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Temporal variability of soil health indicators under annual and perennial continuous living cover: Effects of annual weather and management

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Abstract

Soil health indicators (SHIs) are used to detect changes in soil conditions in response to management. However, management effects must be disentangled from other factors influencing SHI variability. Specifically, how annual weather affects SHI remains poorly understood. To address this gap, we analyzed soil annually for 5 years at 64 sampling points in two silt loam fields, in Minnesota. One field had annual crops (maize, *Zea mays* L.; winter camelina, *Camelina sativa* L. Crantz.; soybean, *Glycine max* L. Merr.; wheat, *Triticum aestivum* L.; and winter barley, *Hordeum vulgare* L.) while the other had perennial crops (alfalfa, *Medicago sativa* L. and Kernza, *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey). We measured mineralizable carbon (MinC), extracellular enzymes, microbial biomass, soil organic carbon, mineral-associated organic matter, and particulate organic matter (POM) at 0- to 15-cm and 15- to 30-cm depth. We present two key findings. First, growing season weather was correlated with annual changes in several indicators. The most striking relationships were between growing season mean air temperature (MAT) and 1-, 4-, and 12-day MinC ($p < 0.001$). MAT was not correlated with 21-day MinC. MAT–MinC correlations were depth-dependent: positive at 0–15 cm ($r = 0.65$–0.68) and negative at 15–30 cm ($r = -0.39$ to -0.60). Second, soil health improved in the perennial field. Most illustrative of this finding were gains in arbuscular mycorrhizal fungi ($p = 0.013$), subsurface MinC, and POM ($p < 0.001$). In summary, we recommend that soil health assessment account for annual weather, and suggest that perennial crops lead to improved soil health.

Abbreviations: AcidP, acid phosphatase; AMF, arbuscular mycorrhizal fungi; ANOVA, analysis of variance; BetaG, β-glucosidase; CEC, cation exchange capacity; CLC, continuous living cover; CV, coefficient of variation; IQR, interquartile range; MAOM, mineral-associated organic matter; MinC, mineralizable carbon; NAG, N-acetylglucosaminidase; PLFA, phospholipid fatty acid; POM, particulate organic matter; SOC, soil organic carbon.

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Plain Language Summary

Farmers and researchers measure soil health using indicators that are sensitive to management changes, but we need to better understand how much these indicators vary from year to year. We collected and analyzed soil for 5 years from the same 64 sample points in two fields under contrasting cropping systems: (1) diversified annual crops and (2) perennial crops. We found that the growing season weather, especially mean air temperature, was often associated with annual measurements of soil health. This association makes it hard to tell if changes in the soil health indicators are due to farming practices or just the weather conditions over the growing season. Soil health improved over 5 years in the perennial crop field, especially in beneficial fungi that help plants get nutrients. However, weather effects on soil tests imply that we need better methods to separate weather-driven changes from true soil health improvements due to management.

1 | INTRODUCTION

Evaluating the effect of agricultural management on soil health is costly and laborious (Nunes et al., 2021). Due to this resource intensity, it is common for practitioners to assess long-term soil health changes by collecting and analyzing soil every 3–5 years (Liebig et al., 2021). However, results from this approach can be confounded by other factors influencing temporal variability of soil health indicators (Crookston et al., 2021; Wuest & Durfee, 2024). A major challenge in soil health assessment is controlling for the degree to which soil health indicators vary through time, independent of management effects. It is critical to account for this indicator-specific variability to assist in the development of rigorous soil health assessments.

To address this challenge, researchers need a clearer understanding of soil health indicators' inherent variability over a range of timescales (Crookston et al., 2021). Previous studies have examined temperature and moisture effects on soil properties at seasonal (short-term; Gonzalez-Maldonado et al., 2025; McDaniel & Grandy, 2016; Piotrowska-Długosz et al., 2019) and climatic (long-term; Jenny, 1994) timescales, but the effects of interannual weather variability (medium-term) on soil properties remain poorly understood. This gap is particularly critical since annual sampling represents the highest practical frequency for most farmers and researchers tracking soil health indicators, yet we lack understanding of how annual weather variability influences these yearly measurements. Quantifying this temporal variability requires appropriate statistical methods, but existing approaches have limitations.

Coefficients of variation (CVs) are often used by researchers to quantify spatial and temporal variability across soil properties (Crookston et al., 2021; Hurisso et al., 2018; Shukla et al., 2004). It has been proposed that soil properties can be categorized as exhibiting low, medium, or high variability based on their CVs (Wilding & Drees, 1983). Additionally, CV can be particularly useful in calculating the appropriate soil sampling density given a desired confidence interval and allowable error (Lawrence et al., 2020; Wilding & Drees, 1983). However, the use of CV for these cases is dependent on an assumed relationship between sample means and sample variances (i.e., Taylor's Power Law; Taylor, 1961) and this relationship has not been systematically evaluated for many soil properties. A more thorough understanding of the mean-variance relationship could help clarify the appropriate uses of CV for evaluating variability in soil sampling schemes. Beyond methodological considerations for quantifying variability, it is essential to understand which soil properties should be monitored and what criteria they should meet.

An enhanced understanding of temporal variability is especially useful in the iterative process to evaluate the best available soil health indicators (Stott, 2019). Ideal soil health indicators should meet the criteria of (1) integrating multiple soil properties and processes, (2) be easy and accessible, (3) correlate with ecosystem processes, and (4) be sensitive to management but not be overly sensitive to short-term weather patterns (Doran & Parkin, 1997). The basis for this weather-sensitivity criterion is the belief that sustained changes in soil health are slow to reach detectable levels, so short-term fluctuations that are not management related can confound analysis interpretation (Doran & Zeiss, 2000; Lehmann et al., 2020; Liptzin et al., 2022).

To meet this sensitivity criterion, researchers have focused on identifying dynamic soil properties that can be harbingers of long-term management–induced change in soil health (Bagnall et al., 2023). Dynamic soil properties are primarily focused on soil biological processes, and include analyses of extracellular enzymes (Dick, 1994), mineralizable carbon

(MinC) (Franzluebbers et al., 2000), and microbial biomass (Sparling, 1997). These properties have been found to respond in the short term to the types of management that lead to long-term soil health gains. For example, long-term studies have found that perennial crops increase soil organic carbon (SOC; Ledo et al., 2020; Siddique et al., 2023). Perennial crops can also improve dynamic properties, including microbial biomass, β-glucosidase (betaG) activity, soil respiration, active carbon, and soil protein (Nunes et al., 2020). Moreover, ideal dynamic properties are highly correlated with desired soil health outcomes (Barthès & Roose, 2002; Culman et al., 2012; Hurisso et al., 2016). The idea that dynamic properties give a glimpse of how a change in management will affect the emergent property (soil health) is central to the current approach to soil health assessment (Culman, Snapp, Green, et al., 2013; Liptzin et al., 2022; Morrow et al., 2016; Stewart et al., 2018; Wade et al., 2022).

However, the practical application of this approach requires parsing management-driven changes in soil from exogenous abiotic factors (i.e., annual weather patterns) that influence soil temporal variability. While there has been considerable research on the sensitivity of soil biology to temperature and precipitation, the findings are not often framed as affecting the use of biological analyses as soil health indicators (Curiel Yuste et al., 2007; Curtin et al., 2012; Lauber et al., 2013; Paul & Frey, 2023). Similarly, it is recognized that climate (i.e., long-term trends in temperature and precipitation) leads to differences in measured soil health indicators (Karlen et al., 2019; Nunes et al., 2021; Rinot et al., 2019). However, investigation into how variation in annual weather patterns affects soil analyses is limited. Accounting for the impact of growing season weather conditions on soil health indicators can improve interpretability of soil health assessment.

For assessment purposes, annual sampling is useful to capture integrative responses to management, but evidence from multiyear studies that collected annual soil sampling is limited (Liebig et al., 2021). Quantifying how annual weather influences soil health indicators is essential for distinguishing between weather-driven fluctuations from management induced effects in soil health assessments. Addressing this challenge can be approached by examining how distinct management systems affect soil under the same weather conditions. There is limited evidence comparing how different management approaches affect soil health indicators' temporal variability at the field-scale. This comparative, observational approach is particularly useful when evaluating emerging management strategies that aim to fundamentally alter soil–plant interactions and temporal patterns of biological activity relative to conventional summer annual cropping systems (Blair et al., 2024).

One such strategy is continuous living cover (CLC), which aims to maintain living roots in the soil through rotations of consecutive annual and/or perennial crops (Reilly et al., 2023). This approach focuses on reducing the fall-to-spring fallow period common in conventional systems with only summer annual crops. Both perennial and annual CLC systems improve soil conditions relative to conventional summer annual crop rotations, which leave the soil uncovered from fall to spring, though through different mechanisms and to different degrees. Perennials contribute greater, deeper, and more persistent root biomass (DuPont et al., 2014; Rakkar et al., 2023), while annual CLC systems maintain fall-to-spring soil cover and increase temporal crop diversity (Congreves et al., 2015; Kaspar & Singer, 2011; Koudahe et al., 2022). However, comprehensive field-scale comparisons of how these contrasting approaches affect soil biological and chemical properties over multiple years remain limited.

To fill these knowledge gaps, we conducted a study in two neighboring fields under annual and perennial CLC management. We measured temporal variability of soil biological and chemical properties through 5 years of annual sampling. We sought to address three questions: (1) How do annual weather patterns influence soil health indicators? (2) How do soil health indicators respond year-to-year and over a 5-year period to annual and perennial CLC management? (3) what are the practical implications of our findings for soil health assessment? To that end, we first used descriptive statistics and coefficients of variation (CV) to assess temporal variability of measured soil properties. We also examined CV in this study to evaluate whether assumptions underlying its use in sampling design are valid across the suite of measured soil properties. Then, we further explored temporal variability through annual change calculations and analysis of variance (ANOVA). Finally, we investigated how growing season weather conditions correlate with annual changes in

Core Ideas

- We assessed annual changes in soil health indicators over 5 years in two fields under annual or perennial crop rotations.
- Growing season mean air temperature and total precipitation were correlated with annual changes in select soil health indicators.
- The relationship between growing season mean air temperature and mineralizable carbon is dependent on soil depth and incubation duration.
- Soil health indicators increased in the perennial cropping field, particularly arbuscular mycorrhizal fungi and subsurface mineralizable carbon.
- Soil health assessment should account for annual weather to avoid conflating effects of weather and management on changes in measured indicators.

