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Long-term storage of air-dried samples compromises water-extractable organic carbon as a soil health indicator

Swarnali Mahmood[1], **Franky Celestin**[1], **Diego Arruda Huggins de Sá Leitão**[1], **Ryan E. Champiny**[1], **Xinlin Wang**[1], **JoAnn B. Donald**[1], **Audrey Plauche**[1], **S. Carolina Córdova**[2], **Christine D. Sprunger**[3], **Gabriel Maltais-Landry**[1], and **Yang Lin**[1]

[1]Department of Soil, Water, and Ecosystem Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, USA
[2]Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
[3]Department of Plant, Soil, and Microbial Sciences, W.K. Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060, USA

Correspondence: Swarnali Mahmood (swarnali.mahmood@gmail.com)

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Abstract. The assessment of soil health relies on sensitive indicators to detect management-induced changes, yet the analytical reliability of these indicators following long-term storage is rarely assessed. We investigated how multi-year storage of air-dried samples influenced the concentrations of several common soil health indicators, including water-extractable organic carbon and nitrogen (WEOC, WEN), mineralizable carbon (Cmin), and permanganate-oxidizable carbon (POX-C), using archived samples from a cover crop experiment. Concentrations of WEOC nearly doubled after three years of storage, while WEN decreased by 19 %. A small but significant 6 % increase in Cmin concentration was also observed. In contrast, POX-C concentrations remained stable, indicating robustness to storage effects. These storage effects were consistent among three treatments with different cover crop species. In addition, WEOC concentrations consistently declined over time in this experiment along with four long-term agricultural sites in the USA, but their bulk soil organic carbon (SOC) or soil organic matter (SOM) did not. These results suggest that multi-year storage of air-dried samples inflates the WEOC pool. Therefore, we caution the use of WEOC as a soil health indicator in archived samples, as the observed variations might reflect storage artifacts rather than genuine management impacts.

1 Introduction

The concept of soil health has emerged as a framework for promoting sustainable soil management (Celestin et al., 2025; Congreves and Wu, 2024; Lehmann et al., 2020; Wood and Blankinship, 2022). Soil health indicators represent the soil's physical, chemical, and biological properties and are used to evaluate its capacity to support key ecosystem functions, including nutrient cycling, water regulation, carbon (C) sequestration, biodiversity habitat, and pollution remediation (Geisseler et al., 2019; Hurisso et al., 2016; Kibblewhite et al., 2008). Effective indicators should be closely linked to soil functions, sensitive to management changes, interpretable for decision-making, and cost-effective (Lehmann et al., 2020). However, indicator selection involves trade-offs. For example, total soil organic carbon (SOC) is a fundamental indicator but responds slowly to short-term management changes (Córdova et al., 2025; Martin and Sprunger, 2022; Nyabami et al., 2024). In contrast, more sensitive indicators such as permanganate-oxidizable carbon (POX-C) and potentially mineralizable carbon (Cmin) represent processed and labile C pools and often respond more rapidly to management or productivity gradients (Bongiorno et al., 2019; Culman et al., 2012; Hurisso et al., 2018; Liptzin et al., 2022; Nyabami et al., 2024). Water-extractable pools, such as water-extractable organic carbon (WEOC), reflect readily available substrates and respond to land use and climate, but are sensitive to environment and sample handling (Liptzin et al., 2022; Waldrip et al., 2022).

Soil processing and storage can systematically bias soil health indicators and affect their repeatability. Air-drying prior to analysis and long-term storage is a standard and widely adopted procedure in soil science for both routine laboratory analyses and soil archiving (Kühnel et al., 2019; Stott, 2019). Despite being classified as air-dried, soils typically retain a small amount of residual water (0.3 %–6 %) that remains adsorbed to mineral surfaces and within fine pores, particularly in clay-rich and organic matter-rich soils. Residual moisture content has been shown to be strongly related to soil texture and organic matter content, reflecting water retained at high matric potentials and on reactive mineral surfaces (Poeplau et al., 2015; Wäldchen et al., 2012). Microbial activity may persist even at the low water contents present in air-dried soils, potentially resulting in continued transformation of labile carbon and nitrogen pools during storage. Although bulk SOC and N pools remain relatively stable during long-term storage (Blake et al., 2000; Kühnel et al., 2019), the quantity and composition of water-extractable pools, including WEOC, water-extractable N (WEN), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON), can be altered by air-drying and freezing processes (Bolan et al., 1996; Jones and Willett, 2006; Kaiser et al., 2001; Rhymes et al., 2021; Sun et al., 2015). For example, WEOC concentrations increase with air-drying temperature (Leeford et al., 2023) and soils archived for decades have shown higher WEOC and WEN than fresh samples (Halvorson et al., 2025). Similarly, Cmin commonly exhibits a pulse following drying and rewetting (the Birch effect), with responses influenced by drying duration, microbial mortality, and extracellular product accumulation (Beem-Miller et al., 2021; De Nobili et al., 2006; Fierer et al., 2021; Mikha et al., 2005; Schimel et al., 1999; Warren, 2016). In contrast, POX-C is generally less sensitive to drying but may still be affected by methodological factors such as soil mass, sieve size, laboratory handling, and SOC content (Gasch et al., 2020; Wade et al., 2020). Despite these advances, the combined effects of management and multi-year storage of air-dried soils on the repeatability and temporal trajectories of soil health indicators remain poorly understood.

