk of salinity and a wide range of low electrical conductivity soils may all have a SMAF soil indicator score of 1.0).

Tables of measured values and SMAF scores are presented as the untransformed mean and standard error to clarify and contextualize measurements, but formal statistical analysis was performed on transformed models, where appropriate. The transformation performed is provided in each table below, alongside the results of the model analysis.

Due to data accessibility constraints, statistical analysis was only performed on the 2021 and 2022 sampling years. However, these results are semi-quantitatively compared to the same analyses performed on soils in 2017 and 2018 [1] to draw general conclusions on trends in soil health indices where statistical inferences are not possible. These soils are comparable, using the same methodologies and sampling locations, but this paper attempts to examine the longer-term state of soil health in the MiG system.

3. Results and Discussion

3.1. Soil Physical Indicators

All mean soil physical indicator characteristics are presented in Table 1. Bulk density increased from 2021 to 2022, particularly in the 5-to-15 cm depth, perhaps as a function of hoof action from grazing cattle [24,43]. The findings were similar to those observed by Shawver et al. [1] on this site. A meta-analysis of 64 studies by Byrnes et al. [24] found that grazing activities significantly increased Bd, yet rotational grazing had a smaller impact than continuous grazing. Byrnes et al. [24] also noted that rotationally grazed systems generally had lower Bd values than continuously grazed ecosystems, with increasing grazing frequency and intensity correlated to increased Bd. While the current field study is generally managed to minimize grazing activity on recently irrigated paddocks, trampling may still increase Bd, limiting root penetration and reducing available water content, particularly in heavy clay soils [25–27]. The average clay content of soil samples from the 5 to 15 cm depth was approximately 34%, and the soils were broadly classified as clays and clay loams, likely increasing the deleterious effect of compaction when this soil is wet and exceeds its plasticity index. Consequently, this increase in Bd at depth resulted in a significant change in the bulk density indicator score (Table 2). Compared to samples taken in 2017 and 2018 (Table A1), the Bd at all depths in 2021 and 2022 appears to have increased slightly over the initial study years, suggesting that prolonged grazing activity has continued to compact the soil over time after transitioning. Furthermore, plant root growth might become impeded at Bd values greater than 1.7 g cm^{-3} [44], as observed in the 5 to 15 cm depth in 2022. Bulk density should continue to be monitored with depth in the future.

The quantity of WSA did not change over year or depth in 2021–2022 (Table 1), yet aggregate stability appeared to be greater than in 2017–2018 (Table A1). Continuous grazing has been shown to reduce soil aggregate stability [25], in large part through the destruction of soil structure, particularly in wet, heavy (i.e., clayey) soils [27]. Results suggest that MiG does not act to the extent that continuous grazing does on WSA. Also playing a role in this system is tillage, or lack thereof. Reduced tillage has been shown to improve shallow soil aggregate stability [45,46], indicating that the opposing effects of lack of tillage and increased grazing action via MiG may result in a somewhat small (albeit positive) change in soil aggregate stability during the study period. An identical response in WSA was observed by Keshavarz et al. [32] in a furrow irrigated continuous corn grazed agroecosystem study under no-till as compared to conventional tillage.

Bulk density and water-stable aggregates are likely to be impacted by additional irrigation inputs in MiG ecosystems that would not be received in traditional, rain-fed pastures. Warren et al. [27] conducted a study of bulk density and soil aggregate stability under varying trampling rates and moisture regimes in silty clay soil, noting that aggregate stability was generally poorer in moist soils, particularly at higher stocking rates. Conversely, bulk density was less sensitive to change as a function of stocking rate in moist soils, perhaps due to the incompressibility of water helping to maintain soil structure during trampling [27]. However, the USDA-NRCS recommends avoiding heavy trampling and field operations while the soil is wet, citing concerns about compaction [26]. Consequently, MiG systems incorporating irrigation require additional planning and oversight to ensure that excessive trampling does not occur during wet conditions to avoid adverse soil physical damage. Thus, in MiG systems such as this, having a contingency plan for relocating grazing animals when fields are extremely wet is suggested, such as a sacrificial area within or near the field [47].