TABLE 1 Soil sampling dates, accumulated growing degree days up to sampling date, and both annual and growing season-only mean air temperature and precipitation values for each year.

Year	Soil sampling date	Growing degree days at soil sampling	Mean annual/growing season air temperature (˚C)	Total annual/growing season precipitation (mm)
2020	April 10	0	7.59/14.51	693/572
2021	April 21	31.7	8.21/14.92	606/474
2022	April 27	4.72	6.33/13.93	647/512
2023	May 5	59.2	8.35/14.77	803/598
2024	May 14	98.6	–	–

Note: Thirty-year annual Normals are 6.8˚C and 867 mm. We define the "growing season" as April 1 to November 30, which has 30-year Normal mean air temperature and precipitation of 13.6˚C and 743 mm. Weather data for 2024 are not reported because this study ended after spring 2024 soil sampling. Growing degree days were calculated starting on January 1 using mean daily temperatures in Celsius and a base temperature of 10˚C.
Source: Minnesota Department of Natural Resources, weather station ID: 217107.

soil health indicators, providing critical insights into the effect of abiotic factors on these analyses. Findings from this study will support refinement of field-scale soil health assessment, as well as offer a rigorous evaluation of how CLC systems affect soil health.

2 | MATERIALS AND METHODS

2.1 | Field site conditions

This experiment was conducted in two fields at the University of Minnesota Rosemount Research and Outreach Center in Rosemount, MN from 2020 to 2024. Thirty-year Normal mean annual air temperature and total precipitation in Rosemount are 6.8˚C and 867 mm, respectively. Weather summary data during the study period are provided in Table 1, and the deviations from the 30-year Normals are provided in Figure 1. The soils in both the perennial rotation field (16 ha; 44˚42′21.6″ N, 93˚04′02.6″ W) and annual rotation field (11 ha; 44˚42′57.3″ N, 93˚04′32.0″ W) are characterized as Waukegan silt loam (fine-silty, mixed, superactive, mesic Typic Hapludolls), a Mollisol formed in loess over glacial till. Both fields have limited topographic variation with the difference between highest and lowest elevation being 2.23 and 1.57 m, in the perennial and annual field, respectively. Prior to the start of this study, the perennial field was in alfalfa (*Medicago sativa* L.) from 2014 to 2016, followed by maize–soybean–maize (*Zea mays* L.; *Glycine max* L.) from 2017 to 2019. The annual field was in soybean in 2014, and in mixed grass–alfalfa from 2015 to 2019.

2.2 | Agronomic management

The perennial field rotation consisted of alfalfa (*M. sativa* L.) followed by Kernza (intermediate wheatgrass; *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey). Alfalfa was planted on April 22, 2020, and harvested twice in the establishment year on July 8 and August 13, 2020, yielding a mean of 3.15 ± 0.72 Mg ha^{-1} dry matter. The field received fertilizer applications on April 21, 2020 (N: 18, S: 20, K: 117 kg ha^{-1}) and September 1, 2020 (Ca: 59, S: 34, K: 118, B: 2 kg ha^{-1}). In 2021, alfalfa was harvested four times: first cut on June 2, second cut on July 8, third cut on August 19, and fourth cut on October 19, producing a total mean yield of 10.13 ± 2.57 Mg ha^{-1} dry matter with no additional fertilizer applications. Three alfalfa cuttings were taken in 2022 on June 1, July 11, and August 10, yielding 7.84 ± 1.62 Mg ha^{-1} dry matter, again with no additional fertilizer inputs. Kernza was planted on August 26, 2022, following tillage and chemical termination of alfalfa. In 2023, Kernza was harvested twice: a summer grain and forage harvest on August 2 and a fall forage-only harvest on November 7, producing 11.00 ± 2.54 Mg ha^{-1} dry matter without additional fertilizer application. In 2024, Kernza received fertilizer on May 16 (N: 96, S: 20 kg ha^{-1}) and was harvested on August 7 (grain and forage) and October 25 (forage-only), yielding 17.15 ± 3.15 Mg ha^{-1} dry matter.

The annual field featured a five-crop rotation including silage maize (*Z. mays* L.), winter camelina (*Camelina sativa* L. Crantz.), soybean (*G. max* L. Merr.), spring wheat (*Triticum aestivum* L.), and winter barley (*Hordeum vulgare* L.). Silage maize was planted on April 30, 2020, received ammonium sulfate fertilizer on April 23, 2020 (rate not recorded), and was harvested on August 25, 2020, producing 24.84 ± 2.16 Mg ha^{-1} dry matter. Winter camelina was planted on September 4, 2020, following the maize harvest. The field received fertilizer on April 6, 2021 (N: 52 kg ha^{-1}), and winter camelina was harvested on June 29, 2021, yielding 5.96 ± 1.83 Mg ha^{-1} dry matter. Soybean was then planted on July 13, 2021, after a July 2021 tillage event, and harvested on November 4, 2021, producing 2.69 ± 0.55 Mg ha^{-1} dry matter without additional fertilizer inputs. The field was fertilized on April 19, 2022 (N: 41 kg ha^{-1}) and tilled on April 27, 2022, and spring wheat was planted the following day on April 28, 2022. Spring wheat was harvested on August 9,

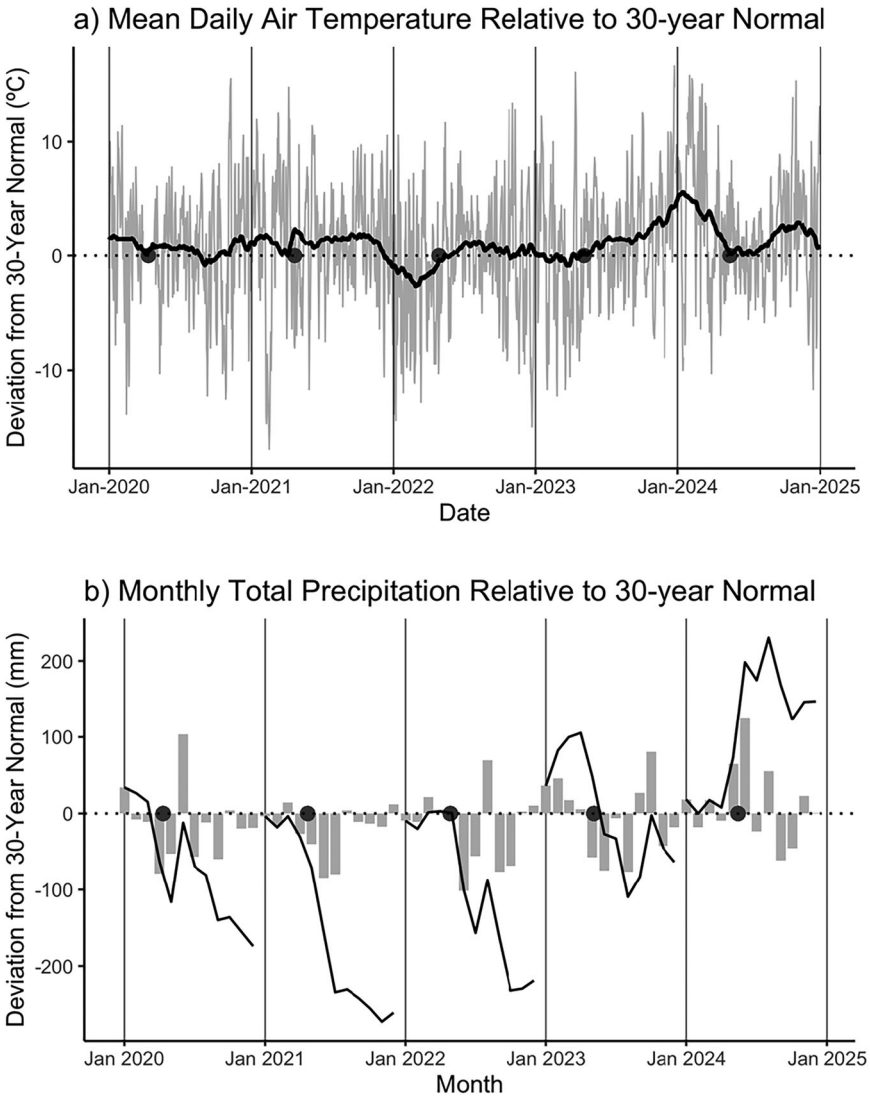


FIGURE 1 Deviations in (a) daily mean air temperature and (b) monthly total precipitation relative to 30-year Normals during study period in Rosemount, MN (weather station ID: 217107). Normals are based on the 30-year period from 1991 to 2020. Solid vertical lines correspond to year-breaks and dots on the zero-line represent soil sampling dates. (a) The background gray line shows the deviation in the daily mean air temperature relative to the 30-year Normal for each day. The black line shows a 120-day rolling average to visualize the trend. (b) Gray columns represent the deviation in the monthly total precipitation relative to Normal. Lines show the annual cumulative precipitation deviation from Normal. *Source*: Minnesota Department of Natural Resources; U.S. National Centers for Environmental Information.