To address these knowledge gaps, we retrieved archived soils from a cover crop management study in Florida and compared the values of WEOC, WEN, Cmin, and POX-C before and after multi-year storage. This Controlled Storage Experiment directly quantified the storage effects by comparing original and reanalyzed measurements from the same archived samples. We hypothesized that multi-year storage would increase the concentrations of WEOC and WEN. We then tested whether these storage effects could be detected in other systems in a Multi-site Validation Experiment. Using archived soils from the Florida study and four long-term agricultural trials, we compared the temporal trajectories of WEOC with those of bulk C in samples collected throughout each experiment. Because bulk C is robust against long-term storage (Blake et al., 2000; Kühnel et al., 2019), we hypothesized that storage effects would cause WEOC trajectories to diverge from those of bulk C.

2 Materials and Methods

2.1 Soil sampling

In the Controlled Storage Experiment, soils were collected in 2019, 2020, and 2021 from a three-year cover crop study at the Field and Fork Farm, University of Florida, Gainesville, FL. Site conditions, soil information, and experimental details are described in Nyabami et al. (2024). We analyzed archived soils from plots planted with pearl millet (*Pennisetum glaucum* (L.) R. Br.), sunn hemp (*Crotalaria juncea* L.), and a mixed treatment consisting of two grasses: sorghum sudangrass, (*Sorghum bicolor* × *S. bicolor* var. sudanense) and pearl millet with two legumes: sunn hemp and cowpea (*Vigna unguiculata* Walp.). The number of replicates for each of the cover crop treatment is four. Surface soils (0–15 cm) were collected each year prior to summer cover crop planting. Soil samples were air-dried in a climate-controlled laboratory on open trays until a constant mass was achieved before sieving and archiving. Samples were then stored in airtight plastic bags at controlled room temperature (i.e., 24 °C) in the lab.

For the Multi-site Validation Experiment, additional soils were obtained from four long-term agricultural studies in the Midwest USA: the W.K. Kellogg Biological Station Long-Term Ecological Research site (KBS LTER) and Great Lakes Bioenergy Research Center (GLBRC) in Hickory Corners, MI; the Northern Great Plains Long-Term Agroecological Research site (NGP LTAR) near Mandan, ND; and the Eastern Nebraska Extension and Education Center (ENREEC) near Ithaca, NE. Site characteristics, including soil texture, establishment year, cropping system, and sampling depth, are summarized in Table 1. The biologically based treatment at KBS LTER is a certified organic system managed without synthetic inputs, receiving N from winter cover crops and using chisel plowing with mechanical weed control (Paul et al., 2015). The KBS GLBRC system consists of perennial switchgrass (*Panicum virgatum*) monocultures. Soils at both KBS sites are moderately fertile, well-drained loamy mesic Typic Hapludalfs derived from Kalamazoo and Oshtemo series and were sampled using hydraulic direct-push samplers. At NGP LTAR, soils from a fallow treatment following spring wheat were collected from Temvik–Wilton silt loam (fine-silty, mixed, superactive, frigid Typic and Pachic Haplustolls) using a Giddings hydraulic probe (Halvorson et al., 2016). At ENREEC, soils from a no-till residue-retained system with corn stover retention were collected from irrigated Tomek silt loam (fine, smectitic, mesic Pachic Argiudoll) and Filbert silt loam (fine, smectitic, mesic Vertic Argialboll) (Schmer et al., 2014). Soil sampling depths differed across these sites, as they were independently managed. After sampling, these soils were air-dried under controlled temperature conditions in the lab to constant mass prior to archival storage.

