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Short-term effects of a heavy dairy manure application on soil chemical and biological indicators in an irrigated semiarid cropping system

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Abstract

Intensive dairy production in southern Idaho is associated with the annual application of manure to croplands. However, a one-time heavy application of manure could alternatively be used as a means to improve soil fertility and health for years or even decades, circumventing the need for frequent applications. To determine if this practice would negatively affect soil properties in the short term, we analyzed chemical and biological indicators of soil health for 2 years after dairy manure incorporation. Soil indicators measured were pH, electrical conductivity, extractable nitrogen (N) and phosphorus, total carbon (C) and N, enzyme activities, net N mineralization, soil organic C, soil protein, active C, ammonia oxidation potential, and particulate organic matter. Manure (with and without synthetic fertilizer) was found to significantly affect chemical and biological indicators in both topsoil (0–15 cm) and subsoil (15–30 cm), but the responses were greater in the subsoil. This can be attributed to the fact that manure was incorporated to approximately 30 cm via moldboard plow.

Abbreviations: ACE, autoclaved citrate extractable soil proteins; AOA, ammonia-oxidizing archaea; AOB, ammonia-oxidizing bacteria; Ctrl, control; EC, electrical conductivity; Fert, fertilizer; Man, manure; ManFert, manure + fertilizer; PAO, potential ammonia oxidation; PCA, principal component analysis; PMN, potentially mineralizable nitrogen; POM, particulate organic matter; POXC, permanganate oxidizable carbon; SOC, soil organic carbon; SOM, soil organic matter; β-GS, β-glucosidase; β-GSA, β-glucosaminidase.

All indicators responded positively to manure, except pH, which decreased slightly in the subsoil in the first year after application. Principal component analysis of chemical and biological indicators, across all years and depths, showed that the first two components explained 62% and 8.5% of the variance. While soil indicators were not adversely affected by manure, silage corn (*Zea mays* L.) yields in year 1 were significantly lower in manured plots, though in year 2, barley grain (*Hordeum vulgare* L.) yields were statistically similar among manure and fertilizer.

1 | INTRODUCTION

Soils are a vital natural resource, which provide essential ecosystem services that support life on the planet, as well as food and fiber production (Amon, 2024). Arid-zone soils in particular are known for producing crops with some of the highest market values and are important for food security (Sojka & Upchurch, 1999). Unfortunately, many soils in semiarid regions have been degraded due to long-term erosion, nutrient losses, and depletion of organic matter (Garcia-Franco et al., 2018). Semiarid soils are unique in that they contain moderate quantities of carbonates, have low soil organic carbon (SOC), and experience strong diurnal temperature fluctuations (Bierer et al., 2021). When these conditions are combined with frequent tillage, irrigation, and fertilization, there is great potential to negatively affect soil physical, chemical, and biological properties (Hills et al., 2020). However, the addition of organic residues to semiarid soils, such as livestock manures, can be beneficial to soil quality and health due to increased SOC (Dungan et al., 2022; O'Brien & Hatfield, 2019; Ozlu et al., 2019). In many cases, livestock manures have improved soil structure, increased infiltration and water-holding capacity, supplied macro- and micro-nutrients, and supported diverse biota (Rayne & Aula, 2020).

In western states, only 12% of total US dairy farms are represented, but they account for nearly 38% of the national milk cow herd due to the large size of these production systems (NASS, 2023). A high percentage of excreted manure is captured as lot scrapings and stockpiled in stacks until conditions are favorable for land application. As a result, large quantities of solid dairy manure are being applied to semiarid soils, which can impact crop quality and cause elevated nitrous oxide emissions (Leytem et al., 2019; Tarkalson et al., 2018). While the long-term effects of manure on soil health are generally understood (E. Liu et al., 2013; Ozlu et al., 2019; Sommerfeldt et al., 1988), more information is needed on the short-term effects of cattle manure. In addition, knowledge surrounding the relationship between manure-derived SOC stabilization and management practices is lacking in semiarid agricultural soils. This and other soil health data are urgently needed given how vulnerable arid-zone soils will be to future climate change (Garcia-Franco et al., 2018). Increasing knowledge of how soils respond to soil management can be used to devise practices that ensure cropland soils are more resilient to extreme weather events.

When livestock manures are incorporated into soil, there is an immediate increase in the amount of recalcitrant and labile organic matter, water-soluble nutrients and salts, and microbial biomass. A very short-term (i.e., days/weeks/months) effect is an increase in microbial activity, resulting in the decomposition of labile organic N compounds and subsequent release of mineral N and gaseous N losses (Aranguren et al., 2021; Calderón et al., 2004; Islam et al., 2021; Kaleem Abbasi et al., 2007; Thomas et al., 2015). In a study using soil from a semiarid cropping system with a 3-year dairy manure history, net N mineralization was 2.9-fold greater on average than in control soils after 49 days of incubation (Cassity-Duffey et al., 2018). Compared to inorganic fertilizers, livestock manures enhance microbial activities because of increased carbon (C) and nitrogen (N) availability (Deng et al., 2006; Lupwayi et al., 2018; Parham et al., 2002). A global meta-analysis revealed that the greatest increases in soil biochemical (e.g., enzyme activities, SOC, microbial biomass C and N) properties occurred with manure or composted manure without inorganic fertilizer use (Liu et al., 2020). Excessive and unbalanced use of inorganic fertilizers has resulted in soil degradation (Karlen & Rice, 2015), thus it makes sense to transition away from our reliance on nonrenewable inorganic fertilizers and promote nutrient cycling and C inputs by using manure resources (Chojnacka et al., 2020; Powers et al., 2019; Xia et al., 2017).

In semiarid southern Idaho, there are approximately 463,000 milk cows and use of their manure in cropping systems is quite common (Leytem et al., 2021). Opportunities are available to utilize manure nutrients in a variety of crops in southern Idaho, thereby bringing manure nutrients more in balance with regional crop demands, as well as increasing soil organic matter (SOM) levels. Since dairy manure is heavy and associated transportation costs are high, a single high-rate manure application event could be used to negate the need for regularly occurring manure applications. This approach is supported by results from Tarkalson et al. (2018), who found a strong legacy effect of dairy manure, with improved soil nutrient status and crop yields up to 7 years after the last manure application. In an effort to better understand this phenomenon in semiarid cropping systems, we established a field study in late 2018 where plots received a single application of dairy manure at a high rate. The objective of this study was to evaluate the initial response of crop and soil properties in the first 2 years following manure application both with and without inorganic fertilizer. It is essential that short-term effects be quantified when applying manure at levels that are above typical agronomic rates, as this could negatively affect soil health and crop production.

Core Ideas

- Chemical and biological indicators responded positively to a heavy dairy manure application in a semiarid cropland.
- Responses were greatest in the subsoil (15–30 cm) due to moldboard plow incorporation of the manure.
- Increased enzyme activities and high mineral N concentrations in the subsoil suggest rapid manure decomposition.
- The indicators were effective in evaluating short-term changes in soil properties under manure fertilization.
- Crop yields were lower in manured plots 1 year after manure application, but not in year 2.

2 | MATERIALS AND METHODS

2.1 | Site description and experimental design

The field site was located at the USDA-ARS Northwest Irrigation and Soils Research Laboratory in Kimberly, Idaho (Latitude 52.518473°, Longitude −114.3783160°). This region of Idaho has a cold semiarid climate (BSk, Köppen climate classification) 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 was a Portneuf silt loam (coarse-silty, mixed, superactive, mesic Durixerollic Calciorthids). The long-term objective of the field site treatments is to evaluate the effects of one-time manure application compared to fertilizer on crop production and soil indicators. The experimental design was a randomized block with five replications and each plot size was 15.2 m × 40.8 m. The treatments included a (a) control, no inorganic fertilizer or manure (Ctrl), (b) inorganic fertilizer based on recommendations (Fert), (c) dairy manure applied in one application (Man), and (d) dairy manure applied in one application and inorganic fertilizer applied based on recommendations (manure + fertilizer [ManFert]). Prior to fall 2018 manure application, this field was chisel plowed followed by roller harrowing, and then planted with silage corn on May 21. No inorganic fertilizer was applied to this field during the 2018 growing season. The corn was harvested during September 12–18, and then dairy manure solids were applied on November 19–21, 2018, at 103 Mg ha^{-1} (dry wt.).