2022, yielding 6.13 ± 2.49 Mg ha^{-1} dry matter. Soybean was planted on May 16, 2023, without fertilizer application, and harvested on September 19, 2023, producing 5.99 ± 1.06 Mg ha^{-1} dry matter. Finally, winter barley was planted on October 2, 2023, received fertilizer on May 16, 2024 (N: 78 kg ha^{-1}), and was harvested on July 1, 2024, yielding 6.17 ± 2.00 Mg ha^{-1} dry matter. All yields are reported as mean dry matter from samples collected at 32 points within the field at each harvest. Fertilizer rates are expressed as nutrient equivalents in kg ha^{-1}.

The three major differences in management between the two fields were (1) crop species, (2) crop residence time, and (3) tillage frequency. The perennial field had 13 harvests of two unique crop species, whereas the annual field had six harvests featuring five unique crop species. Both fields were tilled in fall 2019 to aid in establishment of the experiment. The perennial field was tilled once during the study period in August 2022 during the crop transition from alfalfa to Kernza. The annual field was tilled twice: before winter camelina planting in July 2021 and again before spring wheat planting in April 2022. A winter camelina crop planted in fall 2021 failed to establish due to lack of rainfall and is not reported. Both fields were managed using conventional practices typical for the region, including herbicide for crop termination and weed control (Table S1).

2.3 | Soil sampling methods

We established a systematic sampling scheme to capture spatial variability of soil properties (see Table 2 for a list of properties) within each field while maintaining practical sampling efficiency (Wilding & Drees, 1983). In each field, 32 sampling points were distributed in a pattern of systematically increasing linear distance from a central point, with spacing designed to capture both fine-scale and field-scale variation. In the perennial field (16 ha), distances between sampling points ranged from 18.6 to 509 m, with a mean distance of

TABLE 2 List of analyzed soil health indicators, including abbreviation and units.

Soil health indicator	Abbreviation	Units
Gravimetric soil water content	Grav Water	%
Bulk density	BD	g soil cm^{-3}
pH (1:1 water)	pH	–
Electrical conductivity	EC	dS m^{-1}
Cation exchange capacity	CEC	$cmol_c$ kg^{-1}
Exchangeable magnesium	Mg	ppm
Exchangeable calcium	Ca	ppm
Exchangeable potassium	K	ppm
Phosphorus (Bray)	P	ppm
Nitrate-N	Nitrate	lb $acre^{-1}$
Soil organic carbon	SOC	g C kg $soil^{-1}$
Soil organic carbon stock	SOC Stock	Mg C ha^{-1}
Mineral-associated organic matter carbon	MAOM	g C kg $soil^{-1}$
Particulate organic matter carbon	POM	g C kg $soil^{-1}$
Arbuscular mycorrhizal fungi biomass (PLFA)	AMF	nmol fatty acid g $soil^{-1}$
Fungal biomass (PLFA)	Fungal	nmol fatty acid g $soil^{-1}$
Bacterial biomass (PLFA)	Bacterial	nmol fatty acid g $soil^{-1}$
Total microbial biomass (PLFA)	Microbial	nmol fatty acid g $soil^{-1}$
β-glucosidase activity	BetaG	nmol MUB h^{-1} g $soil^{-1}$
Acid phosphatase activity	AcidP	nmol MUB h^{-1} g $soil^{-1}$
N-acetylglucosaminidase activity	NAG	nmol MUB h^{-1} g $soil^{-1}$
Mineralizable carbon following *-Day incubation	MinC *-Day	mg C kg $soil^{-1}$ day^{-1}

187 m between points. In the annual field (11 ha), these distances ranged from 18 to 435 m, with a mean of 160 m. This design enabled us to sample approximately 2 points per hectare while ensuring adequate representation of within-field variability. The same 32 points were resampled each year, allowing us to track temporal changes while accounting for spatial dependencies through our statistical approach.

Soil samples were collected annually in spring (Table 1) at all 32 points in each field to a depth of 90 cm using a truck outfitted with a 5.08 cm diameter hydraulic soil probe (Giddings Machine Co.). Sampling points were identified using GPS coordinates and cores were collected within 3 m of the GPS coordinates each year. Cores were separated into four depths for analysis: 0–15, 15–30, 30–60, and 60–90 cm. The present study reports findings from analysis of soil in the top two depth increments. Two cores were collected annually at each point. After each core was separated into depth increments, one was left intact to calculate bulk density and the other was subsampled for soil microbial analysis. For the in tact core, each depth increment was transferred to a separate paper bag in the field. In the lab, the soil was then homogenized, sieved to 2 mm, picked free of visible roots, and dried at 35°C until constant mass. For the subsampled core, approximately 20 g of soil was transferred to two separate plastic bags and kept in a cooler until returning to the lab where they were placed in a −20°C freezer. One 20 g subsample was then freeze dried for phospholipid fatty acid (PLFA) analysis and subsequently stored at −20°C. The other 20 g subsample was transferred to a −80°C freezer until analysis of extracellular enzyme activity analysis.

2.4 | Soil analyses

2.4.1 | Soil physical and chemical properties

Bulk density was estimated using soil dry weight and the volume of the probe. Gravimetric soil moisture was either calculated based on wet and dry weights of the intact core (2020−2022) or by drying a 10 g subsample in a 60°C oven until constant mass (2023−2024). Soil chemical analysis was conducted at a service lab (AGVISE Laboratories) using the following methods: exchangeable Mg, Ca, K (Fassel & Kniseley, 1974; Page et al., 1982); pH (1:1 water; Brown, 1998); nitrate-N (Brown, 1998; Willis & Gentry, 1987); phosphorus (Bray; Brown, 1998); electrical conductivity (Brown, 1998; Burt, 2004); and cation exchange capacity (CEC) (Burt, 2004). Dried soil from the intact core was also used for soil texture and soil carbon analysis. Soil texture analysis was completed on soil collected in 2020 by the hydrometer

method (Bouyoucos, 1936). Total SOC was analyzed through dry combustion using a Soli TOC cube (Elementar), which uses ramped thermal analysis to partition organic and inorganic carbon pools. SOC stock was calculated using the SOC concentration, bulk density, and soil depth increment height (Tiessen et al., 1982). While equivalent soil mass calculations are more accurate across multiple depths, we used the simpler fixed depth bulk density method. This approach quantifies changes in soil volume based on management, while maintaining a depth-specific calculation in line with the other data included in this study (Lee et al., 2009).

2.4.2 | Particulate and mineral-associated organic matter

Particulate organic matter (POM) and mineral-associated organic matter (MAOM) fractionation followed methods adapted from Cotrufo et al. (2019) using soil from the intact core described above. Approximately 5.5 g soil was placed in a 50-mL centrifuge tube and oven dried overnight at 55°C. Once dried, samples were weighed and approximately ten 4-mm glass beads were added. Soil was suspended in 30 mL 0.5% sodium hexametaphosphate solution and placed on a reciprocal shaker at 175 rpm for 18 h. The soil solution was then poured over a 53 μm sieve and rinsed thoroughly with deionized water. Material captured on top of the sieve was considered POM, and what passed through the sieve was considered MAOM. Fractionated soil solutions were collected in aluminum pans and dried for 2–3 days in a 55°C oven. Once dried, samples were weighed and confirmed to have met recovery standards of 95%–105% of the pre-fractionation oven-dry soil mass. POM and MAOM material were scraped and ground using a pestle and transferred to 1.5 mL microtubes. To ensure homogeneity, samples were further ground using a Geno/Grinder 2010 (Cole-Palmer). Organic carbon concentration of POM and MAOM was analyzed through dry combustion using a Soli TOC cube (Elementar). Data are reported as POM-C/MAOM-C (g C kg soil^{-1}), which is calculated by multiplying the proportional mass of the POM/MAOM relative to the pre-fractionation oven-dry soil mass by the organic carbon concentration of the sample.

2.4.3 | Soil extracellular enzyme activity

Activity of betaG, acid phosphatase (acidP), and N-acetylglucosaminidase (NAG) were analyzed using methods described in German et al. (2011) and Johnsen and Jacobsen (2008). Soil samples were stored at −80°C until analysis. Approximately 0.5 g field moist soil was suspended in 50 mL of 50 mM Tris buffer that had been adjusted to pH 5.5 to match the mean pH of the soil samples. After sonicating for 5 min, the soil slurry was then pipetted into a 96-well plate to which 1500 μM/L solutions of 4-methylumbelliferyl β-D-glucopyranoside, 4-methylumbelliferyl phosphate, and 4-methylumbelliferyl N-acetyl-β-D-glucosaminide had been added in separate plate assay columns. Additional plate columns contained 4-methylumbelliferone at concentrations of 2.5, 1.25, 0.63, and 0.16 μM L^{-1} for standard curve calculation, as well as substrate blanks, homogenate blanks, and plate blanks. After adding the soil slurry, plates were incubated at room temperature for 1 h. One minute prior to plate reading, 10 μL 1 M NaOH was added to each well to optimize fluorescence. Plates were read with a BioTek Synergy HT microplate reader (BioTek Instruments, Inc.) at 360-nm excitation and 460-nm emission filters. Enzyme activity is reported as nmol MUB h^{-1} g soil^{-1}.

2.4.4 | Mineralizable carbon

MinC was measured after 1, 4, 12, and 21 days of incubation using methods adapted from Sherrod et al. (2012). Approximately 10 g of air-dried soil was added to a 16 oz jar. Deionized water was added to the sample to reach 50% water holding capacity. The jar was then sealed and incubated at 25°C until sampling. At each sampling point, a sample of the head space was collected by inserting a syringe into the jar through a rubber septum. The sample was analyzed using a LI-850 Gas Analyzer (LI-COR Biosciences). After sampling, the jar was temporarily opened to allow for the reentry of atmospheric air before being sealed again and placed in the incubator until the subsequent sampling date. Using the ideal gas law, the mass of soil, and the respective number of days incubated, the carbon dioxide concentration in the jar headspace was converted to reported values in mg C kg soil^{-1} day^{-1}.

