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Response of soil health indicators to long-term dairy manure in a semiarid irrigated cropping system

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Abstract

Dairy manure fertilization is an important practice to return nutrients to agricultural soils, but there is limited knowledge regarding the effect of manure on soil health metrics in semiarid irrigated row crops. The objective of this research was to determine how long-term dairy manure application affects biological and chemical indicators of soil health in a field experiment in southern Idaho. The treatments were no fertilizer, inorganic fertilizer (IF), and dairy manure applied annually or biennially at rates of 17, 35, and 52 Mg ha^{-1} on a dry weight basis. Spring soil samples were collected 7 yr after project initiation at depths of 0–15 cm and 15–30 cm and analyzed with soil health metrics that are commonly used to quantify organic matter pools and biological nutrient cycling via enzyme activities and N transformation rates. Soil organic C increased with increasing application rate and more than doubled after 7 yr of annual manure at the highest application rate. In general, C and N pools, enzyme activities, and N transformation rates were greater at 0–15 cm than deeper in the soil profile. Compared with IF, annual and biennial manure treatments had a significant effect on most indicators at both soil depths, which increased with increasing manure application rate. Because of the strong response of the indicators to dairy manure, all were found to be positively and significantly correlated with each other, suggesting that only a small subset of the metrics tested could potentially be used to evaluate the influence of manure on soil health in the region.

1 | INTRODUCTION

In the United States, approximately 210 Tg of dairy manure are produced annually by lactating cows, much of which is land applied. Cattle manure applications have potential to improve overall soil health and it is not surprising given the high microbial activity and presence of labile C, that livestock manures have a short-term (i.e., weeks to months) effect on soil biological and chemical properties (Antonious et al., 2020; Klapwyk et al., 2006; Tavali et al., 2019). A limited number of studies have researched the long-term effects of cattle manure on soil health indicators (Ozlu et al., 2019), with one study specifically addressing manure use in semiarid irrigated soils (Lupwayi et al., 2019). In these studies, dairy and beef manure were applied annually for 8 and 43 yr, respectively. Enzymatic activities, microbial biomass

Abbreviations: ACE, autoclaved citrate extractable protein; CTL, control; DEA, denitrification enzyme activity; IF, inorganic fertilizer; LTM, long-term manure study; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; PAO, potential ammonia oxidation; PMN, potentially mineralizable nitrogen; POXC, permanganate oxidizable carbon; SOC, soil organic carbon; SOM, soil organic matter.

C (MBC), and/or microbial community structure were significantly influenced in the topsoil (≤15 cm) by the manure when compared with inorganic N fertilizer, with fewer effects on microbial properties found at 15–30 cm (Lupwayi et al., 2018). The work by Lupwayi et al. (2019) also found that 29 yr after discontinued manure application, legacy effects (relative to 43 yr without manure) were observed for some enzyme activities in bulk soils. The legacy effects of manure on soil health indicators can likely be attributed to the release of nutrients, which can occur for long periods of time after the last manure application (Tarkalson et al., 2018).

One of the biggest challenges with long-term crop cultivation practices is the depletion of soil organic matter (SOM) (Reeves, 1997; Tiessen & Stewart, 1983). Soil organic matter is considered one of the most important parameters of soil health, and its depletion can lead to erosion, compaction, decreased fertility, and general degradation (Bauer & Black, 1994; Lehman et al., 2015; X. Liu et al., 2006). The beneficial properties of SOM are numerous, such as improved physical structure, acting as a slow-release plant fertilizer, having a high cation-exchange capacity, supporting diverse biological populations, and improving water-holding capacity (Tate, 1987). Compared with conventional inorganic fertilizer (IF), there is abundant evidence that livestock manures provide additional beneficial effects on soil health and crop productivity (Rayne & Aula, 2020). Long- and short-term studies have demonstrated the ability of manure applications to slow or reverse declining organic matter levels in cropland soils (Dungan et al., 2021; Lin et al., 2019; Schulten & Leinweber, 1991). Because few studies have investigated the response of soil health metrics to long-term cattle manure use in semiarid cropping systems, additional studies are warranted to improve overall knowledge. Semiarid irrigated soils are unique in that they have low SOM (<1%), and the dry climate conditions, along with frequent irrigation intervals that temporarily increase soil moisture, could significantly affect microbial processes relative to more humid regions.

Soil biology is the least understood aspect of soil health and most sensitive to short-term changes, unlike chemical and physical indicators, which require much longer periods before exhibiting the effects of management (Hills et al., 2020; Nelson et al., 2009). One could even make a case that some soil chemical measurements are essentially indicators of biological function. Soil organic C (SOC), for example, is formed and degraded primarily through microbially mediated mechanisms, although it is technically a chemical measurement. Although SOC is considered the leading soil health baseline indicator (Lehman et al., 2015), given that it changes relatively slowly, it is recommended that additional indicators that respond more quickly be used to ensure early changes in soil health are being identified. Some recommended biological and chemical (biochemical) measurements that respond quickly to management are enzyme activities (e.g., β-glucosidase, β-glucosaminidase, phosphatase, and arylsulfatase), permanganate oxidizable C (POXC), and autoclaved citrate extractable (ACE) protein (Moebius-Clune, 2016; Stott, 2019). These analyses together can give information on the decomposition and transformation of key substrates in soil (i.e., C, phosphorus [P], and sulfur [S] cycling and proteins). There are many other biological and chemical indicators that have been used in soil health assessments, but some are considered time and labor intensive for high-throughput laboratories (e.g., MBC, microbial biomass N [MBN], and potentially mineralizable N [PMN]).