2.2 Soil analysis

To evaluate storage effects on soil health indicators, archived soils from the Controlled Storage Experiment were reanalyzed in 2025 using the same protocols as reported previously in Nyabami et al. (2024) and then compared with their values generated in 2021–2022. WEOC, WEN, and Cmin were first analyzed in 2022 across all samples, and POX-C was measured in two batches: 2019–2020 samples were analyzed in 2021, whereas 2021 samples were analyzed in 2022. WEOC and WEN were extracted by shaking 3 g soil with ultrapure water (1 : 10 $m : v$) for 1 h, followed by centrifugation, filtration (Whatman #42), and analysis using a Shimadzu TOC-L/TNM analyzer (Kyoto, Japan). Cmin was determined by rewetting 10 g of air-dried soil and measuring headspace CO_2 accumulation in sealed 236 mL mason jars after 24 h using an LI-830 CO_2 analyzer (LI-COR Environmental, USA). POX-C was quantified by reacting 2.5 g soil with 18 mL ultrapure water and 2 mL 0.02 M $KMnO_4$ for 10 min followed by colorimetric determination (Agilent BioTek Synergy H1 reader, USA). Changes in these indicators were quantified as differences between original (2021–2022) and reanalyzed (2025) values from the same archived samples, thereby isolating storage effects from field-driven temporal variability. The residual moisture content was analyzed using a subset of the archived samples, where 10 g soil was oven dried at 105 °C for 24 h, by when a constant mass was achieved. The water content was then expressed as a percent of the weight of the oven-dried soil samples. Archived soils from the Multi-site Validation Experiment were sieved (< 2 mm), finely ground, and analyzed for SOC using a Costech CHNS elemental analyzer, and WEOC was determined in 2023 using the same extraction protocol described above.

2.3 Data analysis

In the Controlled Storage Experiment, paired t-tests ($\alpha = 0.05$) were used to compare indicator concentrations before and after storage. Percent changes were calculated relative to the first measurement year (2021 or 2022 depending on indicator). Two-way ANOVA evaluated effects of sampling year and cover crop treatment on percent change, with Tukey HSD used for post-hoc comparisons when interactions were significant. In the Multi-site Validation Experiment, we compared the temporal trajectories of the SOM and WEOC values reported in Nyabami et al. (2024) using Pearson correlation analysis between sampling time and soil properties. We repeated this analysis for SOC and WEOC in archived soils from the four long-term agricultural studies (Table 1). These trajectories reflect the combined effects of management practices, natural variability in field conditions, and multi-year storage. Since bulk C is robust against storage, we expected storage effects to cause WEOC trajectories to diverge from those of bulk C. All data analyses were completed in R (version 4.5.1).

3 Results

3.1 Controlled Storage Experiment

Three years of storage significantly altered water-extractable pools (Fig. 1). Across sampling years and cover crop treatments, WEOC increased by 96 % ($p < 0.001$), whereas WEN declined by 19 % ($p < 0.001$). Two-way ANOVA indicated that sampling year, but not cover crop treatment, influenced WEOC percent change ($F_{(2,27)} = 10.96$, $p < 0.001$), with larger increases in 2020 and 2021 than in 2019 (Table S1 in the Supplement). WEN percent change was also driven by sampling year ($F_{(2,27)} = 49.72$, $p < 0.001$) without treatment effects.

Cmin increase was less pronounced compared to WEOC, by 5.7 % ($p < 0.05$), with significant temporal variation ($F_{(2,27)} = 75.94$, $p < 0.001$) but no treatment effect (Table S1). Storage increased Cmin relative to 2021 but slightly decreased it when compared to 2019–2020 values. In contrast, POX-C did not change significantly overall ($p = 0.64$), although a treatment × year interaction was detected ($F_{(4,27)} = 3.54$, $p = 0.019$) with effects limited to specific treatments (Tables S1 and S2). Using a subset of archived samples, the residual moisture contents averaged 0.26 % ($n = 9$; range: 0.20 %–0.32 %; CV = 16 %). This value is consistent with expectations for coarse-textured sandy soils, which retain substantially less residual water than fine-textured soils (Poeplau et al., 2015; Wäldchen et al., 2012).

3.2 Multi-site Validation Experiment

Across the five studies, WEOC showed consistent declines over time whereas bulk SOC/SOM trends varied among sites (Fig. 2). In the Florida study, SOM showed no temporal trend ($r = 0.19$, $p = 0.264$) but WEOC declined significantly over time ($r = -0.60$, $p < 0.001$). Similar patterns occurred across long-term studies: SOC increased slightly over time at KBS LTER ($r = 0.36$, $p < 0.05$), declined marginally at KBS GLBRC ($r = -0.52$, $p < 0.10$), and showed no trend at NGP LTAR or ENREEC, whereas WEOC declined significantly at all sites ($r = -0.73$ to -0.84; $p \leq 0.007$).