2.4.5 | Microbial biomass

PLFAs were analyzed to estimate microbial biomass using methods described in Balser et al. (2019). Briefly, lipids were extracted from a 3 g subsample of freeze-dried soil using a mixture of 0.15 M citrate buffer, chloroform, and methanol, and allowed to phase-separate overnight. An internal standard methyl nonadecanoate (19:0) was added to the soil sample prior to adding the solvents. The chloroform phase was then saponified and methylated to produce fatty acid methyl esters. The saponification reagent was prepared by dissolving 15 g of sodium hydroxide pellets in a 100 mL of 1:1 (v/v) methanol:water solution. The methylation reagent was prepared by mixing 275 mL methanol with 325 mL of 6.0 N hydrochloric acid. Evaporated fatty acid methyl esters samples were suspended in hexane, transferred to a vial fitted

with a glass insert, and allowed to evaporate in the fume hood. Once evaporated, samples were suspended in 20 μL 13:0 internal standard solution (0.5 μg μL^{-1}) and quickly sealed to preserve concentration. Samples were quantified with gas chromatography (Agilent 7890; Agilent Technologies, Inc.). PLFA peaks were identified based on retention times of external standards.

Total lipid biomass was calculated by dividing PLFA peak areas by a standard constant (*k*-value), which was based on the 13:0 peak area of internal 13:0 standards, control, and blank samples with known 13:0 concentration. The adjusted peak area was then divided by the product of the fatty acid molecular weight and soil mass. A linear regression model showed no significant relationship between the *k*-value and the mean total biomass of each batch ($p = 0.98$).

PeakSimple software (SRI Instruments) was used to automate the labeling of 31 PLFA peaks in the chromatograms. Chromatographs were visually reviewed for accuracy of software labeling and adjusted based on instrument drift, as needed. Bacterial community biomass was estimated by averaging the biomass of 12 fatty acids considered to be of bacterial origin: iso 15:0, anteiso 15:0, 16:1ω9c, 16:1ω7c, 16:0 10-methyl, iso 17:0, anteiso 17:0, cyclo 17:0, 16:0 2-hydroxyl, 18:1ω7c, 18:0 10-methyl, cyclo 19:0 (Wilkinson et al., 2002). PLFA 16:1ω5c was used as a biomarker for arbuscular mycorrhizal fungi (AMF) biomass and 18:1ω9c as a biomarker for fungal biomass (Balser & Firestone, 2005; Gutknecht et al., 2012). Twenty-five peaks were used to measure total microbial biomass, which, in addition to those mentioned above, included 12:0, 13:0, 12:0 hydroxyl, 14:0, 15:0, 16:0, 17:0, 18:2, 18:0, 19:0, and 20:0 (Balser & Firestone, 2005; Gutknecht et al., 2012).

2.5 | Data analysis

Data were reviewed for outliers using a two-step approach. First, to identify values that were anomalous within repeated measures at the same collection location, data for each soil health indicator and depth were grouped by field point (i.e., the 64 sample locations; $n = 5$ per point) and screened for outliers and extreme points based on interquartile range (IQR). Points falling outside 1.5x IQR were flagged as "outliers," while those falling outside 3x IQR were flagged as "extreme points." Second, to identify potentially anomalous values relative to others within the same field, data for each indicator and depth were grouped by field-year ($n = 32$ per field-year) and the same IQR screening was applied. A data point was only removed if it met one of the following criteria: (1) flagged as extreme in both grouping approaches, or (2) flagged as extreme in one approach and as an outlier in the other. This method was designed to distinguish analytical errors from legitimate spatial variability by preserving data from plots that were consistently high or low relative to the rest of the field. Of 12,670 data points, 106 (<0.1%) were removed as outliers. There were an additional 23 enzyme data points that were negative, which was considered to result from a near-zero fluorescence. These values were set to a minute value of 1.

For the 0- to 15-cm depth, indicators were analyzed at all 64 field points, except for PLFA, which was conducted on a subset of the same 24 field points each year ($n = 12$ per field). For the 15- to 30-cm depth, PLFA was not analyzed, and extracellular enzymes were analyzed only on the same 24 field points each year, and all other indicators were measured at all 64 points. The subset of 24 points used for PLFA and extracellular enzymes were distributed diagonally in transects across the fields.

For each indicator, field, and depth combination, we calculated mean, standard deviation, and coefficient of variation (CV), temporally, using the five repeated measure values, one for each year sampled (Figure S1; Table S2). Next, we explored the temporal variability in a subset of variables that are commonly used in soil health assessments, including PLFA, enzymes, MinC, SOC, SOC Stock, POM, and MAOM. On this subset of indicators, we also calculated annual change and performed ANOVA to compare mean values of each indicator among years. Annual change calculations address both the magnitude and direction of the variability and ANOVA tests whether indicators changed over 5 years of study. In this way, these two measures of temporal variability can offer insights into the short- and medium-term temporal variability of a suite of soil health indicators. ANOVAs were performed using linear mixed effect models on indicators in each field and depth. This study has pseudo-replication within each field, as we collected multiple observations across space and time from single experimental units (one field per management system). We addressed this by using linear mixed-effects models with a term for sampling point as a random effect to account for nonindependence of repeated measures at each sampling point. We analyzed each field separately, recognizing that direct statistical comparison between fields is not appropriate given the unreplicated management treatments. These models used the sampling year as the fixed effect and sampling location (i.e., point; $n = 32$ per field) as a random effect. When necessary, data used in the ANOVA models were either square root or log-transformed to meet model assumptions of normality and equal variance of residuals. When a significant effect of year ($\alpha = 0.05$) was determined, Tukey's Honestly Significant Difference (HSD) was used for pairwise comparison of means.

Annual change was calculated at the site-level by first calculating the absolute change from the previous year's value ($n = 4$). Relative annual change (i.e., percent change) was found by then dividing by the previous year's value. Linear mixed effect intercept-only models with sampling point as a

random effect were used to determine whether the relative annual change for each indicator was significantly different from zero.

To contend with spatial dependencies in our statistical methods, we first explored spatial variability by calculating a CV for each field, year, depth, and indicator combination (Figure S2). We found indicator spatial CV values to be quite variable from year-to-year, which suggested the lack of consistent spatial pattern within our data (Figure S2). Next, we fit models using Gaussian, exponential, or spherical spatial correlation structures using sampling point latitude and longitude and year as structure form, which allowed for unique coordinates for each repeated annual measurement. We used likelihood ratio tests to compare the three spatial models to the random effect-only model and found the spatial correlation structures never improved goodness of fit ($p = 0.99$–1.0). This pattern suggested that using a term for sampling point as a random effect was sufficiently accounting for spatial dependencies, which led us to use only random effects in the final models.

Pearson's correlation analysis was used to explore how growing season weather correlates to the annual changes in soil health indicators. Mean air temperature and total precipitation were calculated for the period of April 1 (DoY 91) to November 30 (DoY 334) each year using annual weather data detailed above. This date range is approximately the period of above-freezing mean air temperatures based on site-specific 30-year Normals. The 30-year Normal mean air temperature and precipitation for this period are 13.6°C and 743 mm. The growing season total precipitation and mean air temperature values for each year are reported in Table 1. Pearson's correlations were analyzed using either total precipitation or mean air temperature over the growing season as the independent variable. The dependent variable was the absolute annual change in each soil health indicator-depth combination at each sampling point. Absolute annual change, as described above, was calculated as the difference between indicator values measured in consecutive spring soil samples. The weather data used for each correlation corresponded to the growing season that occurred between these two sampling dates. Data from both fields were analyzed together to examine the effect of annual weather on soil health indicators regardless of management.

Due to the variability in crop species' growth habits, physiology, and phenology used in this study, we were unable to define the start and end of the growing season based purely on crop biological conditions. Thus, some subjectivity was used to define growing season length each year using the 30-year Normal period of above-freezing air temperature. To assess the robustness of the correlations to the defined growing season temporal window, we conducted a sensitivity analysis using three growing season periods: the original period (DoY 91–334), an extended period (+20 days: DoY 81–344), and a shortened period (−20 days: DoY 101–324). The mean air temperature (°C) and total precipitation (mm) values for the extended growing season window were: 13.44, 584.45 (2020); 13.85, 522.99 (2021); 12.71, 557.53 (2022); and 13.52, 633.98 (2023). For the shortened window, these values were: 15.18, 572.26 (2020); 15.87, 440.69 (2021); 15.14, 468.63 (2022); and 16.17, 561.85 (2023). Correlation analyses were repeated for each temporal window.

Calculations for enzyme activity, CV, and annual change in soil health indicators are detailed in the Supporting Information. Analysis and data visualization were completed in R studio using R version 4.3.3 (R Core Team, 2024). We primarily used the following R packages in our analysis: *nlme* for linear mixed effects models (Pinheiro et al., 2017), *multcomp* for post hoc pairwise comparisons using Tukey HSD (Hothorn et al., 2016), *tidyverse* for data manipulation and workflow (Wickham et al., 2019), and *correlation* for correlation analysis (Makowski et al., 2022).

3 | RESULTS

3.1 | CV of soil properties

Chemical properties were the most stable through time, with pH exhibiting low temporal CVs at both depths (mean temporal CV: 0.034–0.040; Figure S1). In contrast, biological and plant nutrient properties showed the highest CVs. Nitrate consistently exhibited the highest mean temporal CV at both depths (0.879 at 0–15 cm; 0.919 at 15–30 cm). At 0–15 cm, AMF and fungal biomass followed nitrate at 0.744 and 0.781, respectively. At 15–30 cm, acidP and NAG followed nitrate at 0.795 and 0.612, respectively.

Notably, we found a wide range of temporal CV values across the 64 sampling points. The difference between the minimum and maximum temporal CV observed across all points was as large as 1.275 (fungal), 1.464 (0–15 cm acidP), and 1.734 (15–30 cm nitrate). Properties with low mean temporal CVs also exhibited wide ranges, including 0–15 cm SOC (mean temporal CV: 0.099, range: 0.240), 15–30 cm Ca (mean temporal CV: 0.099, range: 0.340), and 15–30 cm CEC (mean temporal CV: 0.146, range: 0.600). Given this high temporal variability, we next examined whether properties changed consistently over the 5-year period or if changes reflected random fluctuation.