The manure properties were moisture content, 67%; pH, 9.4; electrical conductivity (EC), 15.2 dS m^{-1}; nitrate-nitrogen (NO_3-N), 243 mg kg^{-1}; ammonium-nitrogen (NH_4-N), 95.8 mg kg^{-1}; total C, 209 g kg^{-1}; total N, 15.1 g kg^{-1}; total P, 6.4 g kg^{-1}; and total K, 35.3 g kg^{-1}. The manure was first incorporated by disking (~15-cm depth), followed by moldboard plow (~30-cm depth) within 1 day of application. All plots, including those without manure, received tillage at the same time for consistency. Based on the total N content of the manure, N was applied at approximately 1,555 kg ha^{-1}. In spring 2019, urea was applied to the Fert and ManFert plots at 213 and 106 kg N ha^{-1}, respectively. In spring 2020, urea and monoammonium phosphate were applied to the Fert plots at 100 kg N ha^{-1} and 149 kg P_2O_5 ha^{-1}, respectively, while urea was applied to the ManFert plots at 106 kg N ha^{-1}. The fertilizer was applied at rates based on spring soil test data and University of Idaho and industry crop recommendations (Brown et al., 2010; Robertson & Start, 2003). Inorganic fertilizer was broadcast in the spring before planting and then immediately irrigated with 19 mm of water to incorporate the fertilizer. The study field was planted to corn (*Zea mays* L., Pioneer P9188R) on May 10, 2019, and barley (*Hordeum vulgare* L., Moravian 69) on April 4, 2020. The crops were irrigated with a linear move irrigation system to meet calculated crop evapotranspiration rates (Wright, 1982).

2.2 | Soil sampling

Preplant soil samples were collected on April 23, 2018, April 24, 2019, and March 13, 2020, prior to the application of any inorganic fertilizer. Five soil cores were collected per plot at 0- to 15-cm and 15- to 30-cm depths using a 70-mm diameter hand auger (AMS Inc.). After collection, the cores from each plot were composited by depth and then thoroughly mixed. Field moist subsamples were passed through a 4-mm sieve, and then a portion was immediately placed into a clean sealable plastic bag and refrigerated at 5°C. Soil biological analyses requiring field moist soils were completed within 2 weeks of the sampling time, otherwise samples were frozen at −80°C until the analyses could be performed. The soil moisture was gravimetrically determined by drying at 105°C for 24 h.

2.3 | Soil analysis

The pH and EC were determined in soil mixed with deionized water at ratios of 1:1 and 1:2, respectively. Nitrate-N and NH_4-N were determined after extraction in 2 M KCl (Mulvaney, 1996) using a Lachat QuickChem 8500 Flow Injection Analyzer (Hach Company). Olsen P was determined as sodium bicarbonate extractable P (Olsen, 1954). Total C and N were determined by complete combustion of a soil sample in a FlashSmart NC Soil Analyzer (CE Elantech). The natural abundance of ^{15}N isotope in soil, relative to ^{15}N of air, was determined using a PDZ Europa 20-20 isotope ratio mass spectrometer (Secron Ltd.) at the University of California Davis Stable Isotope Facility. Soil organic C was determined using the Walkley–Black method (Walkley & Black, 1934). Permanganate oxidizable C (POXC) was determined using the protocol developed by Weil et al. (2003). Total particulate organic matter (POM), POM-C, and POM-N were evaluated using the procedure as outlined by Cambardella and Elliott (1992). The autoclaved citrate extractable (ACE) soil proteins assay was a modification of a procedure used to extract proteins from fungi and soil (Wright & Upadhyaya, 1996) and was based on the approach of Moebius-Clune (2016). β-glucosidase (β-GS) and β-glucosaminidase (β-GSA) enzyme activities were performed as described by Eivazi and Tabatabai (1988) with some modifications. Potential ammonia oxidation (PAO), also known as the short-term nitrification assay, was adapted from Hart et al. (1994). Potentially mineralizable nitrogen (PMN) was determined using the 7-day anaerobic laboratory method (Waring & Bremner, 1964). Net N mineralization potential was also determined with 7- and 28-day aerobic methods (Hart et al., 1994).

2.4 | Statistical analysis

All statistical analyses were conducted using SAS software version 9.4 (SAS Institute). Linear mixed models for soil variables were performed using PROC GLIMMIX with year, treatment, and depth as main effects, block as a random effect, and year as a repeated measure. A log normal distribution was used where necessary, and the covariance structure was determined by comparing fit statistics. Analyses on yields were performed for each year separately. The three-way interaction was considered a planned comparison, and multiple comparisons were performed on least squares means with a Bonferroni adjustment if the p-value for the interaction was insignificant. All comparisons were considered statistically significant at $\alpha = 0.05$. Soil ^{15}N was analyzed across depths within each year for only Fert and Man treatments. Principal component analysis (PCA) was performed using JMP software version 17 (SAS Institute) to evaluate the relationship among soil indicators; the analyses included an assessment across all years and soil depths.

TABLE 1 Average crop yields by treatment and year on a dry weight basis.

Treatment	Silage corn (2019) (Mg ha^{-1})	Barley grain (2020) (Mg ha^{-1})
Ctrl	15.5b	3.5b
Fert	17.3a	7.4a
Man	15.9b	8.0a
ManFert	15.1b	7.4a

Note: Means in a column followed by the same letter are not significantly different ($p < 0.05$).

Abbreviations: Ctrl, control; Fert, fertilizer; Man, manure; ManFert, manure + fertilizer.

3 | RESULTS

3.1 | Crop yields

Average yields for silage corn (2019) and barley grain (2020), on a dry matter basis, are presented in Table 1. The silage corn yield from Fert was 17.3 Mg ha^{-1}, while yields from Ctrl, Man, and ManFert were 10% lower on average. Barley grain yields were similar among Fert, Man, and ManFert at about 7.6 Mg ha^{-1}, while the yield from the Ctrl was 54% lower on average.

3.2 | Soil chemical indicators

Soil chemical properties at two depth increments (0–15 cm and 15–30 cm) for spring 2018, 2019, and 2020 are presented in Table 2. The average pH value across all years, depths, and treatments was 8.1 (±0.1). Application of fertilizer and manure treatments in fall 2018 significantly decreased spring 2019 pH values in Man and ManFert at both soil depths but returned to 2018 baseline levels in 2020. Soil pH values for the Ctrl and Fert treatments were similar to the Man and ManFert in 2018 and remained constant over time. Soil salinity, measured as EC, ranged from 0.27 to 1.03 dS m^{-1} across 2019 and 2020. Salinity was twofold- to threefold greater at both depths in 2019 and at 15–30 cm in 2020 in Man and ManFert compared to Ctrl and Fert. Salinity was greater in 2019 than 2020 for Fert, Man and ManFert at 0–15 cm, and Man and ManFert at 15–30 cm. Salinity differences between soil depths within a treatment occurred in Man and ManFert in 2019 and 2020.

Baseline NO_3-N concentrations in 2018 did not differ among treatments at 0–15 cm, but they were significantly greater in Fert than Man at 15–30 cm, and greater at 15–30 cm than 0–15 cm for Ctrl, Fert, and ManFert, indicating minor in-field variation prior to applying fertilizer and manure treatments. Application of fall manure increased spring 2019 NO_3-N at both depths. In 2019, average NO_3-N concentrations were greater in Man and ManFert than Ctrl or Fert at

TABLE 2 Soil pH, electrical conductivity (EC), KCl-extractable nitrate (NO_3^-) and ammonium (NH_4^+), Olsen phosporous (P), and total carbon (C) and nitrogen (N) at two depth increments in the spring.