Core Ideas

- Soil health was investigated in a semiarid crop rotation under long-term dairy manure.
- Commonly used biological and chemical indicators were evaluated.
- Soil organic carbon more than doubled after 7 yr of manure.
- All soil health metrics increased with increasing manure application rate.
- Indicator responses were greater in topsoil than deeper in the soil profile.

Because dairy manure is commonly used in crop production in Idaho, a USDA-ARS field project was established in 2012 to assess the long-term effects of manure on nutrient cycling, greenhouse gas emissions, crop quantity and quality, and microbiome (Leytem et al., 2019, 2020; McKinney et al., 2018). Keeping in step with regional practices, dairy manure was applied annually or biennially to this field site at three representative rates. The objective of the present study was to determine the influence of dairy manure on selected biological and chemical indicators of soil health (Table 1) at two sampling depths (i.e., 0–15 cm and 15–30 cm), 7 yr after the first manure application. Although most evaluations only consider soil properties in the topsoil, we included 15–30 cm to determine the effect of manure deeper in the soil profile. These deeper soils are also found within the rootzone and can be important with respect to nutrient dynamics and plant health. The indicators evaluated were related to the microbial component via microbial biomass (MBC and MBN); SOM dynamics via SOC, POXC, and ACE; enzyme activities involved in the degradation of cellulose (β-glucosidase) and chitin (β-glucosaminidase) and transformations of P (alkaline phosphatase) and S (arylsulfatase); and N transformations via PMN, potential ammonia oxidation (PAO), and denitrification enzyme activity (DEA). Our eventual goal is to select for metrics that are fast, cost effective, and could be used by regional

TABLE 1 Biological and chemical indicators, their purpose, and potential implications when evaluating soil health effects of dairy manure in southern Idaho cropland soils

Indicator/method	Purpose	Implications for soil health and functions
Chemical		
Soil organic C	Indirect measure of soil organic matter (SOM) (58% of SOM)	Main source of energy for soil microorganisms; indicator of C sequestration
Permanganate oxidizable C	Active C; fraction of the SOM pool	This C pool could be different depending on the month and plant growth synchronization, and it can represent simple C sources available due to decomposition processes, substrates from root exudates, and MBC
Biological		
Microbial biomass C and N	Microbial community size; labile C and N sources	Soil processes such as decomposition, C sequestration, N fixation, and nutrient cycling and availability; soil structure
Autoclaved citrate extractable protein	Amount of protein-like substances present in SOM	Major source of N that will become available to plants through mineralization; soil structure
β-glucosidase, β-glucosaminidase, acid/alkaline phosphatase, arylsulfatase	Enzyme activity assays	Predictor of organic matter decomposition; nutrient cycling of C, N, P, and S
Potentially mineralizable N	Capacity of microbial community to mineralize N in organic residues	Integrative indicator of labile N and microbial activity for increasing plant available N
Potential ammonia oxidation	Capacity of microbial community to oxidize ammonium	N dynamics; crop N supply
Denitrification enzyme activity	Capacity of microbial community to reduce nitrate to N gases under anaerobic conditions	N dynamics; loss of plant available N

producers to determine how their soil management practices are affecting soil health.

2 | MATERIALS AND METHODS

2.1 | Field site and treatments

The field site was located at the USDA-ARS Northwest Irrigation and Soils Research Laboratory in Kimberly, ID. This region of southern Idaho has a semiarid climate with a mean annual temperature of 8.9 ˚C and precipitation of 229 mm, consisting of hot dry summers and cool wet winters. Soil at the site is a Portneuf silt loam (coarse-silty, mixed, superactive, mesic Durixerollic Calciorthids) that was sprinkler irrigated with Snake River water. The long-term manure study (LTM) was initiated in 2012 and consisted of a 4-yr rotation of spring wheat (*Triticum aestivum* L.) (2013)–potato (*Solanum tuberosum* L.) (2014)–spring barley (*Hordeum vulgare* L.) (2015)–sugarbeet (*Beta vulgaris* L.) (2016), which was repeated starting in 2017. The experimental design was a randomized complete block with four replications and individual plots sizes of 18.2 m × 12.2 m. The treatments included (a) control, no IF or manure (CTL), (b) IF, (c) dairy manure applied annually at 17 Mg ha^{-1} (17A), 35 Mg ha^{-1} (35A), and 52 Mg ha^{-1} (52A), and (d) dairy manure applied biennially at 17 Mg ha^{-1} (17B), 35 Mg ha^{-1} (35B), and 52 Mg ha^{-1} (52B) on a dry weight basis. The annual manure applications occurred every year from 2012 to 2018; biennial manure applications occurred on even years (i.e., 2012, 2014, 2016, and 2018). Cumulative manure totals in the 17A, 35A, and 52A plots were 119, 245, and 364 Mg ha^{-1} (dry wt.), whereas manure totals in the 17B, 35B, and 52B plots were 68, 140, and 208 Mg ha^{-1} (dry wt.), respectively. The moisture content of the dairy manure was approximately 50% (w/w) at the time of application. Inorganic fertilizer was applied to IF plots and, in some years to select manure plots, at rates based on the University of Idaho recommendations using spring soil test data (Brown et al., 2010; Moore et al., 2009; Stark et al., 2004). Inorganic fertilizers were broadcast in the spring before planting and manure was broadcast in the fall after harvest; both were incorporated within 24 h of application to 15 cm using a tandem disk, followed by roller harrowing. All plots received tillage at the same time for consistency.