The soil physical health index (Table 3) is an average of the individual scores for Bd and WSA (from Table 2). The combined effect of increasing Bd and somewhat constant WSA resulted in a significant decrease in the soil physical health index from 2021 to 2022. The soil physical health index scores, with depth, in 2021 and 2022 are comparable to scores from 2017 and 2018 (Table A1), suggesting that overall changes in soil physical health have not been altered over the past four to five years. These findings do not support our hypothesis that negative changes in physical soil health would occur from continued trampling via hoof pressure.

Table 1. Soil indicator means (± standard error) in 2021 and 2022 with ANOVA results.

Soil Indicator	2021	2022	2021	2022	ANOVA	ANOVA	ANOVA	Transformation
	(0–5 cm Depth)		(5–15 cm Depth)		(Year)	(Depth)	(Year × Depth)	
Physical								
Bd (g cm^{-3})	1.42 ± 0.04	1.56 ± 0.04	1.41 ± 0.05	1.70 ± 0.03	**			
WSA (%)	59.2 ± 4.5	64.3 ± 3.3	61.3 ± 3.3	63.5 ± 3.4				
Biological								
BG (mg pnp kg^{-1} soil hr^{-1})	490 ± 34	839 ± 34	229 ± 21	300 ± 19	** at 0–5 cm	** for both years	**	
MBC (mg g^{-1})	219 ± 15	438 ± 16	137 ± 13	203 ± 10	** at both depths	** for both years	**	
SOC (%)	2.10 ± 0.10	2.38 ± 0.17	2.06 ± 0.09	1.86 ± 0.12		*		
PMN (mg kg^{-1})	15.5 ± 2.8	50.7 ± 4.2	16.1 ± 1.0	42.2 ± 3.4	**			
Chemical								
pH, 1:1	7.87 ± 0.02	8.07 ± 0.04	7.99 ± 0.03	8.05 ± 0.03	** at 0–5 cm		*	
EC, 1:1 (dS m^{-1})	1.27 ± 0.17	1.04 ± 0.16	1.59 ± 0.23	2.19 ± 0.22		** for both years	**	Aligned-Rank
Nutrient								
P (mg kg^{-1})	29.7 ± 3.8	27.0 ± 2.9	14.8 ± 2.0	9.9 ± 1.2		**		Logarithmic
K (mg kg^{-1})	487 ± 50	523 ± 63	281 ± 29	410 ± 59		**		

Significance is denoted with * if significant at 0.05 probability level and ** if significant at the 0.01 probability level. If the interaction term was significant, the result of pairwise comparisons is shown in the ANOVA (Year) and ANOVA (Depth) columns, comparing within a single depth or year, respectively. Transformation performed on outcome variables to fit model assumptions is noted but means and standard error are not transformed. Blank cells indicate a lack of significance or a lack of transformation, respectively. Bd = bulk density; WSA = water stable aggregates; BG = beta-glucosidase activity; MBC = microbial biomass carbon; SOC = soil organic carbon; PMN = potentially mineralizable nitrogen; EC = electrical conductivity; P = plant-available phosphorus; K = plant-available potassium.

Table 2. Mean soil indicator scores (± standard error) in 2021 and 2022 with ANOVA results.

Soil Indicator	2021 (0–5 cm Depth)	2022 (0–5 cm Depth)	2021 (5–15 cm Depth)	2022 (5–15 cm Depth)	ANOVA (Year)	ANOVA (Depth)	ANOVA (Year × Depth)	Transformation
Physical								
Bd	0.47 ± 0.06	0.31 ± 0.02	0.47 ± 0.07	0.24 ± 0.01	**			Aligned-Rank
WSA	0.94 ± 0.03	0.99 ± 0.01	0.96 ± 0.02	0.98 ± 0.02				Aligned-Rank
Biological								
BG	0.80 ± 0.06	0.98 ± 0.01	0.34 ± 0.08	0.48 ± 0.08	*	**		
MBC	0.38 ± 0.07	0.85 ± 0.03	0.18 ± 0.04	0.30 ± 0.05	**	**		Logarithmic
SOC	0.43 ± 0.06	0.53 ± 0.07	0.42 ± 0.06	0.37 ± 0.07				Logarithmic
PMN	0.69 ± 0.10	1.00 ± 0.00	0.89 ± 0.05	1.00 ± 0.00	** at 5–15 cm	* for 2022	*	Aligned-Rank
Chemical								
pH	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	** at 0–5 cm	** for 2021	*	Logarithmic
EC	0.86 ± 0.07	0.93 ± 0.06	0.74 ± 0.09	0.53 ± 0.08		** for both years	**	Aligned-Rank
Nutrient								
P	1.00 ± 0.00	1.00 ± 0.00	0.94 ± 0.03	0.81 ± 0.06	[a]	** for both years	**	Aligned-Rank
K	1.00 ± 0.00	0.99 ± 0.01	0.98 ± 0.01	0.95 ± 0.04				Aligned-Rank