	Treatment	pH	EC (dS m^{-1})	NO_3-N (mg N kg^{-1})	NH_4-N (mg N kg^{-1})	Olsen P (mg P kg^{-1})	Total C (g C kg^{-1})	Total N (g N kg^{-1})	C:N
2018									
0–15 cm	Ctrl	8.09a	0.21aB*	12.2aA *	3.14aC	10.2a	12.2a	0.94a	13.0a
	Fert	8.14a	0.24aB*	13.5aA*	2.89aC	8.8a	12.0a	0.97a	12.4a
	Man	8.18aA	0.25aC	12.6aB	3.08aB	8.9aB	11.8aB	0.92aB	12.8a
	ManFert	8.16aA	0.23aC	13.1aC*	2.88aB	9.3aB	11.7aB	0.96aB	12.2a
15–30 cm	Ctrl	8.10a	0.26aB	19.8abA	3.18aB	9.3a	11.9a	0.90a	13.1a
	Fert	8.19a	0.32a	25.3aA	3.04aB	8.6aA	10.9a	0.90a	12.1a
	Man	8.17aA	0.27aC	14.4bC	2.60aC	8.6aB	11.1aC	0.87aC	12.8a
	ManFert	8.16aA	0.30aC	19.6abC	2.79aC	8.5aB	10.8aB	0.90aB	12.1a
2019									
0–15 cm	Ctrl	8.14a	0.29bA	10.4bA	6.03abB	10.9b	12.6bc	1.01b	12.4a
	Fert	8.11a*	0.32bA	10.8bAB	4.80bB	9.6b	11.8c	1.00b	11.8a
	Man	7.92bB	0.82aA*	60.8aA*	7.38aA	38.5aA	15.0aA*	1.24aA*	12.0a
	ManFert	7.93bB	0.81aA*	57.0aA*	7.23aA	35.6aA	14.1abA*	1.25aA*	11.3a
15–30 cm	Ctrl	8.16a	0.31bA	10.2bB	5.41bA	9.3b	11.9b	0.91b	13.1a
	Fert	8.20a	0.29b	9.4bC	4.96bA	7.9bA	10.9b	0.93b	11.7a
	Man	7.88bB	1.03aA	91.1aA	12.86aA	112.1aA	19.9aA	1.66aA	12.0a
	ManFert	7.91bC	1.03aA	82.8a A	12.74aA	97.4aA	18.7aA	1.60aA	11.7a
2020									
0–15 cm	Ctrl	8.11a	0.27bA	6.3bB	8.30aA	8.5b	12.1a	1.0bc	12.5a
	Fert	8.14a	0.27bAB	8.2bB*	7.30aA	7.2b	11.9a	1.0c	11.9a
	Man	8.16aA	0.38aB*	16.3aB*	6.74aA	36.6aA	13.6aA*	1.1abA*	12.2a
	ManFert	8.12aA	0.45aB*	24.3aB*	7.60aA	26.4aA	13.8aA*	1.2aA*	11.8a
15–30 cm	Ctrl	8.15a	0.29bAB	7.1dC	6.54aA	7.5b	12.0b	0.96c	12.6a
	Fert	8.13a	0.31bAB	13.9cB	6.25aA	5.5bB	11.4b	0.95c	12.0a
	Man	8.09aA	0.63aB	38.4bB	6.29aB	132.1aA	16.2aB	1.34bB	12.1a
	ManFert	8.04aB	0.77aB	61.6aB	7.08aB	84.0aA	20.9aA	1.72aA	12.1a
	Distribution	N	logN	logN	logN	logN	logN	logN	N
	Year (Y)	35.56****	200.03****	65.8****	235.44****	87.7****	42.5****	62.29****	8.37***
	Treatment (T)	4.14**	35.07****	52.1****	4.24**	73.0****	7.3***	31.25****	0.99^{ns}
	Y × T	18.65***	45.13****	62.9****	9.79****	37.8****	14.5****	15.41****	0.77^{ns}
	Depth (D)	0.01^{ns}	101.96****	71.1****	0.30^{ns}	22.5****	8.1**	6.36*	0.32^{ns}
	Y × D	1.28^{ns}	21.91****	8.5***	8.15***	8.5***	10.7****	8.66***	0.41^{ns}
	T × D	3.28*	18.23****	3.8*	2.17^{ns}	17.7****	11.5****	11.89****	0.58^{ns}
	Y × T × D	0.61^{ns}	2.57^{ns}	3.4**	2.00^{ns}	4.2***	3.6**	3.63**	0.29^{ns}

Note: The spring 2018 soils represent baseline conditions prior to any treatment. Lowercase letters are for differences among treatments within a depth and year. Uppercase letters are for differences among years within a depth and treatment. Asterisks are for differences between depths within a treatment and year (indicated at surface depth).
Abbreviations: Ctrl, control; Fert, fertilizer; Man, manure; ManFert, manure + fertilizer.
Distribution: N = normal; LogN = log-normal.
****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns, not significant.

both depths, and significantly greater at 15–30 cm than 0–15 cm for the manured treatments only. Spring 2020 NO_3-N declined in most treatments compared to 2019 values, and treatment differences at 0–15 cm were the same as 2019, but concentrations at 15–30 cm were significantly different among all treatments with the greatest concentration in ManFert and the lowest in Ctrl and were greater at 15–30 cm than at 0–15 cm for all except the Ctrl.

In 2018, baseline NH_4-N concentrations averaged 2.95 mg kg^{-1} across all treatments and depths and increased for all treatments and depths in 2019, where it was greater in Man and ManFert than Fert at 0–15 cm, and in Man and ManFert than both Fert and Ctrl at 15–30 cm. Concentrations increased between 2019 and 2020 for Ctrl and Fert at 0–15 cm and decreased for Man and ManFert at 15–30 cm, but were all still greater than in 2018. In 2020, NH_4-N concentrations were similar among all treatments and soil depths. Baseline Olsen P concentrations averaged 9.0 mg kg^{-1} across all treatments and depths in 2018. Addition of manure with or without fertilizer significantly increased Olsen P at both depths, and fertilizer increased Olsen P at 15–30 cm in 2019 compared to 2018, though concentrations were still greater for manured treatments over Fert or Ctrl. Treatment differences continued into 2020, where concentrations were similar to 2019 except where it declined for Fert at 15–30 cm.

Initial concentrations of total C and total N in 2018 averaged 11.5 and 0.9 g kg^{-1}, respectively, with no differences among treatments or depths. Total C and total N in Ctrl and Fert remained constant over time and soil depths. Addition of manure increased both total C and N in Man and ManFert treatments, significantly more at 15–30 cm than 0–15 cm in 2019. By 2020, treatment differences continued only for total N at 0–15 cm and for both total C and N at 15–30 cm. Despite changes in total C and N with manure application, the C:N ratios (average = 12.2) were found to be statistically similar among all treatments and depths. There was an overall significant year effect ($p < 0.001$), which indicated that C:N was significantly lower in 2019 and 2020 (average = 12.1) than 2018 (average = 12.5).

3.3 | Soil organic, particulate, and active C and N fractions

Average values for soil organic C, and particulate and active C and N fractions are presented in Table 3. In 2018, the baseline data collected for all organic, particulate, and active soil C and N fractions were similar among all treatment plots and soil depths. In 2019, SOC, POXC, and ACE were significantly greater in Man and ManFert than in Ctrl and Fert at both depths, and SOC and POXC were also greater at 15–30 cm than at 0–15 cm for Man and ManFert. From 2018 to 2019, SOC increased in all treatments at both depths, POXC increased only for Man and ManFert at both depths, and ACE increased only in ManFert at 0–15 cm and Man and ManFert at 15–30 cm. In 2020, SOC concentration for Ctrl increased from 2019 (8.63 B vs. 10.15 A) at 0–15 cm, while POXC for ManFert also increased at this depth (0.43 B vs. 0.54 A). Also, 2020 treatment differences at 15–30 cm depth were generally the same, but concentrations at 0–15 cm were only significantly greater in ManFert than Ctrl or Fert.

Total POM and POM-C and POM-N did not differ among treatments or soil depths in 2018, nor did they differ among treatments within the 0–15 cm soil depth in 2019 or 2020, despite a significant increase between 2018 and 2019 for total POM in Man and ManFert and POM-N for Man, or the increase between 2019 and 2020 for POM-C in Ctrl. In 2019, both POM-C and POM-N were significantly greater in Man and ManFert than Ctrl and Fert at 15–30 cm, which was due to a significant increase from 2018. By 2020, POM-C and POM-N were significantly different only between ManFert and Ctrl at 15–30 cm, and ManFert was the only treatment where concentrations were greater at depth.

Based on ^{15}N contents of the urea and dairy manure, as well as accounting for baseline ^{15}N in the Ctrl, the fraction of total soil N that was from those fertilizer sources was determined (Figure 1). In 2019 preplant soils, ≤1.0% of the urea-^{15}N in the Fert plots was attributed to previous year's use of inorganic fertilizer at the field site. However, given the large amount of manure applied in fall 2018, it was found that 45% and 73% of the preplant soil N in 2019 at 0–15 cm and 15–30 cm, respectively, was derived from the manure. The dairy manure applied in 2018 contained about 1.5% N, thus approximately 1555 kg N ha^{-1} was applied to the Man and ManFert plots. In 2020 preplant soils, little to no N could be attributed to urea from the previous year, but manure accounted for 19% and 51% of the N present at 0–15 cm and 15–30 cm, respectively.