Table 1. Site characteristics for long-term agricultural studies.

Site	Location	Soil texture	Depth (cm)	Established	Treatment (No. of replicates)	Cropping system
KBS LTER	MI	Loam	0–25	1989	Biologically based (4)	Corn-soy-wheat
KBS GLBRC	MI	Loam	0–10	2008	Perennial (4)	Switchgrass
NGP LTAR	ND	Silt loam	0–15.2	1993	Fallow (3)	Spring wheat
ENREEC	NE	Silt loam	0–15.2	2000	No-till/residue retained (3)	Continuous corn

4 Discussion

4.1 Soil storage effects differed among soil health indicators

Multi-year storage nearly doubled WEOC concentrations (Fig. 1), suggesting that even a few years of storage could lead to drastic increases in WEOC. This WEOC increase is consistent with studies of both short-term drying and decades-long soil archives (Bolan et al., 1996; Halvorson et al., 2025; Jones and Willett, 2006; Sun et al., 2015). Together these results indicate that WEOC is not a stable C pool, and its concentration could increase with storage time spanning from weeks to decades. These increases likely result from physical disruption, microbial cell lysis, and continued enzymatic depolymerization during drying and storage (Geisseler et al., 2011; Schimel et al., 2017; Sun et al., 2015). Even low levels of residual moisture might support microbial transformation of organic matter. Drying may also alter mineral equilibria and SOM solubility (Bartlett and James, 1980), increasing WEOC when the soil was rewetted. In contrast, after rewetting for analysis, WEN declined during storage (Figs. 1 and S1 in the Supplement), contrary to typical post-rewetting increases reported previously (Halvorson et al., 2025; Jones and Willett, 2006). This decrease may reflect microbial immobilization or sorption of released organic N onto mineral surfaces.

The increase in Cmin after wetting archived air-dried soils (Fig. 1) and significant percent change in 2021 (Figs. 1 and S2) are consistent with the well-known Birch effect, whereby rewetting mobilizes previously protected substrates and stimulates microbial respiration (Beem-Miller et al., 2021; De Nobili et al., 2006; Mikha et al., 2005; Singh et al., 2023; Warren, 2016). However, the POX-C concentrations did not significantly change after soil archiving (Figs. 1 and S2). This finding is consistent with previous results showing that POX-C was unaffected by air-drying (Gasch et al., 2020; Hurisso et al., 2016). This stability likely reflects that POX-C measures a readily oxidizable C pool that is primarily particulate and light-fraction organic matter with phenolic and polyphenolic composition, rather than the labile substrates rapidly consumed by microbes. Recent studies have questioned its interpretation as an indicator of biologically active carbon (Christy et al., 2023; Vellenga et al., 2025; Woodings and Margenot, 2023).

Storage effects on WEOC, WEN, and Cmin were independent of cover crop treatment (Table S1), suggesting that cover crop composition or tissue chemistry did not influence storage effects. For WEOC, storage effects were stronger in soils collected in 2020 and 2021 than those in 2019 (Figs. 2 and S1). We speculate that air-drying introduces a strong artifact on WEOC, but over time this artifact becomes smaller, which helps explain the stronger effects in recent soils. For example, samples collected in 2019 were first analyzed in 2022, meaning that the changes in WEOC occurred after the initial three-year storage period. In contrast, the 2021 samples experienced a shorter interval between collection and first analysis, making them more susceptible to the immediate artifact effects associated with air-drying. For Cmin, we observed positive storage effects in the 2021 samples but negative effects in older samples. It is possible that long-term storage alters microbial community in ways that reduces its ability to utilize organic substrates upon rewetting. Similar effects have been reported in soils after extended drought (Schimel et al., 1999).