3.2 | Changes in soil health indicators over 5 years of sampling in annual and perennial fields

Nearly all soil health indicators changed significantly over the 5-year period, with only three indicator-depth combinations remaining unchanged in both fields: 0–15 cm POM and

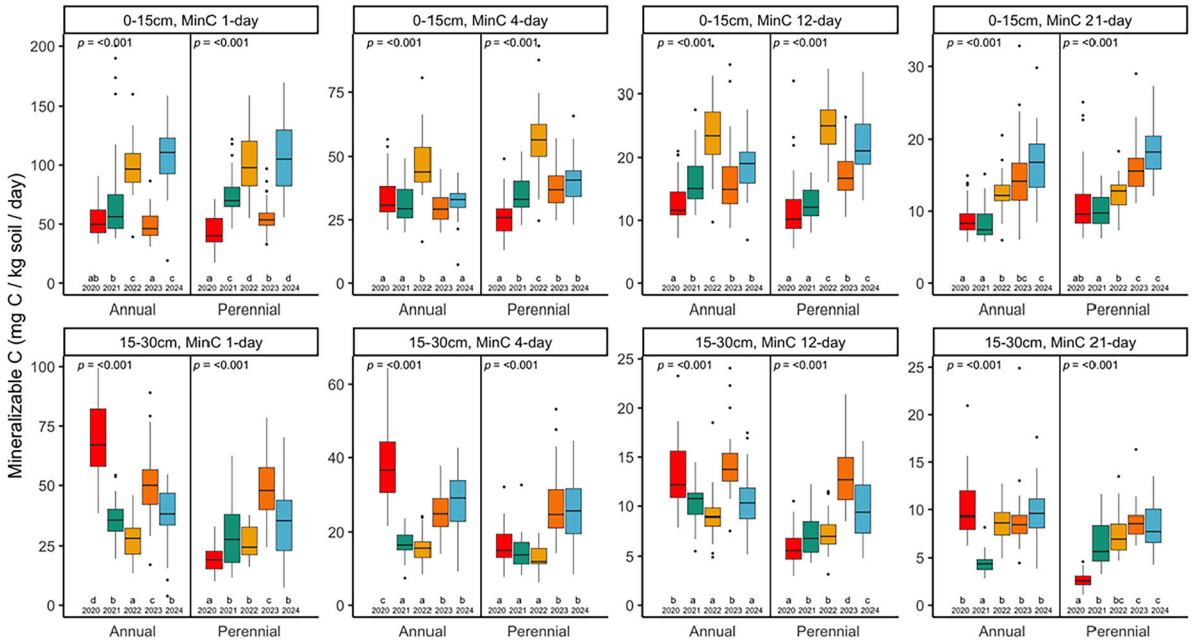


FIGURE 2 Five-year trends in mineralizable carbon (MinC) separated by field (within panel) and depth (rows). Analysis of variance was performed on linear mixed effect models of soil health indicators in each field and depth. These models used the sampling year as the fixed effect and sampling location (i.e., point) as a random effect. Letters reflect pairwise comparison of means using Tukey HSD ($\alpha = 0.05$).

15–30 cm MAOM and SOC (Table S3). Conversely, these same indicators changed significantly over time at the alternate depth (15–30 cm for POM and 0–15 cm for MAOM and SOC; $p = 0.001$–0.047). The direction of the change in POM at 15–30 cm differed between fields. The annual field showed a considerable drop in 15–30 cm POM after 2020 and remained at the lower level ($p = 0.001$), whereas POM at 15–30 cm in the perennial field increased from 2020 levels ($p = 0.047$). Interestingly, in both fields, SOC reached its highest value in 2022 and SOC in both fields followed an overall similar trend through time. SOC concentration did not change at 15–30 cm, but SOC stock changed at all depths and fields ($p = <0.001$–0.016). This pattern suggests stock changes were driven by bulk density increases in later years of the study (Table S2; Figure S3).

The remaining indicator-depth-field combinations showed a significant ($p < 0.05$) effect of year. The strongest changes were observed for MinC, extracellular enzymes, and PLFA groups. MinC changed significantly over time ($p < 0.001$; Figure 2), with similar temporal patterns at 0–15 cm in both fields. At 15–30 cm, however, fields diverged: the annual field declined from 2020 to 2022 while the perennial field maintained or increased MinC during this period.

Extracellular enzymes changed significantly in both fields and depths ($p = 0.001$–0.046; Figure S3). At 0–15 cm, the perennial field showed a positive trajectory while the annual field remained stable until increasing in 2024. At 15–30 cm, patterns differed: steady increases in the annual field versus a more variable pattern (2020–2021 increase, 2022 decline, and 2023–2024 recovery) in the perennial field.

All PLFA groups changed through time ($p = <0.001$–0.011). Across all PLFA groups, the annual field showed a downward trend through time. That downward trend was only interrupted by an increase in 2022. In the perennial field, PLFA groups varied in the 2021–2023 period, but by 2024 they were at a level similar to 2020. The exception to this was fungal biomass, which had a marked decline through time. Between fields, AMF patterns appeared to be the most distinct. AMF had a precipitous decline across years in the annual field but remained stable in the perennial field with the exception of a sharp decline in 2023 that rebounded by 2024. To better understand the direction and magnitude of these changes, we quantified year-to-year differences between consecutive sampling periods.

3.3 | Relative annual change of soil health indicators

Across both management systems, extracellular enzymes and 0–15 cm MinC showed the highest relative annual change of all indicators (Figure 3; Table S4). Extracellular enzymes were highly variable with annual increases of up to 1900% in acidP at 15–30 cm in the annual field. BetaG and NAG had smaller, but still considerable, annual increases in both systems and depths that ranged from 19%–102% ($p = 0.001$–0.007). At 0–15 cm, all four MinC measures increased

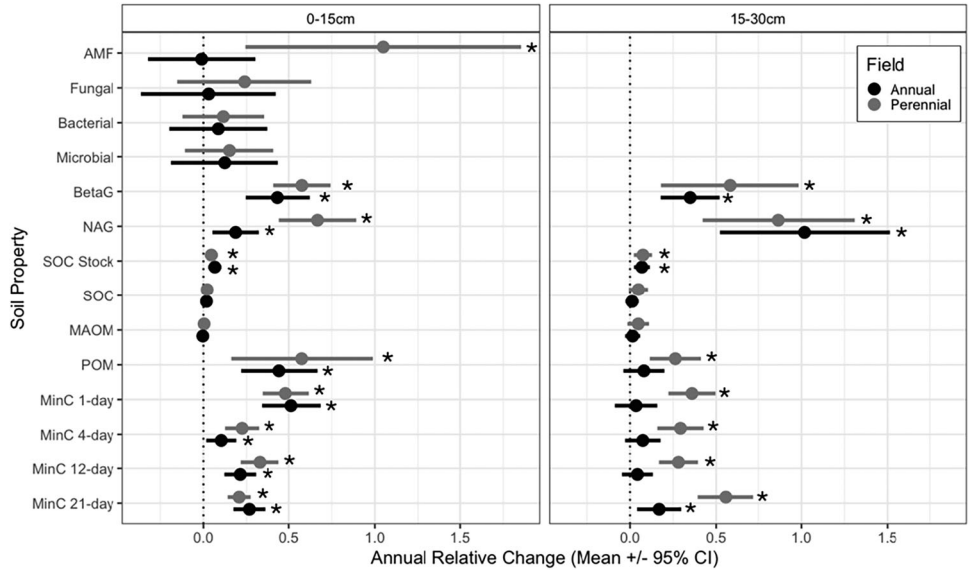


FIGURE 3 Annual relative change in select soil health indicators separated by field and depth. Circles are the mean annual relative change based on four interannual periods (2020–2024) and 32 sampling points in each field, except for N-acetylglucosaminidase activity (NAG) and β-glucosidase activity (betaG) at 15–30 cm, which was analyzed at a subset of 12 sampling points in each field. Acid phosphatase activity (acidP) is not shown due to its effect on axis scaling but is reported in Table S4. Error bars represent a 95% confidence interval (CI). Asterisks identify indicators in each field-depth that had a mean annual relative change that was significantly different from zero based on linear mixed effects intercept-only models with a term for sampling point as the random effect ($\alpha = 0.05$). AMF, arbuscular mycorrhizal fungi; MAOM, mineral-associated organic matter; MinC, mineralizable carbon; POM, particulate organic matter; SOC, soil organic carbon.

annually by 10–51% ($p = 0.001$–0.020), on average. The indicators with the lowest relative annual change were MAOM and SOC. Across depths and management systems, the mean relative annual change in these two indicators ranged from −0.04% to 4.8% ($p = 0.090$–0.810). SOC stock was slightly more variable than SOC concentration at 0–15 cm (4.6%–6.6%, $p = 0.01$–0.018). Interestingly, at 15–30 cm, MAOM and SOC did not change annually in either field (1.2%–4.8%, $p = 0.090$–0.538), but SOC stock changed in both fields annually (6.9%–7.5%, $p = 0.004$–0.006). Similar to the ANOVA finding, this SOC stock change appears largely driven by changes in bulk density.