3.4 | Soil biological indicators

Average values for enzyme activities (β-GS and β-GSA) and N transformation rates determined via PAO and anaerobic(an)/aerobic(ae) net N mineralization assays (PMN_{7an}, PMN_{7ae}, and PMN_{28ae}) are presented in Table 4. In 2018, neither enzyme activities, PAO, and PMN_{7ae} activities differed among treatments for either depth. However, at 15–30 cm, PMN_{7an} and PMN_{28ae} were significantly elevated in Man over ManFert and Man over ManFert and Ctrl, respectively, suggesting some unknown pre-existing field variability. β-glucosidase and β-GSA enzyme activities ranged from 50.5 to 82.6 and 11.1 to 25.8 μg *p*-nitrophenol g^{-1} h^{-1}, respectively. The β-GS activity did not show a response to treatment until 2020, where the activity was greater in ManFert than Fert at 15–30 cm, and activity for ManFert was significantly greater than in 2018. β-glucosaminidase activities were significantly greater in Man than Fert at 0–15 cm in 2019, and greater in Man and ManFert than Fert and Ctrl in 2019 and 2020 at 15–30 cm, where total activities in Man and ManFert were also significantly increased from 2018. In 2019, treatment differences for PAO activities were similar to β-GSA for both depths, but were also significantly greater than activities within the same treatments in 2018. By 2020, PAO declined in all treatments, though differences were maintained with greater PAO for ManFert and Fert than Ctrl at 0–15 cm and

TABLE 3 Soil organic carbon (SOC), permanganate oxidizable carbon (POXC), particulate organic matter (POM), and autoclave citrate extractable protein (ACE) at two depth increments in the spring.

	Treatment	SOC ($g\ C\ kg^{-1}$)	POXC ($g\ C\ kg^{-1}$)	POM ($g\ kg^{-1}$)	POM-C ($g\ C\ kg^{-1}$)	POM-N ($g\ N\ kg^{-1}$)	ACE ($g\ kg^{-1}$)
2018							
0–15 cm	Ctrl	8.31aB	0.24a	4.74a	2.63aB	0.18aB	2.65aA
	Fert	8.02aB	0.23a	4.90a	3.02a	0.21a	2.55aA
	Man	7.86aB	0.20aB	3.27aB	1.69aB	0.12aB	2.68aA
	ManFert	8.04aB	0.22aB	4.31aB	2.33a	0.16a	2.58aB
15–30 cm	Ctrl	7.21aC	0.22a	3.53a	1.80a	0.12a	2.51aA
	Fert	7.00aB	0.20a	4.68a	1.63aB	0.13aB	2.31a
	Man	7.07aB	0.20aB	3.90aB	2.03aB	0.14aB	2.53aC
	ManFert	7.23aB	0.21aC	4.73a	2.21aB	0.15aB	2.40aB
2019							
0–15 cm	Ctrl	9.65bA	0.23b	6.62a	2.35aB	0.19aAB	2.38bAB
	Fert	9.62bA	0.22b	6.81a	2.89a	0.24a	2.39bAB
	Man	11.72aA*	0.30aA*	7.28aA	3.44aAB	0.28aA	3.07aA
	ManFert	11.47aA*	0.31aA*	7.11aA	3.56a	0.30a	3.06aA
15–30 cm	Ctrl	8.63bB	0.21b	4.67a	2.29b	0.17b	2.21bAB
	Fert	8.58bA	0.21b	5.55a	2.23bB	0.17bAB	2.16b
	Man	17.05aA	0.45aA	6.98aA	4.52aA	0.40aA	4.19aA
	ManFert	16.68aA	0.43a B	6.91a	4.79aA	0.40aA	4.04aA
2020							
0–15 cm	Ctrl	9.89bcA	0.22b	5.83a	4.60aA*	0.34aA*	2.11bB
	Fert	9.58cA	0.22b	6.31a	4.24a	0.31a	2.14bB
	Man	11.42abA*	0.28abA*	6.35aA	4.61aA	0.36aA	2.28abB
	ManFert	11.95aA*	0.32aA*	5.99aAB	3.55a*	0.27a*	2.61aA
15–30 cm	Ctrl	10.15cA	0.25b	4.02a	2.15b	0.15b	2.10cB
	Fert	9.53cA	0.23b	5.52a	4.09abA	0.29abA	2.15c
	Man	14.59bA	0.46aA	5.01aAB	3.33abAB	0.26abAB	3.15bB
	ManFert	18.00aA	0.54aA	6.07a	5.09aA	0.40aA	3.95aA
	Distribution	logN	logN	N	N	N	logN
	Year (Y)	163.62****	56.35****	23.59****	21.73****	25.99****	13.82****
	Treatment (T)	26.27****	24.35****	2.28^{ns}	2.87*	4.24**	22.26****
	Y × T	13.09****	16.35****	1.20^{ns}	2.15^{ns}	2.76*	11.63****
	Depth (D)	6.67**	20.48****	6.12*	1.02^{ns}	0.65^{ns}	9.68**
	Y × D	17.43****	14.67****	1.11^{ns}	1.98^{ns}	1.60^{ns}	10.97****
	T × D	14.05****	11.71****	1.94^{ns}	3.93*	4.34**	10.19****
	Y × T × D	3.17**	1.41^{ns}	0.20^{ns}	1.63^{ns}	1.72^{ns}	2.39*

Note: The spring 2018 soils represent baseline conditions prior to any treatment. Lowercase letters are for differences among treatments within a depth and year. Uppercase letters are for differences among years within a depth and treatment. Asterisks are for differences between depths within a treatment and year (indicated at surface depth).
Abbreviations: Ctrl, control; Fert, fertilizer; Man, manure; ManFert, manure + fertilizer.
Distribution: N = normal; LogN = log-normal.
****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns, not significant.

for Man and ManFert than Ctrl and Fert at 15–30 cm. In 2019, PMN_{7an} was significantly greater for Man and ManFert than Ctrl and Fert at both depths and was greater than in 2018 for all treatments at 0–15 cm, but only for Man and ManFert at 15–30 cm. Treatment differences persisted into 2020 for both depths, but compared to 2019, potential mineralization significantly declined for Man and ManFert at 0–15 cm and Man at 15–30 cm, and increased for Fert at 15–30 cm. In 2019, treatment differences for PMN_{7ae} and PMN_{28ae} were absent at 0–15 cm but were significantly greater for Man and ManFert

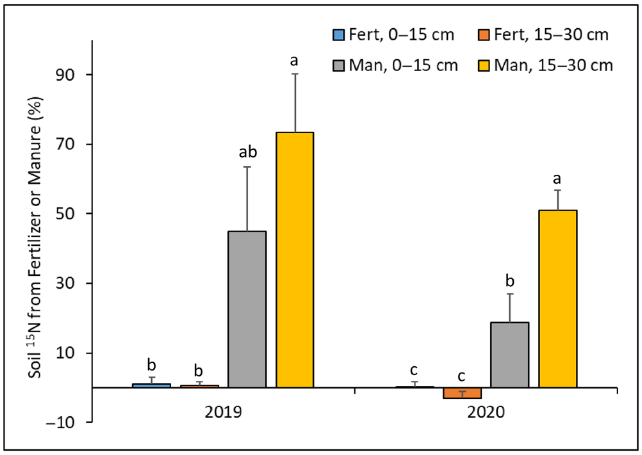

FIGURE 1 The natural abundance of ^{15}N in soil from urea fertilizer (Fert) or dairy manure (Man) at 0–15 cm and 15–30 cm. The manure was applied in fall 2018, while soil samples were collected in spring 2019 and 2020.

than Ctrl, or Ctrl and Fert, at 15–30 cm. In 2020, treatment differences appeared for PMN_{7ae} at 0–15 cm, whereby Man and ManFert were greater than Ctrl, likely because ofa significant decline for Ctrl from 2019. At 15–30 cm, PMN_{7ae} also declined from 2019 to 2020 for Ctrl and Man, but treatment differences were generally the same. In 2019 and 2020, PMN_{28ae} differed among treatments only at 15–30 cm, with ManFert consistently greater than Ctrl and Fert, but changes over time were absent.

3.5 | Principal component analysis

PCA of soil chemical and biological indicators are presented in Figure 2A,B. Across all 3 years and soil depths, the first two components explained 61.8% and 8.5% of the variance. The 2019 and 2020 Man and ManFert treatments at 15–30 cm were clearly separated along PC1 from most other observations, with PC2 contributing to separation of the 0–15 cm observations in 2019. Soil pH was negatively associated with PC1 and most other indicators except C:N ratio, POM, POM-C, and POM-N, which were more generally associated with PC2. These loadings indicated a strong relationship of soil indicators with manure application.