Significance is denoted with * if significant at 0.05 probability level and ** if significant at the 0.01 probability level. If the interaction term was significant, the result of pairwise comparisons is shown in the ANOVA (Year) and ANOVA (Depth) columns, comparing within a single depth or year, respectively. Transformation performed on outcome variables to fit model assumptions is noted but means and standard error are not transformed. Blank cells indicate a lack of significance or a lack of transformation, respectively. Bd = bulk density; WSA = water stable aggregates; BG = beta-glucosidase activity; MBC = microbial biomass carbon; SOC = soil organic carbon; PMN = potentially mineralizable nitrogen; EC = electrical conductivity; P = plant-available phosphorus; K = plant-available potassium. [a] The ANOVA test for P SMAF scores at 0–5 cm was significant with respect to year. However, this is due to exact ties in every single data point, where the Aligned-Rank Transformation is seriously limited [42], and statistical analysis is not warranted. Almost every soil sample in the 0–5 depth scored 1.00 for both years.

Table 3. Mean soil health index scores (± standard error) in 2021 and 2022 with ANOVA results.

Soil Indicator	2021 (0–5 cm Depth)	2022 (0–5 cm Depth)	2021 (5–15 cm Depth)	2022 (5–15 cm Depth)	ANOVA (Year)	ANOVA (Depth)	ANOVA (Year × Depth)	Transformation
Physical	0.70 ± 0.04	0.65 ± 0.01	0.71 ± 0.04	0.61 ± 0.01	*			Logarithmic
Biological	0.58 ± 0.05	0.84 ± 0.02	0.46 ± 0.04	0.54 ± 0.04	** at both depths	** for both years	**	Aligned-Rank
Chemical	0.45 ± 0.03	0.47 ± 0.03	0.38 ± 0.04	0.27 ± 0.04		** for both years	**	Aligned-Rank
Nutrient	1.00 ± 0.00	1.00 ± 0.00	0.96 ± 0.02	0.88 ± 0.05	[a]	** for both years	*	Aligned-Rank
Overall	0.66 ± 0.02	0.76 ± 0.01	0.59 ± 0.03	0.57 ± 0.02	** at 0–5 cm	** for both years	**	Aligned-Rank

Significance is denoted with * if significant at 0.05 probability level and ** if significant at the 0.01 probability level. If the interaction term was significant, the result of pairwise comparisons is shown in the ANOVA (Year) and ANOVA (Depth) columns, comparing within a single depth or year, respectively. Transformation performed on outcome variables to fit model assumptions is noted but means and standard error are not transformed. Blank cells indicate a lack of significance or a lack of transformation, respectively. [a] The ANOVA test for P SMAF scores at 0–5 cm was significant with respect to year. However, this is due to exact ties in over 28 of the 30 data points, a condition where the Aligned-Rank Transformation is seriously limited [42], and statistical analysis is not warranted.

3.2. Soil Biological Indicators

Biological indicators of soil health have been purported to be particularly sensitive to field management and play an important role in soil biogeochemical cycling and other ecosystem services [11,14]. All mean soil biological indicator characteristics are presented in Table 1. B-glucosidase is an enzyme important for cellulose biodegradation, with BG activity often used as an indicator of general microbiome capacity for organic matter assimilation [11,13]. Significant BG activity differences existed between years, depths, and for the year by depth interaction. Generally, it appeared that 2022 had significantly greater BG activity than 2021 in the top 5 cm of soil (Table 1). This dynamic may be due to organic matter and manure inputs in the top portion of soil, which are thought to increase enzyme activity, though the impact of manure on exoenzyme activity has shown mixed results [12,48,49]. Notably, increased soil moisture has been shown to increase enzyme activity [50,51], but the average soil moisture at the time of sampling fell from 19.5% in 2021 to 14.0% in 2022, indicating that other factors were likely playing a role in the increase in BG in 2022 as compared to 2021. Temperature has been positively correlated to enzyme activity [51,52]. However, soils were sampled in late June and May of 2021 and 2022, respectively, and June was warmer than May (average maximum soil temperatures of 27.8 °C compared to 18.6 °C at a depth of 5 cm, respectively; Colorado State University—CoAgMet Station ftc03—CSU-ARDEC; available at: https://coagmet.colostate.edu/ (accessed on 1 July 2023); [53]). Thus, soil temperature certainly does not support the increased BG activity between years. It is possible that the continuation of current management practices was likely the driver of changes in BG activity.