2.2 | Soil collection

Preplant soil samples were collected in late March of 2019 prior to the application of fertilizer. Six soil cores (0–15 cm and 15–30 cm) were collected per plot and composited by depth, then thoroughly mixed. Field moist subsamples were sieved, then a portion was immediately placed into a clean sealable plastic bag and refrigerated at 5 ˚C, whereas the other portion was air dried, then placed into a clean sealable plastic bag. The soil moisture was gravimetrically determined by drying at 105 ˚C for 24 h. Soil biological analyses on field moist soils were completed within 2 wk of the sampling time.

2.3 | Soil analysis

Soil organic C was determined using the Walkley–Black method (Walkley & Black, 1934). Microbial biomass C and N were determined using the chloroform-fumigation extraction method on field moist soil (Brookes et al., 1985; Vance et al., 1987). Permanganate oxidizable C was determined using the protocol developed by Weil et al. (2003). The autoclaved citrate extractable soil proteins assay was a modification of a procedure used to extract proteins from fungi and soil (Wright & Upadhyaya, 1996). The enzyme assays performed were β-glucosidase, β-glucosaminidase, alkaline phosphatase, and arylsulfatase as described by Acosta-Martinez et al. (2018). Potentially mineralizable N was determined using the 7-d anaerobic laboratory method (Waring & Bremner, 1964). Potential ammonia oxidation, also known as the short-term nitrification assay, was adapted from Schmidt and Belser (1994). The acetylene inhibition method was used to perform the denitrification enzyme assay (Hunt et al., 2003; Tiedje, 1994). Complete details for each of the assays, along with modifications, can be found in the Supplemental Information.

2.4 | Statistical analysis

Data were statistically analyzed using the generalized linear mixed model (GLIMMIX) procedure of SAS (SAS Institute) including treatment as a main effect and block as a random effect. Treatment effects were assessed via contrast statements. Contrast statements evaluated control vs. all (where "all" data included all plots receiving treatments), fertilizer vs. annual (fertilizer only vs. annual manure application pooled over rate), fertilizer vs. biennial (fertilizer only vs. biennial manure application pooled over rate), annual vs. biennial (annual and biennial manure applications pooled over rate), and manure rate annual and biennial (linear responses with increasing manure application rate). The CORR procedure was used to determine Pearson correlation coefficients between the soil properties. Statements of statistical significance were based on a P value < .05.

3 | RESULTS

3.1 | Carbon and nitrogen pools

Annual and biennial manure treatments had a significant effect on SOC and POXC concentrations at both soil depths, which increased linearly with increasing manure application rate (Table 2). Compared with IF, SOC concentrations at 0–15 cm were 38–118% and 18–54% greater in the annual and biennial manure treatments, whereas POXC concentrations at 0–15 cm were 26–95% and 3–47% greater, respectively. At 15–30 cm, the percentage increases for SOC and POXC in the annual and biennial manure treatments were similar to those at 0–15 cm. At this depth, however, there was no significant effect of IF vs. biennial manure.

For MBC and MBN concentrations, there was a positive linear response to annual and biennial manure application rates at 0–15 cm, but only a linear response to biennial manure rate at 15–30 cm (Table 2). Compared with IF, MBC concentrations at 0–15 cm were 48–99% and 24–87% greater in the annual and biennial manure treatments, whereas MBN concentrations at 0–15 cm were 21–95% and 17–81% greater, respectively. At 15–30 cm, MBC and MBN concentrations were 12–114% greater than IF in the annual and biennial manure treatments.

Increasing annual and biennial manure application rates caused a positive linear response in ACE protein concentrations at both soil depths (Table 2). Compared with IF, the ACE concentrations at 0–15 cm were 35–104% and 12–50% greater in the annual and biennial manure treatments, whereas ACE concentrations at 15–30 cm were 13–78% and 0–30% greater, respectively. At 15–30 cm, there was no significant effect of IF vs. biennial manure.

For all health metrics in Table 2, there was no significant effect of manure timing (annual vs. biennial application) at either depth.

3.2 | Enzyme activities

Enzyme activities, at both soil depths, had a positive linear relationship with both annual and biennial manure application rates, although there was no effect of manure timing (annual vs. biennial application) at either depth (Table 3). Among the enzymes, β-glucosidase activity was the most sensitive to manure, and activities at 0–15 cm were 85, 175, and 239% greater than with IF at annual application rates of

TABLE 2 Average C and N pool concentrations (± SD) for each treatment at two soil depths