When separated out by year, PCA explained only 39.6% of the total variance for 2018 (Figure 3A,B). However, PC1 separated the observations for the 0- to 15-cm and 15- to 30-cm depths, with the surface samples associated with positive PC1 scores for most soil parameters. Not surprisingly, subsurface 15–30 cm observations were associated with soil NO_3-N, which was the main property exhibiting greater concentration with depth than in 2018 (Table 2). For 2019, PC1 and PC2 explained 69.8% and 7.1% of the variance, respectively, and most soil indicators were positively associated with PC1, whereas pH was generally negatively associated but it had some positive influence on PC2 (Figure 3C,D). All observations for Man and ManFert at 15–30 cm had positive loadings on PC1, supporting a positive outcome from manure application on soil indicators. The C:N ratio was generally orthogonally related to most soil indicators, indicating absence of significant correlations; however, removal of this property from the analysis did not significantly change any loadings or interpretations (data not shown). For 2020, 71.2% of the total variance was explained by the first two components, with Man and ManFert observations at 15–30 cm again positively loading on the PC1 axis (Figure 3E,F). Soil C:N and pH had minimal to moderate negative influence on the two axes, NH_4-N had minimal positive influence on PC2, and the majority of the remaining indicators again were strongly and positively associated with PC1. The others, including POM, POM-C, and POM-N, were positively and strongly associated with PC2.

4 | DISCUSSION

4.1 | Manure application and soil indicators

Few studies have reported short-term effects on chemical and biological properties after a new management practice has been implemented, especially under field conditions in semiarid environments (Kocyigit et al., 2017; Mdlambuzi et al., 2021). The current field study investigated the effect of a single heavy application of dairy manure on crop yields and soil health indicators prior to and for 2 years following manure incorporation in fall 2018. Given the high manure application rate and thickness of the manure (~10 cm) on the soil surface after application, the most effective means to fully incorporate the manure was by moldboard plow. Therefore, both topsoil (0–15 cm) and subsoil (15–30 cm) were assessed, assuming that moldboard plowing would place most of the manure into the subsoil layer. The chemical and biological indicators used to quantify C and N pools and nutrient cycling potentials via enzyme activities and N transformation rates were selected for this study as they are currently used by the scientific community and land managers for soil health evaluations (Moebius-Clune, 2016; Norris et al., 2020; Stott, 2019). In general, biological indicators are sensitive to short-term changes in the soil environment, while physical and chemical indicators typically require longer periods before exhibiting effects of management (Nelson et al., 2009). Livestock manure applications can have short-term effects on soil chemistry (Du et al., 2020; Eghball, 2002; Yilmaz & Alagöz, 2010), thus chemical indicators were deemed useful along with biological indicators to evaluate the short-term effects of manure on soil properties.

TABLE 4 β-Glucosidase (β-GS) and β-glucosaminidase (β-GSA) activities and N transformation rates determined via potential ammonia oxidation (PAO) and 7- and 28-day potentially mineralizable nitrogen (PMN) under anaerobic (an) and aerobic (ae) conditions at two depth increments in the spring.

		β-GS (μg *p*-nitrophenol g^{-1} h^{-1})	β-GSA (μg *p*-nitrophenol g^{-1} h^{-1})	PAO (μg NO_2-N g^{-1} h^{-1})	PMN_{7an} (net mg N kg^{-1})	PMN_{7ae} (net mg N kg^{-1})	PMN_{28ae} (net mg N kg^{-1})
2018							
0–15 cm	Ctrl	65.5a	14.9a	0.49aB	2.9aB	8.9aA	8.9a
	Fert	69.0a	13.7a	0.52aB	3.4aB*	5.7a	16.2a*
	Man	73.2a	15.4a	0.46aB	2.8aC	2.7a	11.8a
	ManFert	65.2a	13.4a	0.51aB	2.9aC*	3.7a	13.6a*
15–30 cm	Ctrl	60.6a	14.2a	0.64aB	1.7abB	4.2aA	7.8b
	Fert	59.6a	12.7a	0.59aB	1.7abB	4.2a	8.1ab
	Man	55.1a	13.0aB	0.61aB	2.7aC	3.4aB	13.7a
	ManFert	64.6aB	12.6aB	0.60aC	1.5bB	2.5aB	6.6b
2019							
0–15 cm	Ctrl	67.2a	13.2ab	0.63abA	6.9bA*	4.7aA	15.6a
	Fert	58.7a	11.6b	0.69bA	6.8bA*	4.4a	14.6a
	Man	67.8a	17.6a*	0.89aA	18.6aA*	6.0a	29.2a
	ManFert	58.8a	15.4ab*	0.80abA	18.7aA	10.9a	31.7a
15–30 cm	Ctrl	50.5a	11.6b	0.82bA	1.9bAB	3.1bA	13.3b
	Fert	52.1a	11.1b	0.88bA	1.5cB	5.0ab	15.2b
	Man	67.4a	25.8aA	1.75aA	31.8aA	15.5aA	49.7a
	ManFert	70.2aAB	23.3aA	1.95aA	30.5aA	15.9aA	40.7a
2020							
0–15 cm	Ctrl	69.3a	12.1a	0.43bB	5.6bA	1.2bB	11.5a
	Fert	61.3a	11.2a	0.57aAB	4.8bAB	2.8ab	15.7a
	Man	73.1a	12.9a*	0.51abB	10.1aB	4.9a	21.7a
	ManFert	76.5a	13.8a*	0.61aB	10.2aB*	3.9a	23.6a
15–30 cm	Ctrl	62.7ab	11.8b	0.39bC	4.2bA	0.6cB	12.1b
	Fert	54.4b	11.7b	0.40bC	3.6bA	2.8b	13.3b
	Man	67.0ab	18.9aA	0.70aB	14.8aB	6.1abB	24.5ab
	ManFert	82.6aA	22.4aA	0.97aB	19.6aA	10.8aA	36.8a
	Distribution	N	logN	logN	logN	logN	logN
	Year (Y)	3.54*	3.13*	140.74****	154.37****	14.94****	39.93****
	Treatment (T)	1.85^{ns}	10.38****	10.84****	149.95****	9.38****	17.35****
	Y x T	1.75^{ns}	6.31****	10.15****	22.04****	4.70***	3.32**
	Depth (D)	5.40*	5.67*	71.47****	14.45***	0.02^{ns}	0.74^{ns}
	Y x D	0.63^{ns}	6.73**	17.70****	7.69***	1.26^{ns}	4.98**
	T x D	2.81*	5.03**	12.70****	15.87****	1.69^{ns}	1.50^{ns}
	Y x T x D	0.78^{ns}	1.74^{ns}	3.24**	4.57****	1.08^{ns}	1.16^{ns}

Note: The spring 2018 soils represent baseline conditions prior to any treatment. Lowercase letters are for differences among treatments within a depth and year. Uppercase letters are for differences among years within a depth and treatment. Asterisks are for differences between depths within a treatment and year (indicated at surface depth).
Abbreviations: Ctrl, control; Fert, fertilizer; Man, manure; ManFert, manure + fertilizer.
Distribution: N = normal; LogN = log-normal.
****$p < 0.0001$; ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns, not significant.