The change in BG activity resulted in an increase in the BG indicator score (Table 2). The BG indicator score increased from 2021 and 2022 and was greater in the 0 to 5 versus 5 to 15 cm depth. The 2021–2022 change in the BG indicator score is noteworthy, but the more impactful story may be that the indicator score has drastically increased from that of the 2017–2018 study (Table A1). This clearly shows that enzyme activity continues to increase in this ecosystem and may have not yet reached a steady state. It is important to note that increases in BG activity are suggestive of both overall biological change [6] and potential increases in SOC accumulation [54].

Continuous increases in BG activity should lead to increases in MBC. Indeed, MBC was greater in 2022 than in 2021 and greater in the 0 to 5 cm versus the 5 to 15 cm depth (Table 1), and the MBC indicator scores responded identically (Table 2). Microbial biomass carbon is typically used as a measure of total microbial population size in soils, with healthier soils typically having larger microbial communities [11]. Both BG and MBC are thought to increase as a function of carbon inputs and manure inputs, particularly in pastures [5,8,48]. This may explain why both of these biological indicators were significantly greater in the 0 to 5 as compared to the 5 to 15 cm depth, as both manure inputs and plant biomass inputs are primarily deposited on the soil surface or in the shallow subsurface, with no tillage or significant soil mixing to move them deeper into the profile. The continued increase in MBC contributes to the evidence that the transition to MiG systems provides significant biological benefits to soils.

Supporting the contention that BG activity may eventually lead to increases in SOC [54], in conjunction with the MBC findings above, SOC has significantly increased in the 0 to 5 as compared to the 5 to 15 cm depth (Table 1). Furthermore, at both depths, SOC was 40 to 82% greater in 2021–2022 than in 2017–2018 (Table A1), indicating that continuous manure inputs under MiG have greatly increased SOC, a dynamic that has been shown by others [16,17,24,55]. The SOC indicator scores showed no significant differences (Table 2), which was similar to those found by Shawver et al. [1] (Table A1). Regardless, the SOC indicator scores in the current study are ~2 times those found in 2017–2018 (Table A1). This also suggests that this ecosystem is improving in terms of SOC accumulation. Future research should continue to monitor potential improvements in SOC content.

Potentially mineralizable nitrogen is a measure of the portion of nitrogen in soil organic matter that is susceptible to be mineralized to plant-available forms, supplementing fertilizer N requirements [56]. For this reason, PMN has been used as an indicator of soil health [11,57]. Potentially mineralizable nitrogen increased from 2021 to 2022 (Table 1), and the PMN indicator score followed an identical response (Table 2). As compared to 2017 and 2018 (Table A1), the 2021 PMN appeared to increase slightly, while the 2022 PMN increased drastically. These changes were likely due to additional factors other than the slow progressive transition to manure-fed systems. Mahal et al. [56] assessed the potential of conservation agriculture to increase PMN in soils, finding that manure-fed systems had higher PMN levels than fields with inorganic N inputs, and no-till systems had greater PMN than conventionally tilled systems. Manure is often rich in organic N that is not yet plant-available but is steadily made available by microbial activity over multiple years. Furthermore, intense tillage is likely to decrease measured PMN, often by increasing the organic matter degradation rate [58,59]. The combined effect of these changes to the no-till, manure-fed MiG system was likely responsible for the increase in PMN over the past several years.

The biological soil health index (Table 3) is an average of the BG, MBC, PMN, and SOC scores from Table 2. All individual indicator scores, except for SOC, improved from 2021 to 2022, resulting in a significant increase in the biological soil health index in 2022 as compared to 2021. Shawver et al. [1] found a similar increase between years, albeit lower than the biological soil health scores found in the current study. Furthermore, BG and MBC had greater indicator scores in the 0 to 5 versus the 5 to 15 cm depth, leading to a greater biological soil health index in the 0 to 5 as compared to the 5 to 15 cm depth. No differences between soil depth were observed in 2017 and 2018 [1]. This supports our hypothesis that biological soil health should continue to improve as manure inputs and minimal soil disturbance support large and active microbial communities.