Treatment	Frequency	SOC	POXC	MBC	MBN	ACE
		g C kg^{-1}	——mg C kg^{-1}——		mg N kg^{-1}	g kg^{-1}
0–15 cm depth						
Control	n/a	7.1 ± 0.7	385 ± 69	180 ± 12	25.6 ± 1.4	2.4 ± 0.2
Fertilizer	n/a	7.9 ± 1.3	433 ± 92	198 ± 16	30.0 ± 2.5	2.6 ± 0.5
17 Mg ha^{-1}	Annual	10.9 ± 0.7	558 ± 55	293 ± 73	36.2 ± 5.6	3.5 ± 0.4
35 Mg ha^{-1}	Annual	13.4 ± 0.8	738 ± 68	340 ± 50	48.6 ± 4.4	4.2 ± 0.3
52 Mg ha^{-1}	Annual	17.2 ± 2.1	862 ± 52	394 ± 31	58.5 ± 4.3	5.3 ± 0.6
17 Mg ha^{-1}	Biennial	9.3 ± 0.8	456 ± 69	245 ± 51	35.2 ± 9.3	2.9 ± 0.3
35 Mg ha^{-1}	Biennial	10.9 ± 0.5	522 ± 72	296 ± 39	41.8 ± 5.7	3.4 ± 0.3
52 Mg ha^{-1}	Biennial	12.2 ± 0.7	650 ± 23	371 ± 34	54.3 ± 5.4	3.9 ± 0.5
P value		<.0001	<.0001	<.0001	<.0001	<.0001
Ctrl vs. all		***	***	***	***	***
Fert vs. annual		***	***	***	***	***
Fert vs. biennial		***	**	***	***	**
Annual vs. biennial						
Manure rate, annual		***	***	**	***	***
Manure rate, biennial		**	***	***	***	**
15–30 cm depth						
Control	n/a	6.0 ± 0.6	274 ± 45	103 ± 39	15.0 ± 4.7	2.0 ± 0.1
Fertilizer	n/a	7.1 ± 1.1	335 ± 104	134 ± 36	15.8 ± 7.4	2.3 ± 0.4
17 Mg ha^{-1}	Annual	8.5 ± 0.7	401 ± 65	175 ± 59	21.3 ± 3.6	2.6 ± 0.2
35 Mg ha^{-1}	Annual	11.7 ± 1.7	561 ± 42	234 ± 50	32.2 ± 6.4	3.4 ± 0.5
52 Mg ha^{-1}	Annual	14.3 ± 3.1	673 ± 93	242 ± 46	33.8 ± 6.3	4.1 ± 0.7
17 Mg ha^{-1}	Biennial	7.3 ± 1.1	342 ± 59	150 ± 51	20.0 ± 7.7	2.3 ± 0.4
35 Mg ha^{-1}	Biennial	8.3 ± 0.7	370 ± 58	172 ± 43	20.1 ± 5.4	2.6 ± 0.3
52 Mg ha^{-1}	Biennial	10.4 ± 1.5	510 ± 117	219 ± 72	24.4 ± 9.7	3.0 ± 0.3
P value		<.0001	<.0001	.0005	.0018	<.0001
Ctrl vs. all		***	***	***	*	***
Fert vs. annual		***	***	***	**	***
Fert vs. biennial				*	*	
Annual vs. biennial						
Manure rate, annual		***	***			***
Manure rate, biennial		**	**	***	**	*

Note. SOC, soil organic C; POXC, permanganate oxidizable C; MBC, microbial biomass C; MBN, microbial biomass N; ACE, autoclaved citrate extractable protein.
*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

17, 35, and 52 Mg ha^{-1}, respectively. With biennial manure treatment, the respective β-glucosidase activities were lower, but still 24, 71, and 133% greater than the IF treatment. The β-glucosaminidase response was intermediate, with activities at 0–15 cm in annual and biennial manure treatments being 32–204% and 21–105% greater than IF, respectively. Alkaline phosphatase and arylsulfatase were the least responsive to the manure treatments. At 0–15 cm, alkaline phosphatase activities in annual and biennial treatments were 15–84% and 5–44% greater than IF, whereas arylsulfatase activities were 45–74% and 13–42% greater, respectively. These same trends in enzyme activities occurred at the 15-to-30-cm depth, although they were slightly lower overall.

3.3 | Nitrogen transformation rates

Potentially mineralizable N increased linearly with annual manure application rate at 0–15 cm and with annual and biennial manure application rate at 15–30 cm (Table 4). However, PMN was not affected by manure timing (annual vs. biennial)

TABLE 3 Average β-glucosidase, β-glucosaminidase, alkaline phosphatase, and arylsulfatase activities (± SD) for each treatment at two soils depths