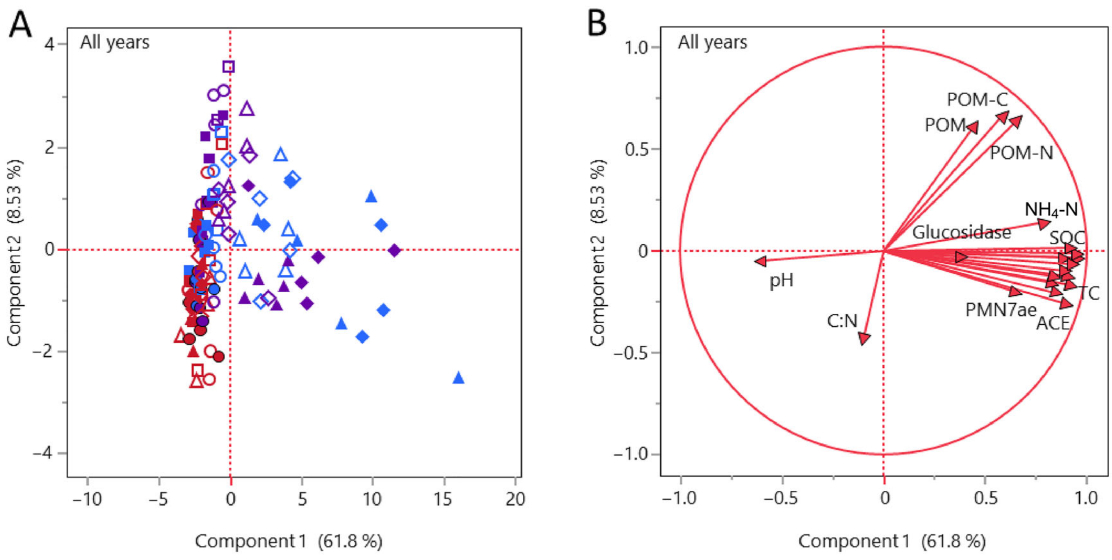

FIGURE 2 Principal component analysis of soil chemical and biological indicators showing scores for all observations (A) and loadings (B) across all years and depths. Open and closed icons indicate 0- to 15-cm and 15- to 30-cm soil depths, respectively. Circles = Ctrl, squares = Fert, triangles = Man, and diamonds = ManFert. ACE, autoclaved citrate extractable soil proteins; Ctrl, control; Fert, fertilizer; Man, manure; ManFert, manure + fertilizer; PMN, potentially mineralizable nitrogen; POM, particulate organic matter; SOC, soil organic carbon; TC, total carbon.

4.2 | pH and electrical conductivity

In the present study, results demonstrated that a single manure application significantly influenced soil chemistry at both soil depths, with the greatest changes at 15–30 cm. In 2019, at 15–30 cm, there was a significant decrease in pH (8.2–7.9) and a doubling of NH_4-N and total N in the manured soils. Soil pH, which had a high negative load on PCA component 1 and negative correlation with most other variables, was strongly associated with the non-manure-treated soils. Although the pH of the dairy manure used in the present study was 9.4, the slight decrease in soil pH that was observed can likely be attributed to the release of hydrogen ions (H^+) during the nitrification of NH_4^+ to NO_3^- (Paul, 2007). In contrast, Du et al. (2020) showed that short-term (<3 years) manure application did not have a significant effect on soil pH compared to medium-term (3–10 years) and long-term (>10 years) manure applications. In the latter cases, only significant increases in soil pH were found to occur with manure in acidic soils and warm and arid climates.

Given the chemical composition of manure, application in excess of crop requirements can cause a buildup of salts and nutrients in the soil (Moore & Ippolito, 2009). At the high manure application rate used in the present study, an immediate and exacerbated increase in soluble salts, N, and P would be expected and was confirmed by significant increases at depth of incorporation. Comparatively, a significant buildup of salts occurred when manure was repeatedly applied to semiarid soils, even under irrigated conditions (Chang et al., 1990; Eghball, 2002). In arid-zone soils, soluble salts can accumulate and remain near the soil surface, especially if saline irrigation water is applied (Ortiz & Jin, 2021). In contrast, Turner et al. (2010) reported that when beef cattle manure was applied annually at 4–37 Mg ha^{-1} for 5 years in a semiarid irrigated, conventionally tilled, cropping system under continuous corn, no significant increases in soil EC occurred at 0–15 cm. The authors speculated that the low EC was related to the transport of mobile soluble salts out of this depth during sampling time. ECs in the present study were substantially lower than 4 dS m^{-1}, where values above this can serve as an indication of salinity problems and are often associated with reduced crop growth (Richards, 1954). Based on the work of Maas and Hoffman (1977), EC thresholds (1:1 extract) for corn and barley were determined to be 1.0–1.2 and 4.5–5.7 dS m^{-1}, respectively. Therefore, it is possible that elevated salinity levels caused corn yields to be lower in the manured plot in the present study. However, we observed that manured plots had higher weed counts in the first year after manure application (data not shown), thus weeds could have contributed to suppressed corn yields.

4.3 | Soil organic carbon

In the Du et al. (2020) meta-analysis, it was reported that the greatest increases in SOC (as well as total N and available N and P) under manure application occurred in soils with low SOC (i.e., 6–12 g C kg^{-1}), regardless of soil texture, crop type, N application rate, and land-use type. Although these increases only occurred when the duration of manure was >10 years. Similarly, Chen et al. (2018) reported that SOC increases were greatest when manure was applied >20

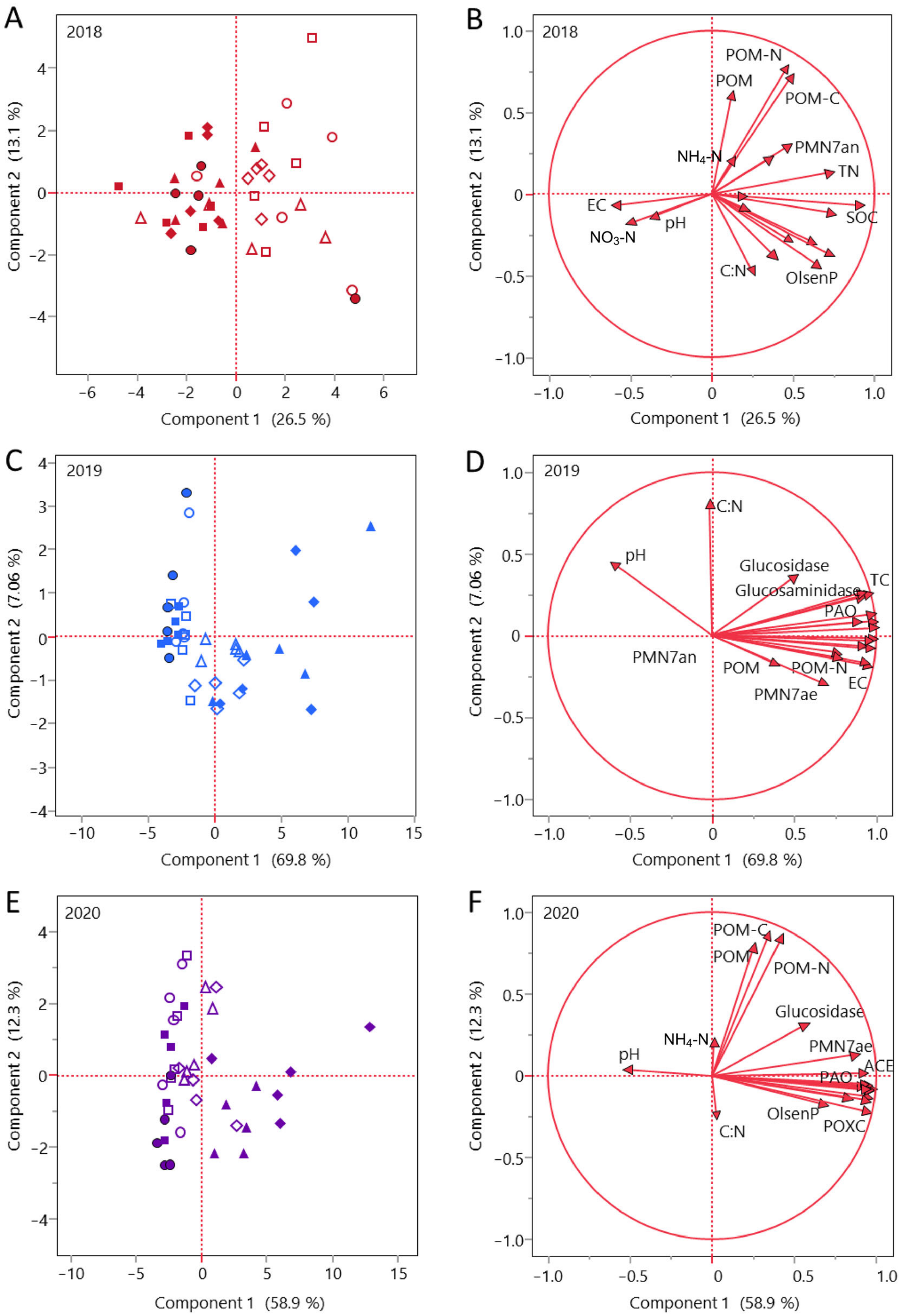

FIGURE 3 Principal component analysis of soil chemical and biological indicators showing scores for all observations and loadings as follows: (A) and (B), for 2018; (C) and (D), for 2019; and (E) and (F), for 2020. Open and closed icons indicate 0- to 15-cm and 15- to 30-cm soil depths, respectively. Circles = Ctrl, squares = Fert, triangles = Man, and diamonds = ManFert. ACE, autoclaved citrate extractable soil proteins; Ctrl, control; EC, electrical conductivity; Fert, fertilizer; Man, manure; ManFert, manure + fertilizer; PAO, potential ammonia oxidation; PMN, potentially mineralizable nitrogen; POM, particulate organic matter; POXC, permanganate oxidizable carbon; SOC, soil organic carbon; TC, total carbon; TN, total nitrogen.

years to soils with low initial SOC (<10 g C kg^{-1}). In the present study, the average SOC concentration across depths in 2018 was 7.6 g C kg^{-1}, but with manure application, SOC, total N, and available N and P increased across both years and depths by 86%, 51%, 225%, and 678%, respectively, compared to baseline values. While total C was not evaluated by Du et al. (2020), we found in the present study that it increased by 43% on average in manured soils, which is in line with increases of SOC and total N.