3.3. Soil Chemical Indicators

All mean soil chemical indicator characteristics (i.e., pH and EC) are presented in Table 1. Soil pH is considered a master variable for biochemical reactions, nutrient availability and toxicity, and other important soil functions [11]. Although significant differences existed with respect to soil pH (Table 1), pH differed only by 0.1 to 0.2 pH units and thus may be inconsequential with respect to altering soil biogeochemical reactions. Given the relatively high $CaCO_3$ and clay content of these soils (~8 and 34%, respectively), there is a large buffering capacity to resist change in soil pH [1,60]. Shawver et al. [1] found similar soil pH values as in the current study (Table A1). Continuous grazing and perennial grasslands have been shown to have mixed effects on pH over time [61–63], likely as a function of initial pH and the effect of changing soil inputs. Moreover, these calcareous soils are typical of the region, and producers are well-acquainted with high-pH-tolerant forage varieties. The relatively high pH of these soils resulted in a relatively low pH indicator score (Table 2), yet this is more telling of the lack of SMAF algorithm curves to fit high-pH-tolerant forage varieties than actual soil health degradation. Further SMAF algorithm development for high pH soils, such as in this study, is warranted.

While high pH may not be a concern for most producers, EC is a significant risk in this region of the western US, particularly as high irrigation requirements may result in steady salinization of agricultural land [64]. Electrical conductivity differed across depth in the current study (Table 1), and subsequently, a depth effect was observed in the EC indicator score (Table 2). Perennial grassland has been shown to produce increased salinity, perhaps by increasing surface evapotranspiration and reducing salt leaching further into the soil profile [65]; manure additions have also been shown to increase EC due to manure-borne salts [22]. However, compared to EC measurements in 2017 and 2018 (Table A1), salinity has generally decreased across all depths, perhaps due to irrigation inputs that leached fertilizer and manure-borne salts deeper into the soil profile. Year-to-year measurement of EC may vary as a function of spatially heterogeneous manure inputs, but the general trend appears to be that the conversion from row crops with inorganic fertilizer inputs to this perennial, animal-based pasture system has decreased EC. It is important to note that all

EC measurements were well below those values that would be of concern for crop growth (~4.0 dS m^{-1}; [66]).

There was a significant increase in the chemical soil health index score as a function of depth and, to a lesser extent, year, driven primarily by the reduction in EC in the soil surface (Table 3). The soil chemical health index scores in the current study tend to represent an improvement over soil chemical health index scores determined in 2017 and 2018 (Table A1). This indicates a positive effect of the decrease in EC over time. In contrast to our hypothesis of no change in chemical soil health, this indicates that long-term decreases in EC may contribute to improving soil health in perennial pasture systems.

3.4. Soil Nutrient Indicators

All mean soil nutrient indicator characteristics (i.e., extractable P and K) are presented in Table 1. Nutrient indicators of soil health are key elements to understanding the capacity of soils to sustain highly productive forage biomass. Neither P nor K showed significant changes in plant availability between 2021 and 2022, but both showed significantly increased concentrations in the top 5 cm of soil compared to the 5 to 15 cm depth. The difference in concentration of both nutrients across depths may be indicative of the significant impact that manure inputs on the soil surface have on soil fertility, as manure and urine are known to be rich in both P and K and likely remain near the soil surface [22,23,67], though top-dressing of phosphate fertilizers likely also played a role. Phosphorus remaining near the soil surface led to greater plant-available P concentrations in the 0 to 5 versus 5 to 15 cm depth, leading to a significant increase in the P index score in the 0 to 5 cm as compared to the 5 to 15 cm depth (Table 2). Pairwise comparisons of years within the top 5 cm for the P index score did produce a significant difference between 2021 and 2022, but this was omitted from Table 2 for brevity's sake. Every soil sample in the top 5 cm of soil scored 1.00 for P index, and the detected difference was due to statistical limitations of assigning ranks under the Aligned-Rank Transformation procedure to a dataset that is entirely ties [42]. The amount of plant-available K in both depths was adequate for plant growth, resulting in no difference in the K indicator score (Table 2). In both years, 22 kg ha^{-1} of P was applied, while no K was applied, as K is generally plentiful in Colorado soils and thus is rarely a concern for plant growth [23]. Chemical P inputs from monoammonium phosphate supplement manure inputs of P to a degree that deficiency is not a concern, again indicated by high SMAF scores.