Treatment	Frequency	β-Glucosidase	β-Glucosaminidase	Alkaline phosphatase	Arylsulfatase
			μg *p*-nitrophenol g^{-1} h^{-1}		
0–15 cm depth					
Control	n/a	88 ± 17	15.4 ± 2.4	189 ± 19	13.2 ± 1.5
Fertilizer	n/a	120 ± 53	15.8 ± 6.1	204 ± 40	17.4 ± 3.3
17 Mg ha^{-1}	Annual	223 ± 30	20.9 ± 2.0	234 ± 23	25.2 ± 4.3
35 Mg ha^{-1}	Annual	330 ± 25	36.6 ± 8.5	275 ± 24	30.9 ± 3.5
52 Mg ha^{-1}	Annual	407 ± 20	48.0 ± 13.5	375 ± 27	30.4 ± 4.2
17 Mg ha^{-1}	Biennial	149 ± 26	19.1 ± 3.7	214 ± 22	19.8 ± 2.4
35 Mg ha^{-1}	Biennial	205 ± 37	23.9 ± 4.8	264 ± 41	22.3 ± 3.1
52 Mg ha^{-1}	Biennial	280 ± 15	32.4 ± 9.8	294 ± 36	24.7 ± 2.2
P value		<.0001	<.0001	<.0001	<.0001
Ctrl vs. all		***	***	***	***
Fert vs. annual		***	***	***	***
Fert vs. biennial		***	*	***	**
Annual vs. biennial					
Manure rate, annual		***	***	***	*
Manure rate, biennial		***	**	***	*
15–30 cm depth					
Control	n/a	55 ± 6	10.0 ± 2.1	157 ± 22	13.5 ± 1.7
Fertilizer	n/a	89 ± 38	11.5 ± 1.4	169 ± 9	16.5 ± 1.8
17 Mg ha^{-1}	Annual	156 ± 43	13.2 ± 2.4	173 ± 20	21.1 ± 1.2
35 Mg ha^{-1}	Annual	244 ± 45	21.9 ± 2.8	220 ± 22	23.6 ± 1.2
52 Mg ha^{-1}	Annual	297 ± 85	26.6 ± 7.6	269 ± 59	27.5 ± 3.6
17 Mg ha^{-1}	Biennial	92 ± 30	11.5 ± 3.0	167 ± 38	17.0 ± 1.4
35 Mg ha^{-1}	Biennial	125 ± 27	16.0 ± 5.6	209 ± 43	21.3 ± 1.7
52 Mg ha^{-1}	Biennial	197 ± 55	25.2 ± 2.5	253 ± 31	24.3 ± 4.0
P value		<.0001	<.0001	.0004	<.0001
Ctrl vs. all		***	***	**	***
Fert vs. annual		***	***	*	***
Fert vs. biennial		***	*	*	**
Annual vs. biennial					
Manure rate, annual		***	***	***	***
Manure rate, biennial		*	***	**	***

*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

at 0–15 cm, but it was affected at 15–30 cm. Compared with IF, PMN at 0–15 and 15–30 cm was 44–83% and 70–149% greater in the annual manure treatments, respectively.

Potential ammonia oxidation rates increased linearly with annual manure application rate at both soil depths (Table 4). Compared with IF, PAO at 0–15 cm was 25–88% greater in the annual manure treatment.

Denitrification enzyme activities, at both soil depths, had a positive linear relationship with both annual and biennial manure application rates, although there was no effect of manure timing (annual vs. biennial application) at either depth (Table 4). Compared with IF, DEA at 0–15 cm was 60–130% and 16–95% greater in the annual and biennial manure treatments, respectively, whereas DEA at 15–30 cm was 51–140% greater in the annual manure treatment.

There was no significant effect of manure timing (annual vs. biennial) on N transformation metrics with the exception

TABLE 4 Average nitrogen transformation rates (± SD) determined via potentially mineralizable N (PMN), potential ammonia oxidation (PAO), and denitrification enzyme activity (DEA) for each treatment at two soil depths

Treatment	Frequency	PMN	PAO	DEA
		μg NH_4–N g^{-1}	μg NO_2–N g^{-1} h^{-1}	μg N_2O-N g^{-1} h^{-1}
0–15 cm depth				
Control	n/a	14.7 ± 5.8	0.4 ± 0.1	66 ± 29
Fertilizer	n/a	22.7 ± 5.3	0.8 ± 0.1	82 ± 20
17 Mg ha^{-1}	Annual	32.8 ± 3.1	1.0 ± 0.1	131 ± 25
35 Mg ha^{-1}	Annual	35.3 ± 3.1	1.1 ± 0.2	144 ± 10
52 Mg ha^{-1}	Annual	41.5 ± 9.0	1.5 ± 0.3	189 ± 6
17 Mg ha^{-1}	Biennial	24.0 ± 4.5	0.9 ± 0.3	95 ± 27
35 Mg ha^{-1}	Biennial	31.3 ± 5.8	1.0 ± 0.1	117 ± 24
52 Mg ha^{-1}	Biennial	29.1 ± 9.2	1.2 ± 0.2	160 ± 39
P value		<.0001	<.0001	<.0001
Ctrl vs. all		***	***	***
Fert vs. annual		***	**	***
Fert vs. biennial				**
Annual vs. biennial				
Manure rate, annual		*	***	**
Manure rate, biennial				***
15–30 cm depth				
Control	n/a	7.5 ± 2.48	0.2 ± 0.1	21.7 ± 8.7
Fertilizer	n/a	13.0 ± 8.28	0.6 ± 0.2	40.3 ± 13.5
17 Mg ha^{-1}	Annual	22.1 ± 9.02	0.6 ± 0.1	60.7 ± 11.3
35 Mg ha^{-1}	Annual	23.7 ± 6.14	0.6 ± 0.3	85.4 ± 18.4
52 Mg ha^{-1}	Annual	32.4 ± 11.9	0.9 ± 0.2	96.6 ± 23.4
17 Mg ha^{-1}	Biennial	10.2 ± 7.2	0.5 ± 0.1	37.0 ± 17.4
35 Mg ha^{-1}	Biennial	13.7 ± 6.81	0.6 ± 0.1	47.9 ± 4.5
52 Mg ha^{-1}	Biennial	19.5 ± 2.32	0.8 ± 0.3	90.8 ± 26.1
P value		<.0001	.0036	<.0001
Ctrl vs. all		***	***	***
Fert vs. annual		***		***
Fert vs. biennial				
Annual vs. biennial		*		
Manure rate, annual		*	*	**
Manure rate, biennial		*		***

*Significant at the .05 probability level.
**Significant at the .01 probability level.
***Significant at the .001 probability level.

of PMN at 15–30 cm, where biennial applications had, on average, 44% lower PMN.