The most notable differences between the two management systems were 0–15 cm AMF biomass, 15–30 cm MinC, 15–30 cm POM, and 0–15 cm acidP and NAG. In the perennial field, AMF had a mean relative annual change of over 100% ($p = 0.013$), whereas that indicator did not change in the annual system (−1%, $p = 0.950$). At 15–30 cm, three of four MinC measures had large mean relative annual changes ranging from 28%–36% ($p < 0.001$) in the perennial field, and did not change in the annual field (3%–7%; $p = 0.160$–0.580). Similarly, 15–30 cm POM, on average, increased by 26% each year ($p < 0.001$) in the perennial field, but did not change in the annual field (8%, $p = 0.180$). The 15–30 cm 21-day MinC did increase, on average, in both fields, but to a greater extent in the perennial field (56%; $p < 0.001$) compared to the annual field (17%; $p = 0.011$). Similarly, both NAG and acidP had positive annual changes in both fields at 0–15 cm, but differed in the magnitude. NAG at 0–15 cm increased on average by 67% ($p < 0.001$) annually in the perennial field compared to 19% ($p = 0.007$) in the annual field. AcidP at 0–15 cm had a remarkable mean annual change of 949% ($p = 0.012$) in the perennial field compared to 69% ($p = 0.004$) in the annual field. To determine whether this temporal variability was driven by annual weather patterns, we examined correlations between growing season conditions and changes in soil health indicators.

3.4 | Correlation between annual weather and year-to-year change in soil health indicators

Growing season mean air temperature was strongly correlated with annual changes in several soil health indicators, particularly MinC ($p = < 0.001$–0.035) and PLFA groups ($p = < 0.001$–0.04) (Figure 4). For MinC, these correlations were depth-dependent and incubation-length dependent, with 21-day incubations showing no temperature correlation (Table 3). At 0–15 cm, 1-, 4-, and 12-day MinC were all positively correlated with growing season mean air temperature ($r = 0.65$–0.68, $p < 0.001$). At 15–30 cm, the direction of these correlations was negative ($r = -0.39$ to -0.60, $p < 0.001$). Total microbial, AMF, and bacterial biomass were also pos-

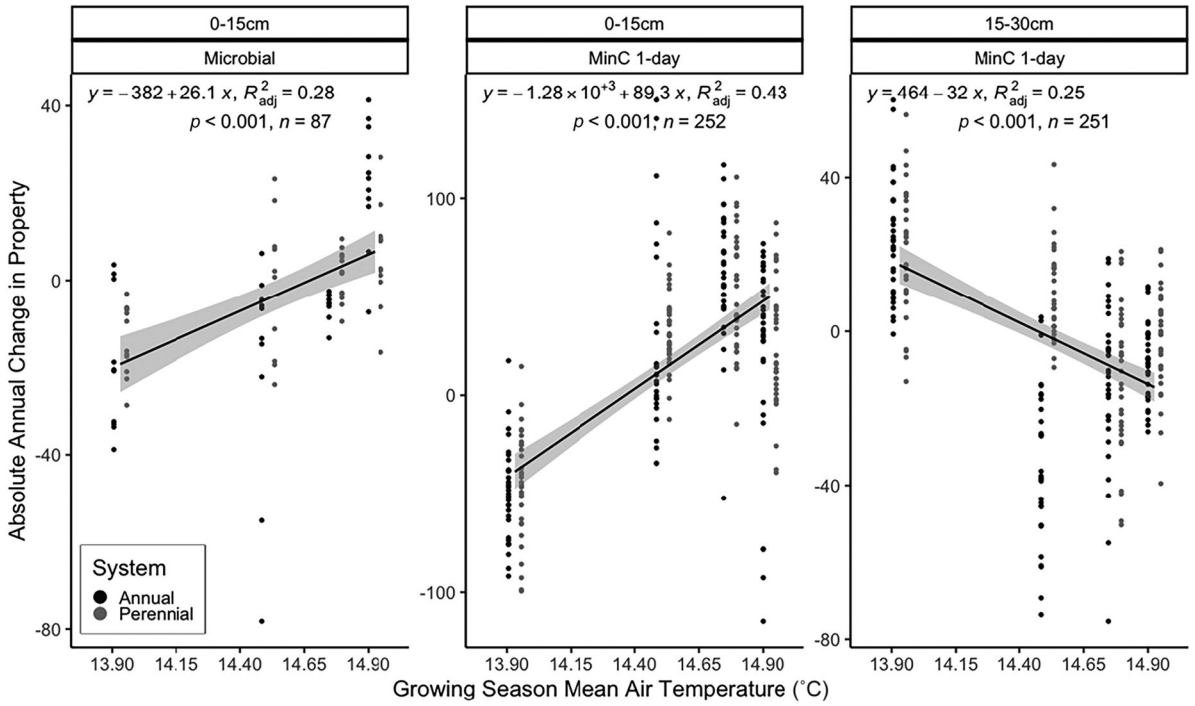


FIGURE 4 Relationships between growing season mean air temperature and the absolute annual change in select soil health indicators: 0–15 cm total microbial biomass, 0–15 cm mineralizable carbon (MinC) 1-day, and 15–30 cm MinC 1-day. Growing season is defined as April 1 (DoY 91) to November 30 (DoY 334), which approximates the annual period of above-freezing mean air temperatures. Both fields are combined for the correlation analysis but are visualized separately by color. The 30-year Normal mean air temperature for this period is 13.6°C. Units of microbial lipid biomass are in nmol fatty acid g soil^{-1} and MinC units are in mg C kg soil^{-1} day^{-1}. Trend lines and summary statistics are produced using the ggpmisc package in R.

itively correlated with growing season mean air temperature (r = 0.40–0.53, p = < 0.001–0.020). At both depths, SOC stock had a weak negative correlation with air temperature (r = −0.29, $p \leq 0.001$), but air temperature did not have a significant correlation with SOC concentration.

There were weaker but significant correlations with growing season total precipitation. At 15–30 cm, all three extracellular enzymes were positively correlated with precipitation (r = 0.38–0.55, p = <0.001–0.04). At 0–15 cm, only acidP was correlated with precipitation ($r = 0.31$, $p < 0.001$). MinC 1-, 4-, and 12-day at 15–30 cm were negatively correlated with precipitation (r = −0.24 to −0.37, p = <0.001–0.03). As with correlations with air temperature, the direction of the MinC 1-day correlation changed with depth as the 0–15 cm value was positively correlated with precipitation (r = 0.40, $p < 0.001$). Finally, change in MAOM at 0–15 cm was negatively correlated with precipitation ($r = -0.24$, $p = 0.027$).

To determine the effect of the defined growing season temporal window on these results, we performed separate correlation analyses using three growing season definitions: shortened (DoY 101–324), extended (DoY 81–344), and the original definition (DoY 91–334). Results of this analysis demonstrate the consistent relationships between air temperature and both MinC and microbial biomass regardless of the defined growing season window (Figure S4). At 0–15 cm, MinC 1-, 4-, and 12-day maintained strong positive correlations with air temperature across all growing season windows (r = 0.39–0.70, $p < 0.001$). The same was generally true for 15–30 cm MinC, where strong negative correlations with air temperature for 1- and 12-day incubations persisted across all temporal windows (r = −0.39 to −0.60, $p < 0.001$). However, 15–30 cm MinC 4-day was not correlated with air temperature in the shortened window but was correlated in the extended and original windows (r = −0.39 to −0.44, $p < 0.001$). MinC 21-day was not correlated with air mean air temperature at either depth in any window. Microbial biomass carbon showed consistent positive correlations with air temperature (r = 0.43–0.53, $p < 0.001$). In contrast, several variables exhibited sensitivity to the growing period window definition. With precipitation, this included 0–15 cm betaG, 15–30 cm acidP, 15–30 cm MinC 4-day, 0–15 cm MinC 21-day, 15–30 cm MinC 21-day, 0–15 cm MAOM, and 0–15 cm SOC stock. With air temperature, in addition to 15–30 cm MinC 4-day, inconsistent correlations were observed in 0–15 cm NAG, AMF and bacterial biomass, and 0–15 cm SOC stock.

4 | DISCUSSION

This study provides important insights into the interannual temporal variability of soil health indicators. Over 5 years, we

TABLE 3 Pearson's correlation analysis between growing season weather and annual changes in soil health indicators.

Indicator + depth	*r*	*p*-value	*n*
Growing season total precipitation			
AcidP 0–15 cm	0.31	<0.001	254
BetaG 15–30 cm	0.43	0.006	93
NAG 15–30 cm	0.55	<0.001	95
MinC 1-day 0–15 cm	0.40	<0.001	252
MinC 1-day 15–30 cm	−0.30	<0.001	251
MinC 4-day 15–30 cm	−0.24	0.035	256
MinC 12-day 15–30 cm	−0.37	<0.001	252
MAOM 0–15 cm	−0.24	0.031	256
Growing season mean air temperature			
MinC 1-day 0–15 cm	0.66	<0.001	252
MinC 1-day 15–30 cm	−0.50	<0.001	251
MinC 4-day 0–15 cm	0.65	<0.001	248
MinC 4-day 15–30 cm	−0.39	<0.001	256
MinC 12-day 0–15 cm	0.68	<0.001	250
MinC 12-day 15–30 cm	−0.60	<0.001	252
Microbial 0–15 cm	0.53	<0.001	87
AMF 0–15 cm	0.40	0.040	88
Bacterial 0–15 cm	0.41	0.027	88
SOC stock 0–15 cm	−0.29	<0.001	250
SOC stock 15–30 cm	−0.29	0.001	253

Note: Results of correlations between the absolute annual change of soil health indicators and either growing season mean air temperature or total precipitation, including correlation coefficient (r), p-value, and number of observations (n). Only significant ($\alpha = 0.05$) correlations are shown. The growing season was defined as April 1 (DoY 91) to November 30 (DoY 334). This is approximately the period of above-freezing mean air temperatures based on 30-year Normals. Data from both fields were analyzed together to examine the effect of weather on indicators regardless of management.

Abbreviations: acidP, acid phosphatase activity; AMF, arbuscular mycorrhizal fungi; betaG, β-glucosidase activity; MAOM, mineral-associated organic matter; MinC, mineralizable carbon; NAG, N-acetylglucosaminidase activity; SOC, soil organic carbon.

conducted annual soil sampling across 64 points within two fields under contrasting management systems. We address three key questions: (1) How does annual weather influence soil health indicators? (2) How does annual and perennial management affect soil health indicators year-to-year and over 5 years? (3) what are the practical implications for soil health assessment?