It is worth noting that the 0 to 5 cm soil P concentrations have increased four- to five-fold as compared to the 2017–2018 P concentrations (Table A1). Due to both manure inputs and the use of phosphorous fertilizer, it is difficult to elucidate if increasing P concentrations are due to fertilizer or manure inputs. However, as the same quantity of fertilizer P was applied in both 2021 and 2022 (22 kg ha^{-1}), and P concentrations decreased slightly, manure inputs alone may not be enough to support highly productive MiG systems, and producers should continue to regularly test soils to determine nutrient needs. Given the near 1% slope of the field, there is not a large concern over P mobilization risk to nearby waters, though mobilization risk was not directly measured in this study and was inferred from the SMAF curve algorithms that punish extremely high P concentrations on sloped fields (i.e., >70 mg kg^{-1}; [11]). While P runoff is not explicitly a concern in this field, the rapid increase in P concentration is a reminder that producers transitioning to systems with large manure inputs should manage herd movements thoughtfully to avoid localized deposition of nutrient-rich manure near receiving water bodies and to carefully balance the combination of fertilizer P and manure P to minimize mobilization risk [23,68].

The effect of decreasing P concentrations with depth led to a decrease in the nutrient content index score from 2021 to 2022 in the top 5 cm but an observable increase since 2017–2018 (Table A1); a similar finding was observed by Shawver et al. [1]. Shawver et al. [1] hypothesized that future monitoring would show that the nutrient status would increase over several years given further manure inputs; the current study proves that hypothesis as correct. However, due to multiple sources of P in the system, it is difficult to identify the source of nutrient status changes. Regardless, the decrease in nutrient content does not support our hypothesis of increasing nutrient content from manure inputs from 2021 to 2022.

3.5. Overall Soil Health

The SMAF averages together the above 10 indicator scores to produce an overall soil health index (Table 3). While biological scores tended to improve from 2021 to 2022 (Table 2) of the nonbiological indicators, only two—pH and Bd—changed from 2021 to 2022, and the change in Bd could be considered deleterious to soil health.

Meanwhile, though sampling depth had a significant impact on just four soil indicators (BG, MBC, EC, and plant-available P; Table 2), these differences led to a significant improvement in overall soil health in the 0-to-5 as compared to the 5-to-15 cm depth (Table 3). These differences were likely driven by manure inputs on the soil surface. A similar finding was noted by Shawver et al. [1] (Table A1). Every biological indicator, except for SOC, showed significant improvement from 2021 to 2022, particularly in the 0 to 5 cm depth. When comparing the current findings to those from 2017 and 2018, in addition to positive changes in biological characteristics mentioned above, SOC appears to have increased from ~1% to ~2% (40 to 82% greater), a change that was predicted via increasing BG activity and MBC by Shawver et al. [1]. Furthermore, compared to 2017 and 2018, the overall soil health scores seemed to increase, driven largely by biological soil health and nutrient status improvements. This supports our hypothesis that overall soil health would improve under MiG systems, as biological soil health improvements outweigh physical soil health degradation.

It is important to note that not all soil health changes are positive as a function of conversion from conventional agricultural practices to MiG. Bulk density increased significantly from 2021 to 2022, and Bd values were greater than in the initial 2017–2018 study. Trampling action, paired with removal of tillage from the management system, seemed to have had an expected negative effect on Bd. The management of MiG systems needs to consider removing animals from wet soils to lessen the effects of hoof pressure on soil bulk density. Opposite, the water-stable aggregates percentage appeared to increase from 2017/2018 to 2021/2022, indicating that perhaps the positive impact of the transition to perennial no-till pastureland under MiG may balance the negative effect of trampling in terms of physical soil health. One of the more interesting findings may be that changes to soil health and nutrient status in these systems are relatively quick, and significant changes occurred in just a few years. This finding provides additional insight into past research comparing intense cropping systems and managed grasslands decades into an established management scheme, highlighting the need for future work to study transitioning landscapes. Building upon the preliminary work by Shawver et al. [1], these overall results provide additional evidence that irrigated, perennial pasture MiG systems have the capacity to significantly improve soil health following decades of successive cropping, providing promising insight for future environmental sustainability efforts in livestock agriculture.