3.4 | Correlation coefficients between soil properties

Pearson correlation coefficients (r) between the different soil biological and chemical properties are shown in Table 5. The relationships were strong, with coefficients ranging from .60 to .96. All correlations were significant at $P < .0001$.

4 | DISCUSSION

4.1 | Manure effect on soil carbon and nitrogen pools

The present study distinguished the benefits of using dairy manure compared with that of IF on SOM dynamics. In the top 30 cm, strong linear relationships between cumulative manure inputs and absolute SOC stock differences were determined in the annual (R^2 = .86) and biennial (R^2 = .78)

TABLE 5 Pearson correlation coefficients for the biological and chemical properties

Property	AP[a]	BGA	AS	PMN	PAO	DEA	SOC	POXC	MBC	MBN	ACE
BG	.876[b]	.840	.873	.813	.777	.849	.956	.942	.766	.720	.942
AP	–	.829	.735	.695	.783	.869	.871	.846	.764	.745	.875
BGA	–	–	.685	.775	.739	.775	.861	.851	.761	.760	.895
AS	–	–	–	.663	.763	.746	.836	.842	.714	.600	.797
PMN	–	–	–	–	.703	.736	.812	.813	.703	.687	.831
PAO	–	–	–	–	–	.859	.776	.795	.851	.745	.799
DEA	–	–	–	–	–	–	.834	.872	.860	.848	.846
SOC	–	–	–	–	–	–	–	.946	.748	.680	.960
POXC	–	–	–	–	–	–	–	–	.787	.741	.942
MBC	–	–	–	–	–	–	–	–	–	.909	.753
MBN	–	–	–	–	–	–	–	–	–	–	.731

[a]BG, β-glucosidase; AP, alkaline phosphatase; BGA, β-glucosaminidase; AS, arylsulfatase; PMN, potentially mineralizable N; PAO, potential ammonium oxidation; DEA, denitrification enzyme activity; SOC, soil organic C; POXC, permanganate oxidizable C; MBC, microbial biomass C; MBN, microbial biomass N; ACE, autoclaved citrate extractable protein.

[b]All correlations were significant at $P < .0001$.

manure-treated soils (data not shown). Although bulk density was not measured at Year 7 of this study, stock calculations were made using a factor of 1.3 based on previous work conducted at the site. In a meta-analysis performed by Maillard and Angers (2014), significant linear relationships (R^2 = .53–.59) between cumulative manure C inputs and absolute SOC stock differences down to 30 cm were found. Gross and Glaser (2021) performed a meta-analysis as well and found a significant linear correlation between cumulative manure C input and SOC stock difference, but it was a weaker relationship (R^2 = .087). Alternatively, some studies have reported no significant changes in SOC following years of liquid hog manure and broiler litter application (Angers et al., 2010; Franzluebbers et al., 2001). Globally, manure use in agricultural soils has been calculated to increase SOC stocks by 35%, on average (Gross & Glaser, 2021).

The greatest C increase in the present study occurred at the highest annual manure rate, where the average SOM concentration was estimated to be 2.7% (based on a bulk density of 1.3 g cm^{-3} and assumption that C makes up 58% of SOM). In the top 30 cm, SOM accumulated at approximately 0.20% yr^{-1} in treatment 52A (i.e., ∼78 Mg wet manure ha^{-1} yr^{-1}), with incrementally less SOM accumulation with decreasing manure application rate. In semiarid Lethbridge, Canada, Sommerfeldt et al. (1988) reported that feedlot manure applied annually for 11 yr at rates of 30–90 and 60–180 Mg ha^{-1} (wet wt.) in nonirrigated and irrigated soils, respectively, caused significant SOM increases in the top 30 cm for the first 8 yr regardless of tillage regime, and they were closely correlated to the total amount of manure applied. By Year 11, the rate of SOM accumulation was 0.03 and 0.12% yr^{-1} at the 30 and 180 Mg ha^{-1} application rates, respectively. In arid Brawley, CA, up to 540 Mg ha^{-1} (wet wt.) of feedlot manure was applied to soil over 2 or 3 yr, initially causing SOM concentrations to reach 3% (from 0.9%) in the top 30 cm, which then declined to 1.8% by Year 9 of the study; the average rate of SOM accumulation was 0.1% yr^{-1} (Meek et al., 1982).

Both POXC and MBC concentrations were found to increase with increasing dairy manure application rate. Furthermore, POXC was strongly correlated with SOC (r = .946) and MBC (r = .787), as well as being positively correlated with all other soil biological and chemical properties. Many studies have found positive relationships between POXC and MBC (Melero, López-Garrido, Madejón, et al., 2009; Melero, López-Garrido, Murillo, & Moreno, 2009; White et al., 2020), which can be expected since both methods are chemical extractions of labile soil C (Weil et al., 2003). Positive relationships between POXC and SOC have also been reported (Caudle et al., 2020; Melero, López-Garrido, Madejón, et al., 2009; Morrow et al., 2016). The interest in microbial biomass is related to its function to serve as a pool of nutrients, its role in soil structure and stabilization, and its function as an ecological marker (Smith & Paul, 1990). However, MBC via chloroform fumigation is a laborious and expensive method to perform outside of research settings, compared with the inexpensive and rapid POXC method (Culman et al., 2012). The active C method that was streamlined by Weil et al. (2003) showed that POXC was more sensitive to management effects than SOC and more closely related to MBC, substrate-induced respiration, soluble carbohydrate, and aggregation. In an examination of soils from 12 studies across the United States, Culman et al. (2012) demonstrated that POXC was equally as sensitive as particulate organic C, MBC, and SOC in detecting differences in soils due to management or environmental factors.