4.2 Storage effects explain divergent WEOC and SOC trajectories

The Multi-site Validation Experiment further suggests that storage effects observed in the Controlled Storage Experiment are not unique to a single soil or management system. Across all five sites, WEOC decreased consistently with sample age (Fig. 2), regardless of their corresponding trends in SOC or SOM. This divergence in temporal trajectories between WEOC and bulk C is indicative of an additional process influencing WEOC measurements beyond actual changes in soil C. The Controlled Storage Experiment provides a plausible explanation for this pattern by demonstrating that prolonged storage increased WEOC concentrations. As a result, the oldest archived samples, which experienced the longest storage durations before analysis, would be expected to exhibit the greatest storage-induced increases, producing an apparent decline in WEOC over time. Although the observational nature of our data does not permit storage effects to be isolated from all other factors, the consistent divergence between WEOC and bulk C across multiple studies supports the hypothesis that storage artifacts can substantially bias retrospective analyses of archived soils. This finding is consistent with the short-term effect of air-drying (Sun

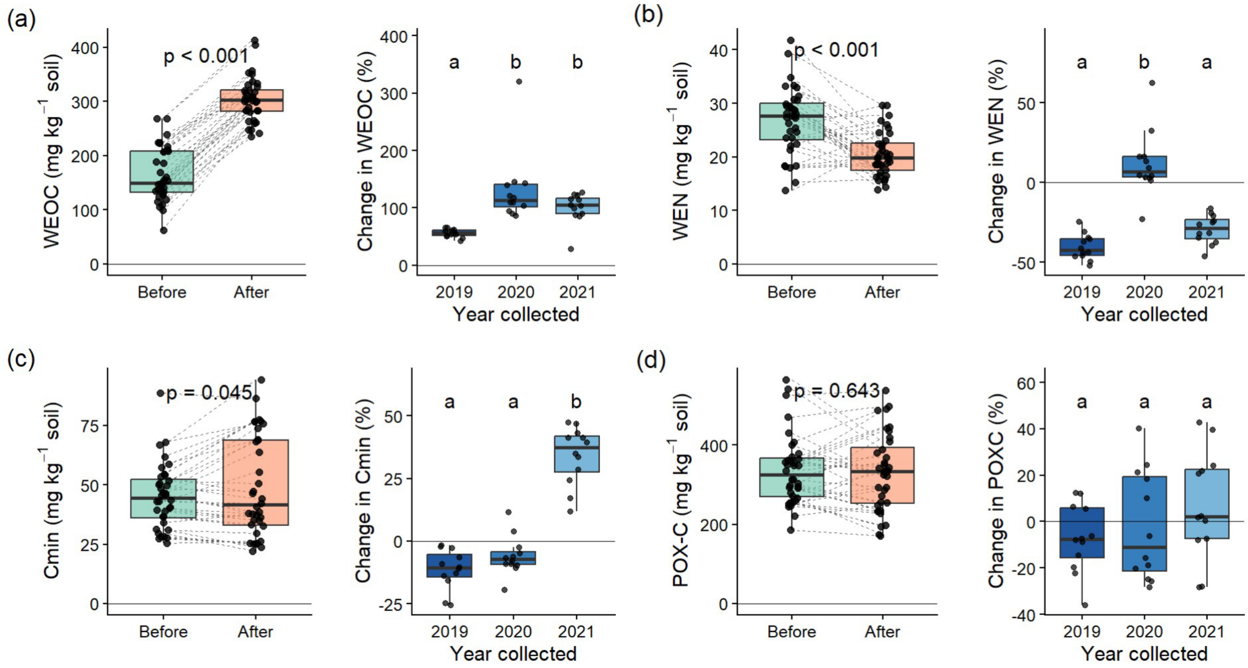

Figure 1. Soil storage increased WEOC **(a)** and Cmin **(c)**, and decreased WEN **(b)** concentrations, while no significant change was observed for POX-C **(d)** concentrations, where the paired observations are represented by dotted lines, and p-values from paired t-tests are also shown. Percent change analysis revealed that POX-C concentrations were stable, while other indicators changed year-to-year, where years sharing the same lowercase letter are not significantly different (Tukey HSD, $\alpha = 0.05$).