We present two primary findings. First, growing season weather patterns, particularly mean air temperature, are strongly correlated with annual changes in soil health indicators. This weather sensitivity presents an interpretive challenge, suggesting that soil health assessment protocols should account for annual weather conditions. Second, the perennial cropping system showed improvements in soil health indicators over the 5-year period.

4.1 | Soil health indicators are highly correlated with annual weather

Our study reveals that growing season weather is correlated with annual changes in several soil health indicators, presenting a fundamental challenge for assessment and interpretation. Growing season air temperature is highly correlated with both MinC ($p < 0.001$) and microbial biomass ($p = < 0.001$–0.04). These correlations were generally robust to the defined growing season window (Figure S4). To our knowledge, this is among the first quantitative demonstrations of how annual weather influences year-to-year shifts in soil health indicators. Given the increasing use of dynamic soil properties in soil health assessment, it is critical to account for the potential for annual weather to affect soil health indicator variability (Stott, 2019). MinC, in particular, has been adopted in major soil health frameworks, and this temperature-sensitivity finding complicates its interpretability (Bagnall et al., 2023; Liptzin et al., 2022; Moebius-Clune, 2016).

The temperature-MinC relationship is depth-dependent. At the surface (0–15 cm), warmer growing seasons increase MinC ($r = 0.65$–0.68, $p < 0.001$), while at the subsurface depth (15–30 cm), the relationship reverses ($r = -0.39$ to -0.60, $p < 0.001$). There could be multiple mechanisms driving the overall sensitivity of MinC to air temperature effects. Abiotically, temperature could directly affect decomposition rates through the "regulatory gate" concept, where warming

increases substrate availability (Brookes et al., 2017; Kemmitt et al., 2008; Zhou et al., 2020). Biotically, temperature enhances microbial activity, which could spur organic matter decomposition and microbial population growth (Burns et al., 2013; Schimel & Weintraub, 2003; C. Wang et al., 2021). Our finding that microbial biomass also correlates positively with temperature ($r = 0.40$–0.53, $p < 0.05$) supports a biotic element, though the causality remains unclear. Alternatively, plant-mediated pathways may be the primary factor, where temperature affects crop productivity and subsequently drives soil carbon cycling through rhizosphere processes (Finzi et al., 2015; Huo et al., 2017; Kuzyakov, 2010). The depth-dependent differences in the temperature-MinC relationship are less clear and there are limited studies on MinC response to experimental or observational warming. Abiotic and biotic factors, including root mass, soil moisture, and microbial abundance, differ along a soil profile (Fierer et al., 2003; Kautz et al., 2013; Rakkar et al., 2023; Wu et al., 2002). The distribution of these factors along a profile may vary with warming, which could result in depth-dependent responses in carbon cycling (B. Sun et al., 2025; Zosso et al., 2021). Further investigation is needed to explore the pathways driving this observation.

The air temperature-MinC relationship is dependent on incubation duration. Shorter incubation periods (1-, 4-, and 12-day) show strong temperature correlations, while 21-day MinC is not correlated. This relationship suggests that more labile substrate becomes increasingly available as annual temperatures rise. However, the same is not true for the availability of more recalcitrant substrate, which appear temperature agnostic. In this discussion, we focus on the implications of this finding for soil health assessment, but recognize it may also offer insights into the sensitivity of organic matter pools to climate warming (Bosatta & Agren, 1999; Lehmann & Kleber, 2015; Liáng et al., 2023). As a process-based assay, MinC reflects both substrate supply and microbial activity. It indicates both the substrate available to be metabolized and the microbial capacity to metabolize the available substrate. However, MinC is believed to be primarily driven by substrate availability, more so than microbial pool size (W. Wang et al., 2003), which we corroborate based on a lack of correlations between MinC and microbial biomass ($p = 0.8$–1; data not shown). Within the first 48 h of incubation, the composition of substrate shifts to be less readily-mineralizable by having fewer N-containing compounds and more lignin-like material (Laffely et al., 2020). After 21 days of incubation, the remaining substrate left un-metabolized is likely more recalcitrant compounds that microbes consume with lower efficiency (Cotrufo et al., 2013). This incubation length dependence offers a potential solution for improving interpretability of MinC within soil health assessment. Extending MinC incubation periods may reduce weather sensitivity, as 21-day MinC likely reflects more recalcitrant carbon substrate that is less responsive to interannual weather variability. Despite this, our findings suggest that 21-day MinC remains sensitive to management. At 15–30 cm depth, 21-day MinC increased by a mean of 56% annually ($p < 0.001$) in the perennial field compared to 17% ($p = 0.011$) in the annual field. This result suggests longer MinC incubations may be sensitive to management, but not annual weather. However, the optimal incubation duration likely varies across soil types and climates, which requires further methodological evaluation and validation.

Our findings suggest that the current approach to monitoring some soil health indicators may not meet a key criterion: they should be sensitive to management but not significantly affected by short-term weather patterns (Doran and Parkin, 1996). The general advice for annual soil sampling is to collect at the same time of year to control for seasonal variation in soil health indicators (Liebig et al., 2021; Liptzin et al., 2022). Our findings suggest that even when this advice is followed, there is an additional distinct influence of annual weather on soil health indicators, and that this influence is soil depth dependent. Given that annual sampling is likely the highest frequency practically achievable for most farmers and researchers, this annual weather sensitivity represents a major confounding factor in soil health assessments that has been largely overlooked.

We propose three potential solutions to this issue. First, soil from a nearby reference field experiencing identical weather could be analyzed in conjunction with a "soil health management" field, which would enable the separation of weather versus management signals in the data. Previous work has shown this is possible given that management effects on soil health indicators are detectable even as they vary with seasonal weather patterns (Culman, Snapp, Ollenburger, et al., 2013; Gonzalez-Maldonado et al., 2025). Second, adjustment factors could be developed based on site-specific annual weather patterns. This approach could function similarly to existing methods of adjusting soil health scores based on site-specific edaphic and regional climate factors (e.g., Soil Health Assessment Protocol and Evaluation tool; Nunes et al., 2021). In effect, this would broaden soil health score adjustment from site-specific to site-year-specific by including annual weather when developing interpretation curves. However, data from many environments representing a range of climate and edaphic characteristics are needed to develop this approach. Finally, for MinC analyses, incubation periods could be extended to 21 days, though determining the optimal incubation duration will require further methodological evaluation and validation across diverse soil types and climates.

4.2 | Soil health indicators increased under perennial management

Over 5 years of monitoring, the perennial field showed greater improvements in soil health indicators compared to the diversified annual field. These fields represent single, unreplicated observations with distinct site histories and management legacies. Nevertheless, the contrasting temporal patterns of measured indicators provide insights into potential management system differences. The most striking difference was in AMF, which declined precipitously in the annual system (ANOVA: $p < 0.001$) while doubling annually in the perennial system (100% mean annual change, $p = 0.013$). This difference is particularly significant given AMF's critical roles in nutrient and water provisioning, climate resilience, yield formation, and soil aggregation (Abrar et al., 2024; Benitez-Alfonso et al., 2023; Rillig, 2004; Smith & Smith, 2011), though field-specific factors may also contribute to these observed temporal patterns.

Our field observations suggest that, while tillage plays a role in AMF biomass, cropping system may also be an important driver, likely due to differences in belowground biomass (Bowles et al., 2017; Säle et al., 2015). In the annual field, AMF declined steadily despite only two tillage events over 5 years (July 2021 and April 2022). Previous investigation in no-till annual cropping systems found that AMF did not recover in the 2 years following one-time tillage (Wortmann et al., 2008). Conversely, the perennial field showed AMF recovery within 1 year following the single tillage event during the alfalfa-to-Kernza transition. This finding suggests that perennial crops may create more favorable conditions for rapid AMF reestablishment, likely through greater belowground biomass and/or a longer active period of living biomass (Duchene et al., 2020; Primieri et al., 2022). In the perennial crop field, aside from AMF, total microbial and bacterial biomass did not change from 2020 baseline data, but fungal biomass did decrease ($p = 0.001$). These findings align with previous studies that found that AMF benefited as much or more from perennial crops than other microbial functional groups (Bergquist et al., 2025; Duchene et al., 2020; Rakkar et al., 2023; Udawatta et al., 2024).

The simultaneous increase in NAG activity and AMF in surface soils in the perennial field point to improved plant–microbe interactions (Figure 5). NAG degrades chitin, a major component of fungal cell walls and arthropod exoskeletons. This reaction produces N-acetylglucosamine, which serves as both carbon and nitrogen substrate for microbes and has been shown to improve crop growth (Sinsabaugh et al., 2008; J. Sun et al., 2022). Recent evidence shows that AMF, while unable to release extracellular enzymes itself, can elicit NAG release from soil bacteria to orchestrate chitin breakdown, resulting in organic nitrogen that AMF then supplies to plant symbionts (Bukovská et al., 2021; Rozmoš et al., 2022). The observation that both NAG and AMF increased in the perennial but not the annual field during the study period suggests perennial crops may be more effective at cultivating plant–microbe nutrient acquisition strategies in surface soils (Boerner, 1992; Primieri et al., 2022). However, we acknowledge that field-specific factors could also be contributing to these observations.

The contrasting responses of labile carbon pools at 15–30 cm depth between the two fields provide evidence consistent with management effects. In the annual field, POM and shorter-term MinC (1-, 4-, and 12-day) declined from baseline levels, while these same indicators increased substantially in the perennial field. The two fields showed markedly different annual relative changes for both 15–30 cm POM (26% vs. 8%) and MinC (28%–36% vs. 3%–7%), with greater gains in the perennial field.