Microbial biomass N and ACE protein represent organic N pools that can be used by microbes to support their growth or by plants after being mineralized (Bonde et al., 1988; Hurisso et al., 2018; Rillig et al., 2007). Available N, as determined via the 7-d anaerobic laboratory method, was found to measure N released primarily from the microbial biomass (Myrold, 1987). Like MBC, studies have reported that the application of manures in agricultural systems resulted in increases in MBN (Kallenbach & Grandy, 2011; Ma et al., 2020; Ren et al., 2019). In the present study, long-term annual and biennial dairy manure applications significantly increased MBN by up to 114% when compared with IF. A meta-analysis based on intensively managed cropping systems in China found that various livestock manures increased MBN by 55% across all observations relative to IF, with the greatest MBN increase of 75% induced by cattle manure (Ren et al., 2019). These increases were found to be higher than a 27% increase in MBN calculated via a meta-analysis based on a global dataset of manure amendments relative to IF (Kallenbach & Grandy, 2011). Both meta-analyses demonstrated that manure amendments, especially cattle manure, were beneficial in helping microbial communities recover from long-term use of IF. Soil protein concentrations are also influenced by livestock manure applications (Halder et al., 2021; Zhang et al., 2014), and in the present study, ACE was significantly increased by up to 104% and it was highly correlated (r = .73–.96) with all other biological and chemical indicators. In semiarid soils under annual crops in California, ACE was found to be highly correlated with total N, particulate organic N, soluble protein, and POXC, but it was not found to be a good predictor of N mineralization potential in soil with low total N (Geisseler et al., 2019). Although soil protein contributes to the formation and maintenance of soil aggregates (Wright et al., 1999; Wright & Upadhyaya, 1998), long-term use of livestock manures has been associated with both negative (Guo et al., 2018; Xie et al., 2015) and positive (Bertagnoli et al., 2020) impacts on soil structure.

4.2 | Manure effect on soil enzyme activities

When soil is amended with livestock manure, significant changes in microbial populations, biodiversity, and enzyme activities take place (Köninger et al., 2021; Larkin et al., 2005; S. Liu et al., 2020). Soil enzymes can react more rapidly than other variables to changes in soil management, thus they can be useful as early indicators of biological change (Acosta-Martinez et al., 2018; Bandick & Dick, 1999; Stott et al., 2010). Organic fertilizers, like manure, with a high content of easily decomposable organic C can lead to rapid growth of soil microorganisms, resulting in higher microbial biomass and enzyme activities, which are often positively correlated with application rate (Chang et al., 2007; Hou et al., 2012; Lalande et al., 2000; Parham et al., 2002). Among the enzyme activities measured in the present study, β-glucosidase and β-glucosaminidase were the most sensitive to manure addition and activities were up to 238 and 204% greater than IF, respectively. Considering the essential nature of enzymes for nutrient cycling, the general consensus is that higher enzyme activities are present in "healthier" soils, although some data suggests that higher activities can also be associated with resource-limiting conditions (Borase et al., 2020). The major unanswered question to date is how much enzyme activity is necessary for a soil to be considered healthy? Our research suggests that the enzyme activities found in annually treated soils at the highest manure rate could represent upper levels for healthy soils in southern Idaho. Despite 7 yr of heavy and long-term manure application, we would classify our field soils as being healthy since they support crops at equivalent yields and quality to those grown with IF, without detriment to soil quality. Continued manure application, however, could eventually bring about high salt concentrations that, for example, will eventually affect both plant growth and microbial activities.

4.3 | Manure effect on soil nitrogen dynamics

Potentially mineralizable N measures the capacity of the soil microbial community to convert N in organic residues to ammonium (NH_4^+), which is plant available. This indicator is included in the USDA Soil Management Assessment Framework because of its relation to nutrient availability and the theorized relationship between microbial activity and plant productivity (Andrews et al., 2004). Relative to IF, dairy manure in the present study increased PMN by up to 149%. Based on results from the PMN assay, anywhere from 43 kg ha^{-1} (CTL) to 144 kg ha^{-1} (52A) of NH_4–N was predicted to be available in the top 30 cm. Our results are in agreement with other researchers who observed large increases in PMN when cattle manure was applied to long-term field plots (Ndayegamiye & Cote, 1989; Nyiraneza et al., 2009; Sharifi et al., 2011; Whalen et al., 2001). In a meta-analysis conducted to assess conservation agricultural practices on PMN, soils receiving manure had higher PMN than those receiving compost or inorganic N fertilizer (Mahal et al., 2018).