A direct comparison of our results to other short-term studies was quite challenging, as many semiarid cropping system studies only addressed the long-term application of cattle manure or legacy effects after long-term annual or biennial manure applications (Chang et al., 1990; Eghball, 2002; Hao & Chang, 2003; Obour et al., 2017). Yilmaz and Alagöz (2010) conducted a short-term greenhouse study with a semiarid clay soil (pH = 6.8) from Turkey that was amended with solid farmyard manure at application rates of 0–40 Mg ha^{-1} (dry wt.) and then incubated for 7 months. The manure significantly increased SOC, total N, and EC by as much as 57%, 110%, and 44%, respectively, at the 40 Mg ha^{-1} application rate. We applied substantially more manure (≥2.6-fold) in the present study, and SOC concentrations increased by 86% on average at 0–30 cm following application, compared to spring 2018 measurements. In a long-term context, on similar silt loam soils near the same location as in the present study, application of manure solids applied annually for 7 years at 52 Mg ha^{-1} (dry wt.) increased SOC by 140% at 0–30 cm over the 7 years (Dungan et al., 2022). Similarly, in a semiarid soil in Nebraska, application of beef cattle manure at 27 Mg ha^{-1} year^{-1} (wet wt.) for 71 years to conventionally tilled and irrigated corn, increased SOC by about 90% at 0–30 cm and also reduced compaction and increased water retention compared to a control without manure (Blanco-Canqui et al., 2015). In calcareous and non-calcareous semiarid soils, dairy manure did not improve physical properties over the short term (21 days) with a small-scale study reporting reduced soil structure and infiltration when non-composted and composted manure was applied at an approximate dry rate of 5 Mg ha^{-1} (Goldberg et al., 2020). While short-term studies identify immediate impacts on specific processes that occur in soils, only long-term data provide confirmation that these effects are having a significant influence on soils over time (Angers et al., 2010).

4.4 | Labile carbon and nitrogen fractions

POXC, also called active C, is an indicator of the SOC fraction that could be readily available for use by the soil microbial community. The labile fractions of SOC are important since they provide energy for the soil food web, thus greatly influence nutrient cycles and biologically related soil properties (Weil et al., 2003). Changes in the labile C fraction can be used as an early indication of soil degradation or improvement in response to management practices (Culman et al., 2012). In the present study, POXC was significantly greater in the manured soils at both depths and years following application. The POXC accounted for 2.3%–3.2% of the SOC, which is close to values reported by others, although not in irrigated semiarid cropping systems with manure (Culman et al., 2012; Margenot et al., 2017). In dryland cropping and grassland systems, POXC was found to be sensitive to SOC as induced by manure additions over short and long time scales (Miles & Brown, 2011; Mirsky et al., 2008). In the current study, POXC was strongly correlated with a number of soil health indicators, as has been found in other studies with indicators such as mineralizable C, soluble carbohydrates, microbial biomass C, and various enzyme activities (Culman et al., 2012; Dungan et al., 2022; Melero et al., 2009; Morrow et al., 2016; Weil et al., 2003). Compared to mineralizable C (flush of CO_2 released in a short-term aerobic incubation), Hurisso et al. (2016) suggested that POXC better reflected practices that promote organic matter stabilization or accumulation, thus it could be a useful indicator of long-term soil C sequestration.

Detecting short-term and medium-term changes in SOM can be challenging, unless labile C fractions are monitored since they are subject to greater turnover rates compared to more recalcitrant C fractions (Liang et al., 2012). Like POXC, soil POM is another labile C fraction that responds quickly to changes in management practices, thus it can be used as an early and sensitive indicator of SOM changes (Chatterjee et al., 2017). Based on numerous long-term studies, livestock manure is more effective than inorganic fertilizer in increasing POM (Banger et al., 2010; Liang et al., 2012; Mikha et al., 2017; Rudrappa et al., 2006). Further, research demonstrating animal manures significantly contributed to POM-C pools more than mineral fertilizer inputs indicated POM likely represents recent C inputs from crop and manure residues (Aoyama et al., 1999; Liang et al., 2012). As the POM decomposes, it breaks down into particulate sizes <53 μm, then it is stabilized by various chemical and physical mechanisms into mineral-associated organic matter (Six et al., 2002). According to Aoyama et al. (1999), manure-derived organic matter first enters the soil primarily as particulate material, and then during decomposition it is transformed within aggregates into mineral-associated organic matter that helps stabilize aggregates. In the present study, total POM, POM-C, and POM-N were unaffected by fertilizer or manure application at the 0- to 15-cm depth, but POM-C and POM-N fractions were increased over Ctrl and Fert at 15–30 cm, which can likely be attributed to deep tillage of the dairy manure. In contrast, Rudrappa et al. (2006) determined POM-C was greater at 0–15 cm with total content decreasing with increasing depth following three decades of farmyard manure (15 Mg ha^{-1} year^{-1}) plus inorganic fertilizer (NPK) disked to 15 cm. Similarly, Mikha et al. (2017) found that POM was about 17% greater at 0–15 cm than at 15–30 cm with two decades of cattle manure (27 Mg ha^{-1} $year^{-1}$) application under moldboard plowing (25-cm depth) in a furrow-irrigated semiarid soil. They speculated that moldboard plowing promoted plant residue and manure decomposition, thus resulting in higher POM-C in the surface layer.

Soil POM-N and ACE both represent labile N fractions of SOM that microbes can mineralize and make available for plants. In the former case, the N associated with POM is mainly derived from plant residues, while in the latter case, the N associated with proteins represents a wide range of organic sources (Hurisso et al., 2018; Moebius-Clune, 2016; Six et al., 2001). Understanding the N supply in SOM is important for assessing the ability of soil to support microbial populations and their many important ecosystem functions, such as nutrient cycling, residue decomposition, pathogen suppression, and many others (Lehman et al., 2015). In addition, soil protein is a potentially informative indicator of soil health since it has been positively correlated with soil aggregation (Fine et al., 2017; Rillig et al., 2007; Wright & Upadhyaya, 1998). In the present study, ACE was more responsive to manure treatment than POM-N contents, with significantly greater amounts of ACE in the Man and ManFert plots at both depths and years following application. In contrast, POM-N was only found to be significantly greater in the manure-treated soils at 15–30 cm in 2019. In a California study, a strong correlation between ACE and POM-N (r = 0.93), POXC (r = 0.96), and total N (r = 0.93) and a weaker correlation between ACE and N mineralization (r = 0.46) was found in soils from 57 fields with no cover crop, manure, or compost for at least 3 years (Geisseler et al., 2019). These relationships were apparent in the PCA analysis for 2019, but by 2020, total and fractions of POM were weakly related to ACE.

4.5 | Soil nitrogen isotope composition

We investigated the N isotope composition ($d^{15}N$) of soil to determine the amount of manure N remaining in the soil following its application. Tracking N losses is difficult because N is highly mobile in the environment, with the largest losses occurring through gaseous emissions (NH_3, N_2O, and N_2) and leaching (NO_3^-). However, measurement of stable isotopes at natural abundance levels is a potentially useful tool to determine the fate of manure applied to fields. In a field experiment in Denmark, $d^{15}N$ values were greater in soil treated with long-term livestock manure from 1923 to 2000 (cattle slurry starting in 1973) compared to unfertilized and inorganic fertilizer treatments (Bol et al., 2005). Using field-scale measurements of $d^{15}N$ at natural abundance, Snider et al. (2017) reported that 56% of NH_4-N in spring-applied liquid dairy manure was presumably lost by NH_3 volatilization within 3 days, and then 95% of remaining NH_4-N was converted within 3 weeks to NO_3^- and N_2O by nitrification and denitrification. In our study, the abundance of $d^{15}N$ in total N revealed that approximately 117 ± 40% and 68 ± 23% of the solid dairy manure N was present 5 and 16 months after application, respectively. The increase in ^{15}N could be due to the preferential loss of ^{14}N during NH_3 volatilization, which enriches the ^{15}N content of residual N in the soil (Bateman & Kelly, 2007). One would not expect substantial losses of manure N within several months of application, especially when the manure was applied in the late fall and cold weather conditions during winter inhibit mineralization, as well as it being incorporated deep into the soil after application to mitigate ammonia volatilization. In the case of plots treated with inorganic fertilizer, $d^{15}N$ abundances indicate that very little of the N (<1.0%) was present in the soil 1 year after application. A single application of inorganic fertilizer or cattle slurry is known to have little residual N effect on subsequent crops (Fuchs et al., 2023; Schröder et al., 2005; Smith & Chalk, 2018), while livestock manure solids can have a substantial residual N effect in long-term studies, especially with repeated manure applications (Tarkalson et al., 2018; Webb et al., 2013).