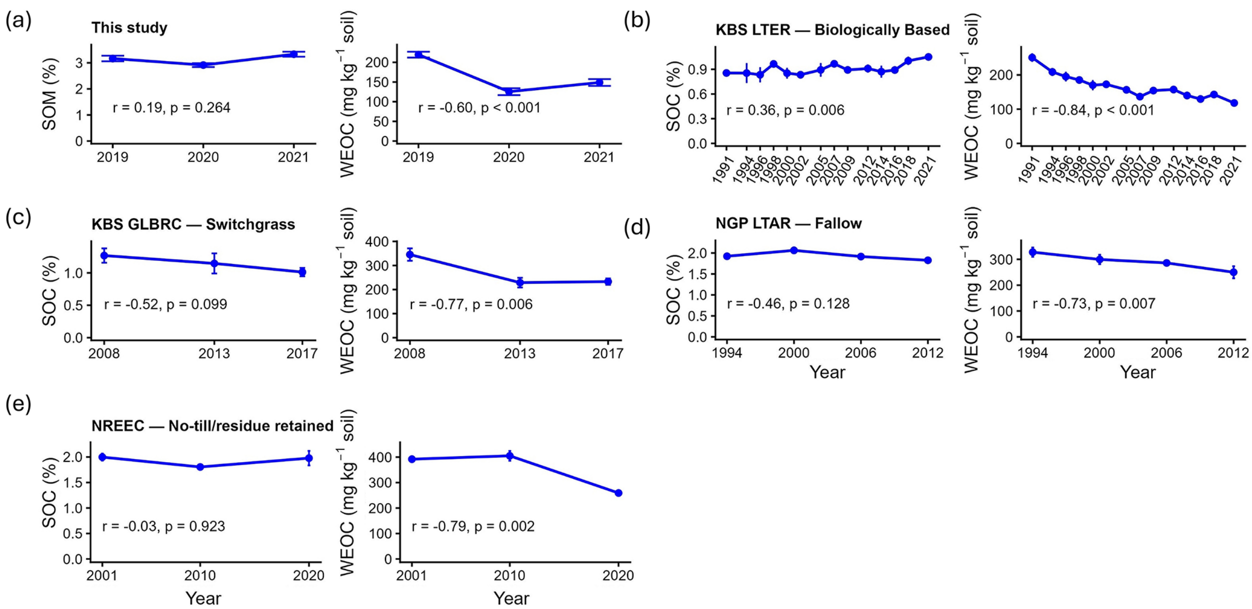

Figure 2. The WEOC showed decreasing trends in all the studies, while the SOC trend shows similar decreasing trends for the KBS GLBRC, and significant increase for the KBS LTER, other SOC or SOM trends were stable. The Pearson correlation coefficients (r) with the corresponding p-values are shown.

et al., 2015) and the effects of decadal storage (Halvorson et al., 2025; Waldrip et al., 2022). Nevertheless, there might not be a complete decoupling between the temporal trends of bulk C and WEOC, as both showed declining trends at the KBS GLBRC (Fig. 2c).

4.3 Implications for soil assessment

Soil archives are valuable resources for measuring the long-term impacts of management practices and guiding the development of novel soil health indicators, as analytical methods are proposed and refined over time (Bergh et al., 2022). However, storage-induced changes in these indicators can obscure the interpretation of temporal trajectories associated with management. Our finding indicates that labile carbon indicators may be more susceptible to storage-induced changes than bulk carbon measurements and underscores the importance of accounting for sample storage history when interpreting long-term soil health trends from archived soils. The consistent increase of WEOC concentrations after multi-year storage compromises its use as a soil health indicator in archived soils. For these labile indicators, drying at controlled temperature and/or cold storage could be further explored. We also caution against using WEOC to infer long-term trajectories in C storage or composition, as storage artifacts likely vary with time. In contrast, POX-C pool remained stable during multi-year storage, underscoring its potential for assessing long-term management impacts in archived soils. This offers new insights into the ongoing debate on the interpretability and practicality of POX-C as a soil health indicator (Gasch et al., 2020; Margenot et al., 2024; Woodings and Margenot, 2023).

As soil health datasets are often constructed from samples collected over extended periods, temporal disparities in storage time introduce a potential artifact. Prolonged storage, even under controlled conditions, can alter microbial community structure and activity, potentially leading to the continued mineralization of labile carbon pools or, conversely, the release of organic carbon through cell lysis. Our unique findings about the potential influence of sample storage artifact effects on WEOC concentrations suggest that this labile carbon pool should be analyzed immediately upon collection.

5 Conclusions

This study demonstrates that the selection of soil health indicators for use with archived, air-dried samples requires careful consideration of their susceptibility to storage artifacts. Doubling of WEOC after multi-year storage undermined its reliability as a soil health indicator when using stored samples. The consistent increase in WEOC over time across diverse management systems indicates that its temporal trend in archives is confounded, making it an unreliable proxy for long-term bulk C dynamics. In contrast, the stability of POX-C over multi-year storage underscores its utility for retrospective studies. Therefore, our results caution against the use of storage-sensitive indicators like WEOC in archived samples.

Data availability. Data required for reproducing the statistical analyses and figures are available at https://doi.org/10.5281/zenodo.22696632 (Mahmood et al., 2026).

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