The annual field's surface soil gains coupled with subsurface soil losses of labile carbon likely reflects the impact of both tillage and root biomass. This interpretation is supported by the temporal patterns: the annual field's 15–30 cm MinC was initially higher than 0–15 cm values in 2020, but declined sharply following tillage in 2021 and 2022. This significant loss of MinC in subsurface soil is likely driven by the reintroduction of tillage to this field after 5 years of mixed grass-alfalfa hay production. Carbon and other soil nutrients have been found to stratify in soil layers following a period of tillage cessation, which can rapidly de-stratify once tillage is resumed (Deiss et al., 2021; Kay & VandenBygaart, 2002). Additionally, likely due to limited distribution of belowground biomass to deeper soil layers, the annual field did not regain this lost subsurface carbon. Conversely, the perennial field showed consistent gains in MinC at both depths, suggesting that perennial crop roots contribute carbon substrate in both surface and subsurface soil.

Despite the perennial field showing greater increases in multiple soil health indicators, the diversified annual field showed improvements relative to baseline as well. Enzyme activities, SOC stocks, and surface MinC all increased, suggesting that low-tillage, diversified annual cropping systems can enhance soil health, albeit to a lesser extent than we observed in the perennial crop field. We cannot isolate management effects from field-specific factors. However, our field observations support previous controlled studies that suggest perennial crops improve soil biological properties more than annual cropping systems (Augarten et al., 2023; Glover et al., 2010; Nunes et al., 2018; Sprunger et al., 2019).

4.3 | CV as a suboptimal measure of soil variability

Our analysis reveals fundamental issues with using CV as a measure of soil property variability. Temporal CVs for individual properties in our study vary dramatically across sam-

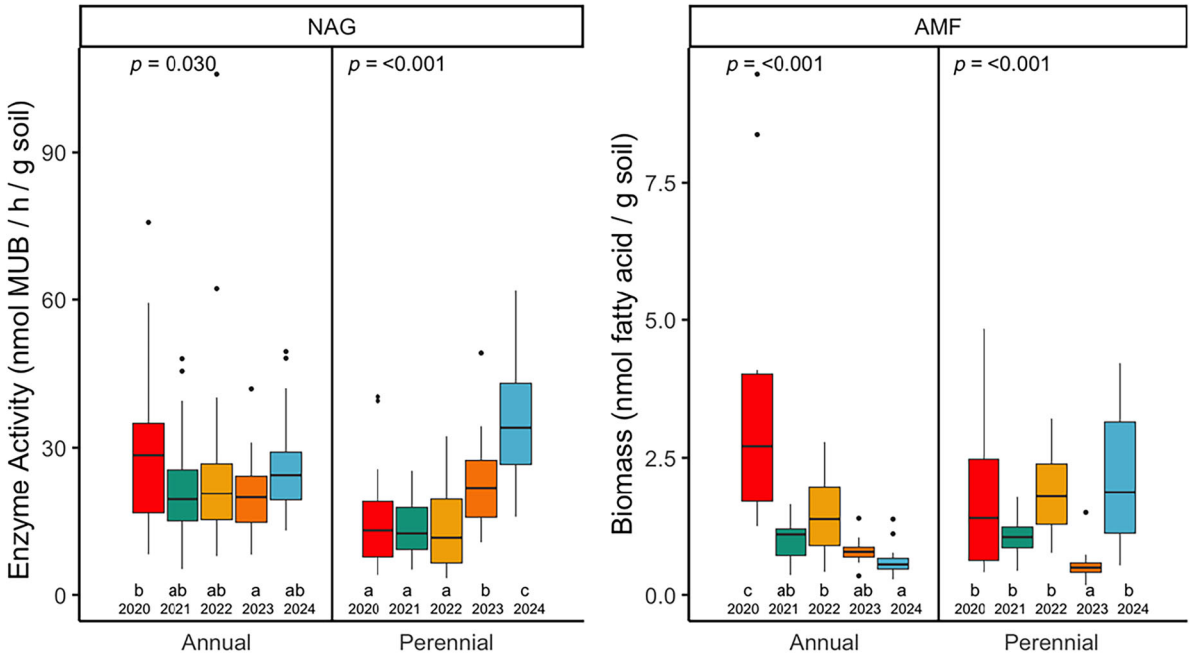


FIGURE 5 Five-year trend in 0–15 cm N-acetylglucosaminidase activity (NAG) and arbuscular mycorrhizal fungi biomass (AMF) separated by field (within panel). Analysis of variance was performed on linear mixed effect models on soil health indicators in each field and depth. These models used the sampling year as the fixed effect and sampling location (i.e., point) as a random effect. Letters reflect pairwise comparison of means using Tukey HSD ($\alpha = 0.05$).

pling points, even within the same field (Figure S1). This wide range could stem from sampling and analytical error, and/or stochasticity. It could also point to systematic differences in mean-variance relationships. If CV is to be reflective of the inherent variability of a soil property, it should remain reasonably consistent across measurement units. Our finding that CVs vary considerably between sampling points suggests that CV may be dependent on the sample mean value at each point. This echoes findings from crop yield stability research where the use of CV has been scrutinized (Döring et al., 2015).

The power law relationship between variance and mean (i.e., log(variance) ~ log(mean)) determines CV utility. If the log-regression slope (b) equals 2, variance scales proportionally with mean and CV provides a useful comparison tool (Döring et al., 2015). However, slopes significantly different from 2 create systematic bias, making CV appear larger ($b > 2$) or smaller ($b < 2$) as mean values increase. While our dataset lacks the mean range needed for definitive conclusions, we find evidence of both "amplifying" ($b > 2$) and "regulating" ($b < 2$) mean-variance relationships across properties whether calculating CVs over time or over space (data not shown). Future soil variability research should systematically explore mean-variance relationships across soil types, climates, and temporal and spatial scales. CV can be a useful tool for determining soil sampling spatial design and density (Lawrence et al., 2020; Wilding & Drees, 1983). However, if mean and variance do not scale proportionally, these calculations may produce misleading estimates.

4.4 | Practical implications for soil health assessment

Our study provides practical insights for farmers, soil health practitioners, and researchers. For farmers considering CLC systems, our field-scale observations across 64 sampling points over 5 years demonstrate that perennial crops offer soil health benefits. This intensive sampling at field scales (11–16 ha) provides insights into soil health dynamics under realistic farming conditions that complement findings from replicated plot-scale experiments (Augarten et al., 2023; Sprunger et al., 2020). While diversified annual rotations do also provide some improvements to soil health, those effects are limited to primarily surface soil.

For soil health assessment, practitioners should be aware that some indicators may reflect effects of both management and annual weather. Assessment protocols should consider incorporating strategies to account for the effects of annual weather through reference field comparisons, extended MinC incubations, or weather-adjustment factors. For researchers, our findings highlight the need to separate weather-driven from management-driven changes in soil health indicators. Long-term studies with annual sampling across diverse environments will be essential for developing well-validated soil health assessment frameworks. The goal of these frameworks should be to isolate effects of management from other factors affecting temporal variability of selected indicators.

4.5 | Limitations and future directions

Our field-scale design prioritizes spatial and temporal resolution over treatment replication. This pseudo-replicated design limits causal inference about management effects. However, our mixed-effects modeling approach and intensive sampling provide through characterization of temporal variability within each cropping system. The paired field approach with differing management histories provides valuable observational evidence of soil health indicators response under contrasting management systems.

The management histories of the two fields add important context to our findings. The annual field's legacy of 5 years of mixed grass-alfalfa may have elevated certain indicators (e.g., AMF, 15–30 cm MinC) that fell dramatically following the transition to annual crops. Conversely, the perennial field transitioned from a 3-year maize–soybean–maize rotation (2017–2019) back to perennial management, having previously been in alfalfa from 2014 to 2016. These legacy effects cannot be separated from the management systems applied during our observational study period. However, they reflect realistic on-farm transitions—fields often rotate between perennial and annual crops—and provide insights into soil health indicator responses during these transitions. With this context, our findings likely represent not just pure management effects, but the dynamics of soil health decline and recovery following management changes, which has practical relevance for farmers considering system transitions.

The study period captured relatively warm and dry conditions compared to 30-year Normals (Figure 1), which may have limited observable changes in stable carbon pools. Continued monitoring could clarify whether MAOM and SOC would respond to perennial management given sufficient time, or if weather constraints fundamentally limit carbon accumulation potential. Future research should further explore annual weather-soil health indicators across broader climatic gradients and soil types. This additional data would enable development of well calibrated adjustment factors for soil health indicators. There is also a need to better understand the shifts in chemical composition of substrate as MinC incubation duration increases. Previous work has explored these shifts over 48 h (Laffely et al., 2020), but our findings suggest that there are still meaningful changes in substrate composition occurring between 12 and 21 days of incubation. Additionally, mechanistic studies are needed to parse abiotic, biotic, and plant-mediated pathways driving weather sensitivity in soil processes.

5 | CONCLUSIONS

This study suggests that CLC cropping systems, particularly those including perennial crops, provide measureable soil health benefits. However, annual weather patterns also mediate soil processes and associated soil health indicators, such as microbial biomass and MinC. Since annual sampling is the practical standard for high-frequency soil health monitoring, our findings highlight a critical need for weather-adjusted interpretation of annual soil health measurements. Without accounting for the annual weather context, soil health assessments may misattribute annual weather effects to management effects. This interpretation could lead to erroneous conclusions about the degree to which management decisions are driving changes in soil health. Finally, we identified the potential for CV to be a misleading representation of variability of soil properties and suggest caution when interpreting these values.

AUTHOR CONTRIBUTIONS

Jacob Kundert: Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing—original draft; writing—review and editing. **Jacob Jungers**: Conceptualization; funding acquisition; investigation; methodology; resources; supervision; writing—review and editing. **Joshua Gamble**: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—review and editing. **Galen Bergquist**: Data curation; formal analysis; investigation; methodology; writing—review and editing. **Jessica Gutknecht**: Conceptualization; funding acquisition; investigation; methodology; resources; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

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