Ammonium produced during mineralization of organic matter is acted upon by autotrophic nitrifiers that convert it to nitrite and nitrate (NO_3^-), with NO_3^- being a major source of inorganic N taken up by higher plants. The short-term nitrification assay is used as an indicator of the potential activity of the nitrifying population present in the soil at the time of sampling. The present study showed that dairy manure increased PAO rates by up to 80%, thus indicating that more plant-available NO_3^- will be present in the manure-treated soils when compared with IF. Tao et al. (2017) reported that

PAO rates were positively correlated with ammonia-oxidizing bacteria abundance in a calcareous agricultural soil, which was highly influenced by manure fertilization. Nyberg et al. (2006) found that PAO rates increased after amending soil microcosms with cattle manure or swine manure, compared with unfertilized soil. In a long-term field experiment where inorganic and organic fertilizers were applied biennially for 46 yr, it was found that cattle manure produced the highest PAO rates compared with sewage sludge, IF, and unfertilized soil (Enwall et al., 2007).

Denitrification is of concern because N is lost via the production of the N gases N_2O, NO, and N_2, with N_2O being a potent greenhouse gas and ozone-depleting substance (Venterea et al., 2012). Results from a field study where plots were treated with liquid cattle manure or solid beef cattle manure for 2 yr, demonstrated that DEA was largely determined by C availability over a 49-d period (Tenuta et al., 2000). It should be noted that the DEA assay measures the potential for denitrification and it is not always closely related to the actual denitrification rate (Bergstrom & Beauchamp, 1993). In the present study, dairy manure applications increased the DEA by up to 140%. Given that manure applications increase SOC and inorganic N concentrations, it was expected that there would be a positive relationship between DEA and manure application rate. Our results are supported by field measurements of N_2O emissions at LTM, where there was a positive correlation between cumulative N_2O emissions and manure application rate (Leytem et al., 2019). However, long-term application of organic fertilizers (e.g., composted livestock manure), and the associated buildup on SOC, can contribute to the immobilization of inorganic N, as well as alter the denitrifier community to favor N_2 production, resulting in lower N_2O emissions (Bhowmik et al., 2016; Lazcano et al., 2021).

4.4 | Soil health benefits of manure versus environmental implications

Arid-zone soils of the western United States generally have very low SOM and are ranked as having low soil quality, despite the fact that many are known for producing crops with some of the highest market values per acre of cropland (Sojka & Upchurch, 1999). The conclusion that could be drawn from our results is that manured soil is "healthier" than soil treated with IF, as a common interpretation for most soil health metrics is that "more is better." However, dairy manure will probably not provide the same physical, chemical, and biological benefits as humified SOM over the short term. Although not presented in this report, annual manure applications in LTM since 2012 have not significantly affected the bulk density down to 10 cm, nor have they led to increases in water infiltration (Jenifer Yost, University of Idaho, personal communication, 2022). This can likely be attributed to the fact that the soils are disked twice annually (i.e., before planting and to incorporate the fall manure), thus probably not allowing for the development of more stable aggregates that might occur under conservation tillage practices.

From a soil fertility standpoint, one of the major benefits of using livestock manure in cropping systems is that it gradually releases essential plant nutrients compared with IF. In 2019, when the soils were collected for this study, the crop was barley and grain yields were statistically similar between the fertilizer and manure treatments (unpublished data, 2019). In years previous to 2019, the yields for wheat, potato, barley, and sugarbeet were statistically similar between IF and manure treatments. The dairy manure, which was applied in the fall, does provide nutrients at levels necessary to achieve optimal crop growth, but the unintended consequence of applying high rates of manure has been a gradual increase of N and P in the top 30 cm compared with IF (unpublished data, 2019). Soil P accumulation has exceeded the environmental thresholds set by the Idaho State Department of Agriculture and would dictate that P-based nutrient management planning be adopted to reduce this accumulation of P over time. Applying manure to meet crop P requirements is a good practice for preventing the accumulation, leaching, and runoff of N, P, and other salts and protect environmental quality (Leytem et al., 2021).

5 | CONCLUSIONS

Our results provide additional evidence that commonly used biological and chemical indicators of soil health are highly influenced by long-term dairy manure use in semiarid irrigated soils, at typical application rates used in southern Idaho. The expected outcome of amending soil with manure for several years was the accumulation of SOC, which doubled at the highest annual application rate. Compared with IF, manure had a significant effect on the indicator responses, which was more pronounced in the top 15 cm. In addition, most of the soil health metrics were found to increase with increasing annual and biennial manure application rate. Despite the fact that all metrics were numerically greater in annually treated soils, there was no significant effect of manure application timing (annual vs. biennial) at both soil depths. Furthermore, given the strong positive correlations between all biological and chemical indicators, it is possible that one or just a few of the many metrics performed in this study could have been used to evaluate the cumulative effects of manure in these semiarid soils. Although β-glucosidase was the most sensitive indicator, based upon our particular study conditions and experience, we recommend that the POXC assay be used over most others since it can be performed very rapidly using air-dried soils and at a relatively low cost per sample.

The future utility of the POXC assay to assess soil health in our region will be undertaken by analyzing cropland soils with and without manure under a wide range of management practices.

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AUTHOR CONTRIBUTIONS

Robert S. Dungan: Conceptualization; Formal analysis; Investigation; Methodology; Supervision; Validation; Writing – review & editing. Chad W. McKinney: Data curation; Formal analysis; Writing – original draft. Veronica Acosta-Martinez: Formal analysis; Writing – review & editing. April B. Leytem: Validation; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

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