4.6 | Nitrogen transformations

Despite the fact that long-term applications of manure are generally required to improve soil physical properties (Rayne & Aula, 2020), manure is an excellent soil conditioner and N fertilizer, thus it is a fundamental component of integrated livestock-crop production systems. Regardless of climate, total N concentrations generally increase with increasing application rate when manure is applied annually (Angers et al., 2010; Cavalcante et al., 2020; Chang et al., 1991; Leytem et al., 2019; Ozlu et al., 2022; Schlegel et al., 2015), with one study showing no significant effect on total N when cattle manure solids were applied biennially for 9 years (Ndayegamiye & Cote, 1989). As the organic residues are decomposed in soil, there is a release of mineral N in the form of NH_4^+, which is plant available. We used both anaerobic and aerobic assays to estimate microbial conversion of organic N to NH_4^+ or $NH_4^+ + NO_3^-$, respectively. The 7-day anaerobic and 28-day aerobic methods consistently produced significantly higher N mineralization in manure-treated soils, which is in agreement with results from short- and long-term field studies using cattle manure (Eghball, 2000; Ndayegamiye & Cote, 1989; Nyiraneza et al., 2009; Sharifi et al., 2011). Using archived semiarid soils from Lethbridge, Canada, that received beef cattle manure annually for 25 years at rates ranging from 0 to 180 Mg ha^{-1} (wet wt.), a direct relationship was found between the amount of mineralized N and manure application rate (Whalen et al., 2001). Compared to inorganic fertilizer, Dungan et al. (2022) found that biennial and annual applications of dairy manure to a semiarid soil for 7 years increased PMN by up to 149%. In a meta-analysis conducted using 43 peer-reviewed studies, Mahal et al. (2018) reported that soils receiving manure had higher PMN than those receiving inorganic N fertilizer or compost.

The short-term nitrification assay or PAO was used to assess the nitrification potential in the soil at the time of sampling. Ammonia oxidation is carried out by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), thus the PAO assay can also be used to detect any negative effects agronomic treatments may have on these microorganisms and the critical role they play in soil N cycling (Abubaker et al., 2015; Nyberg et al., 2004; Risberg et al., 2017). While AOA are generally more abundant than AOB (Leininger et al., 2006; O'Sullivan et al., 2013), Tao et al. (2017) reported that nitrification rates were positively correlated with the abundance of AOB when a calcareous soil in an arid region of China was treated with cattle manure annually over a 4-year period. In a long-term field experiment in Sweden, cattle manure applied biennially for 46 years produced the highest PAO rates compared with inorganic fertilizer and sewage sludge (Enwall et al., 2007). In the present study, PAO was significantly greater by up to twofold in the manured soils, which supports the fact that we found the greatest NO_3^- concentrations in those treatments as well. Given the large amount of total N applied to the manured plots, one would expect higher concentrations of NH_4^+ after mineralization, thus these treatments should have higher PAO compared to Ctrl and Fert.

4.7 | Enzyme activities

Enzyme activity assays, such as β-GS and β-GSA, can provide early detection of changes in soil health because they respond quickly to management changes and environmental factors much sooner than other soil quality parameters (Bandick & Dick, 1999; Stott, 2019). β-GS (cellulose degradation) and β-GSA (chitin degradation) are generally used to represent C and C/N cycling, respectively, and can be used as a predictor of organic matter decomposition. The addition of manure to soil, as well as other organic amendments, strongly influences the soil's potential for enzyme-mediated substrate catalysis that controls nutrient availability and SOM quality and quantity (Acosta-Martinez et al., 2011). As expected, field studies have reported short-term positive effects of manure incorporation on soil enzyme activities when compared to inorganic fertilizer (Lazcano et al., 2013; Marcote et al., 2001; Mdlambuzi et al., 2021; Sandhu et al., 2019). Similarly, in the present study, β-GS activities were significantly elevated in the ManFert treatment, while β-GSA activities were elevated in both Man and ManFert treatments. This response was largely found at 15–30 cm, with smaller effects at 0–15 cm.

Across both years following application, β-GSA was more sensitive to the influence of manure, as the activities were 2-fold greater than in Fert as compared to 1.3-fold greater for β-GS. In another field study in southern Idaho, β-GS was found to be slightly more responsive than β-GSA to the long-term application of dairy manure, consisting of several annual manure applications with incorporation to 15 cm (Dungan et al., 2022). Regardless of enzyme sensitivities, both β-GS and β-GSA can be effectively utilized to evaluate the influence of manure treatment on soil C and N dynamics, even when the last manure application was decades earlier (Lupwayi et al., 2019). In contrast, Lupwayi et al. (2018) reported that β-GS activity was greater at 0–15 cm than deeper in the soil profile, which can be attributed to the fact that cattle manure was incorporated into the topsoil. In most studies where manure is incorporated into soil at different application rates, the enzyme activities are found to be positively correlated with increasing rate (Acosta-Martinez et al., 2011; Dungan et al., 2022; Khorsandi & Nourbakhsh, 2007), although a quadratic response to manure application rate was described in a long-term study by Nyiraneza et al. (2018).

5 | CONCLUSIONS

As visualized in the PCA biplots, our research demonstrated that incorporating a high rate of dairy manure (with or without fertilizer) into a semiarid calcareous soil significantly influenced most chemical and biological indicators in the first 2 years after manure application. These effects were observed in both the topsoil and subsoil, but the responses were greatest in the subsoil, which was attributed to moldboard plowing of manure. The indicators responded positively to manure, except in the case of pH, which decreased slightly in the subsoil in the first year after application. Deep incorporation of the manure (i.e., inversion tillage via moldboard plow) might provide numerous soil health benefits, with the main benefit being conservation of organic C, but increased enzymatic activities and high mineral N concentrations in the subsoil suggested that manure decomposition was occurring at a rapid rate. Manure application is often the primary way to increase SOC in low organic matter semiarid soils, though few studies have also investigated the effects of incorporation with inversion tillage. Although manure improved most soil indicators in this short-term study, long-term sampling will be required to determine the longevity of the SOC, as well as the continued release of mineral N and benefits to crop quality and yields. Since a single heavy application of dairy manure did not adversely impact soil indicators, it can be tentatively recommended as a management strategy. The major benefit of using dairy manure in cropping systems is that it gradually releases essential plant nutrients compared with synthetic fertilizers. While the manure did provide N at concentrations necessary for optimal crop growth, the associated salts may have caused a reduction in corn yield. Regardless, our results support use of chemical and biological indicators to evaluate short-term changes in soil properties under manure fertilization. However, given that the indicators responded similarly under the conditions of our study, the information is likely redundant. Therefore, we suggest that only a small group of representative indicators be utilized to avoid this redundancy and minimize additional analytical costs.

AUTHOR CONTRIBUTIONS

R. S. Dungan: Conceptualization; data curation; formal analysis; investigation; resources; visualization; writing—original draft; writing—review and editing. **V. Acosta-Martinez**: Conceptualization; resources. **R. M. Lehman**: Conceptualization; formal analysis; resources. **D. K. Manter**: Conceptualization; writing—review and editing. **M. M. Mikha**: Formal analysis; resources; writing—review and editing. **C. L. Reardon**: Formal analysis; resources. **D. D. Tarkalson**: Formal analysis; investigation; resources; writing—review and editing. **K. S. Veum**: Formal analysis; resources. **S. L. Weyers**: Formal analysis; resources; visualization; writing—review and editing. **P. M. White Jr**.: Conceptualization; formal analysis; resources.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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