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Appendix A

Table A1. Raw soil indicator measurements, soil indicator scores, and soil health index scores from 2017 and 2018. Table adapted from Shawver et al. [1] (see publication for statistical analysis of these results).

Soil Health Indicators	2017 (0–5 cm Depth)	2018 (0–5 cm Depth)	2017 (5–15 cm Depth)	2018 (5–15 cm Depth)
Physical				
Bd (g cm^{-3})	1.15 ± 0.05	1.52 ± 0.05	1.29 ± 0.04	1.59 ± 0.04
WSA (%)	40.1 ± 3.9	44.3 ± 6.1	54.3 ± 3.8	55.6 ± 5.6
Biological				
BG (mg pnp kg^{-1} soil hr^{-1})	65.3 ± 2.8	84.9 ± 4.2	66.9 ± 3.3	70.2 ± 5.3
MBC (mg g^{-1})	122 ± 6	355 ± 25	136 ± 9	271 ± 22
SOC (%)	1.24 ± 0.06	1.31 ± 0.08	1.21 ± 0.09	1.33 ± 0.06
PMN (mg kg^{-1})	11.8 ± 1.9	17.3 ± 1.0	11.2 ± 1.7	14.9 ± 1.0
Chemical				
pH, 1:1	8.00 ± 0.02	8.17 ± 0.03	7.90 ± 0.02	8.05 ± 0.02
EC, 1:1 (dS m^{-1})	1.96 ± 0.25	1.12 ± 0.25	2.94 ± 0.20	2.52 ± 0.23
Nutrient				
P (mg kg^{-1})	11.8 ± 1.1	6.9 ± 0.9	8.0 ± 1.0	5.7 ± 0.9
K (mg kg^{-1})	175 ± 9	351 ± 25	172 ± 13	186 ± 21
Soil Health Indicator Scores	**2017 (0–5 cm depth)**	**2018 (0–5 cm depth)**	**2017 (5–15 cm depth)**	**2018 (5–15 cm depth)**
Physical				
Bd	0.81 ± 0.06	0.37 ± 0.04	0.61 ± 0.07	0.31 ± 0.05
WSA	0.77 ± 0.05	0.76 ± 0.08	0.93 ± 0.02	0.88 ± 0.06
Biological				
BG	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
MBC	0.17 ± 0.05	0.67 ± 0.06	0.17 ± 0.03	0.49 ± 0.08
SOC	0.19 ± 0.04	0.20 ± 0.05	0.20 ± 0.06	0.21 ± 0.04
PMN	0.64 ± 0.10	0.97 ± 0.01	0.56 ± 0.09	0.90 ± 0.04
Chemical				
pH	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
EC	0.62 ± 0.09	0.87 ± 0.09	0.25 ± 0.06	0.43 ± 0.07
Nutrient				
P	0.92 ± 0.03	0.62 ± 0.07	0.71 ± 0.08	0.47 ± 0.08
K	0.92 ± 0.03	0.99 ± 0.00	0.93 ± 0.02	0.91 ± 0.04
Soil Health Indices	**2017 (0–5 cm depth)**	**2018 (0–5 cm depth)**	**2017 (5–15 cm depth)**	**2018 (5–15 cm depth)**
Physical	0.79 ± 0.04	0.56 ± 0.05	0.77 ± 0.04	0.59 ± 0.04
Biological	0.26 ± 0.04	0.48 ± 0.03	0.25 ± 0.03	0.42 ± 0.03
Chemical	0.32 ± 0.04	0.44 ± 0.04	0.14 ± 0.03	0.22 ± 0.04
Nutrient	0.94 ± 0.02	0.81 ± 0.03	0.82 ± 0.04	0.69 ± 0.05
Overall	0.51 ± 0.03	0.55 ± 0.02	0.45 ± 0.02	0.47 ± 0.02

All values presented as mean ± standard error. Bd = bulk density; WSA = water stable aggregates; BG = beta-glucosidase activity; MBC = microbial biomass carbon; SOC = soil organic carbon; PMN = potentially mineralizable nitrogen; EC = electrical conductivity; P = plant-available phosphorus; K = plant-available potassium.

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