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

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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 and four long-term agricultural sites in the USA, but 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

35 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. Although bulk SOC and N pools remain relatively stable during long-term storage (Blake et al., 2000; Kühnel et al., 2019), the quantity and

40 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 (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

45 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). Drying temperature and vegetation type can further modify these responses (Leeford et al., 2023). 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

50 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. We then compared the temporal trend of WEOC with that of bulk C using these air-dried archived soils, as well as those from four long-term agricultural experiment sites in the Midwest, USA. This study aimed to address two questions: (1) How do soil health

55 indicator values vary after storage? (2) Can the temporal trend of water-extractable C pool capture the trend of bulk C?

2 Materials and Methods

2.1 Soil sampling

Soils for the cover crop study were collected in 2019, 2020, and 2021 from a three-year cover crop experiment at the Field and Fork Farm, University of Florida, Gainesville, FL. The site includes Lake Sand (hyperthermic, coated Typic

60 Quartzipsamments) and Arredondo fine sand (loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults) with 0–8% slope and a subtropical climate with mean annual precipitation of 1270 mm (Nyabami et al., 2024). The site was previously a bahiagrass (*Paspalum notatum* Flüggé) and native grass meadow before conversion to an agricultural research field in May 2019. Experimental details are described in Nyabami et al. (2024). We analyzed archived soils from plots planted with pearl

65 millet (*Pennisetum glaucum* (L.) R. Br.), sunn hemp (*Crotalaria juncea* L.), and a mixed cover crop consisting of two grasses (sorghum sudangrass, *Sorghum bicolor* × *S. bicolor* var. sudanense; pearl millet) and two legumes (sunn hemp and cowpea, *Vigna unguiculata* Walp.). Surface soils (0–15 cm) were collected each year prior to summer cover crop planting, air-dried, and stored in sealed airtight plastic bags at controlled room temperature (i.e., 24°C) in the lab.

Additional soils were obtained from four long-term agricultural experiments in the Midwest USA: the W.K. Kellogg Biological Station Long-Term Ecological Research Site (KBS LTER) and Great Lakes Bioenergy Research Center
70 (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
75 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
80 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.

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

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

2.2 Soil analysis

85 To evaluate storage effects on soil health indicators, archived soils were reanalyzed in 2025 using the same protocols as reported previously in Nyabami et al. (2024). These measurements were compared with values generated in 2021–2022. WEOC, WEN, and Cmin were first analyzed in 2022 across all samples, whereas POX-C was measured earlier for the 2019–2020 samples (2021) and for the 2021 samples 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
90 analyzer (Kyoto, Japan). Potentially mineralizable C (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). Archived soils from long-
term experiments were sieved (<2 mm), finely ground, and analyzed for SOC using a Costech CHNS elemental analyzer.
95   WEOC was determined using the same extraction protocol described above.

2.3 Data analysis

Paired t-tests (α = 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
100   interactions were significant.

We also compared the temporal trends of SOM and WEOC using Pearson correlation analysis between sampling time and
soil properties values reported in Nyabami et al. (2024). We repeated this analysis for SOC and WEOC in archived soils
from the four long-term agricultural experiments (Table 1). All data analyses were completed in R (version 4.5.1).

3 Results

105   **3.1 Changes in soil health indicator concentrations**

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 (Supplementary Table S1). WEN percent change was also driven by sampling year
110   ($F_{(2,27)}$ = 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 (Supplementary Table S1). Storage increased Cmin in 2021 but slightly decreased
it in 2019–2020. 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 (Supplementary Tables S1 and S2).

115   **3.2 Trends of bulk C and WEOC**

Across the five studies, WEOC showed consistent temporal declines in archived datasets whereas bulk SOC/SOM trends
varied among sites (Fig. 2). In the cover crop experiment, SOM showed no temporal trend (r = 0.19, p = 0.264) but WEOC
declined significantly (r = −0.60, p < 0.001). Similar patterns occurred across long-term experiments: SOC increased slightly
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
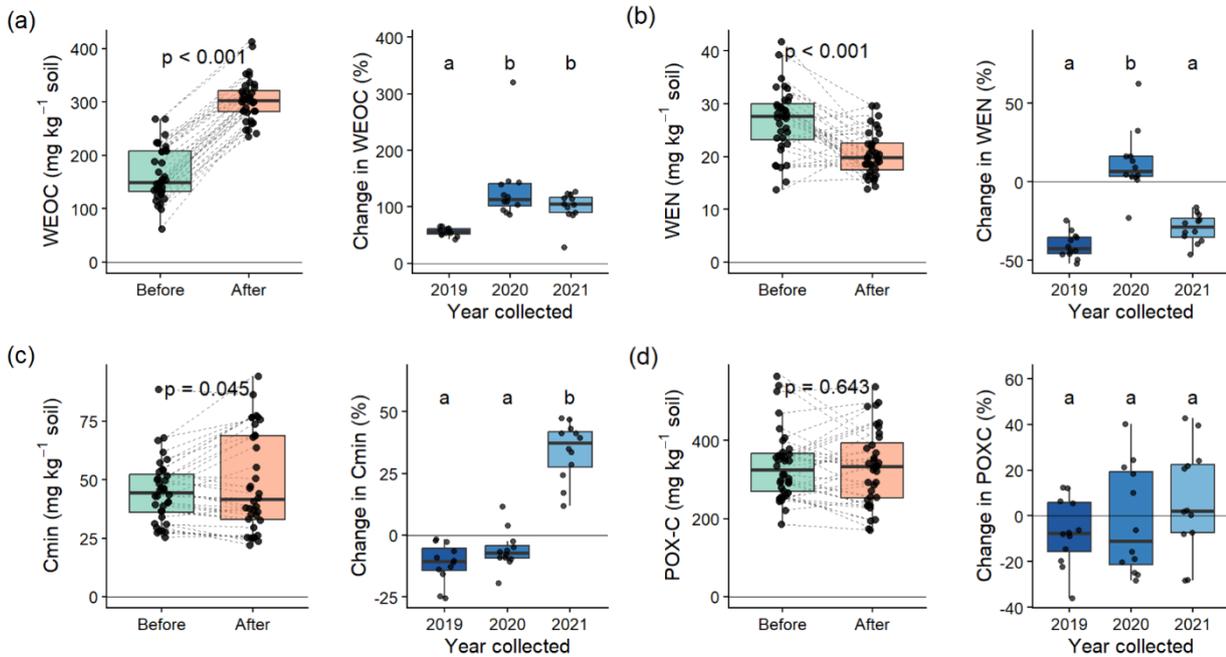120   LTAR or ENREEC, whereas WEOC declined significantly at all sites (r = −0.73 to −0.84; p ≤ 0.007).

Figure 1: Soil storage increased WEOC and Cmin, and decreased WEN concentrations, while no significant change was observed for POX-C concentrations, where the paired observations are shown 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).
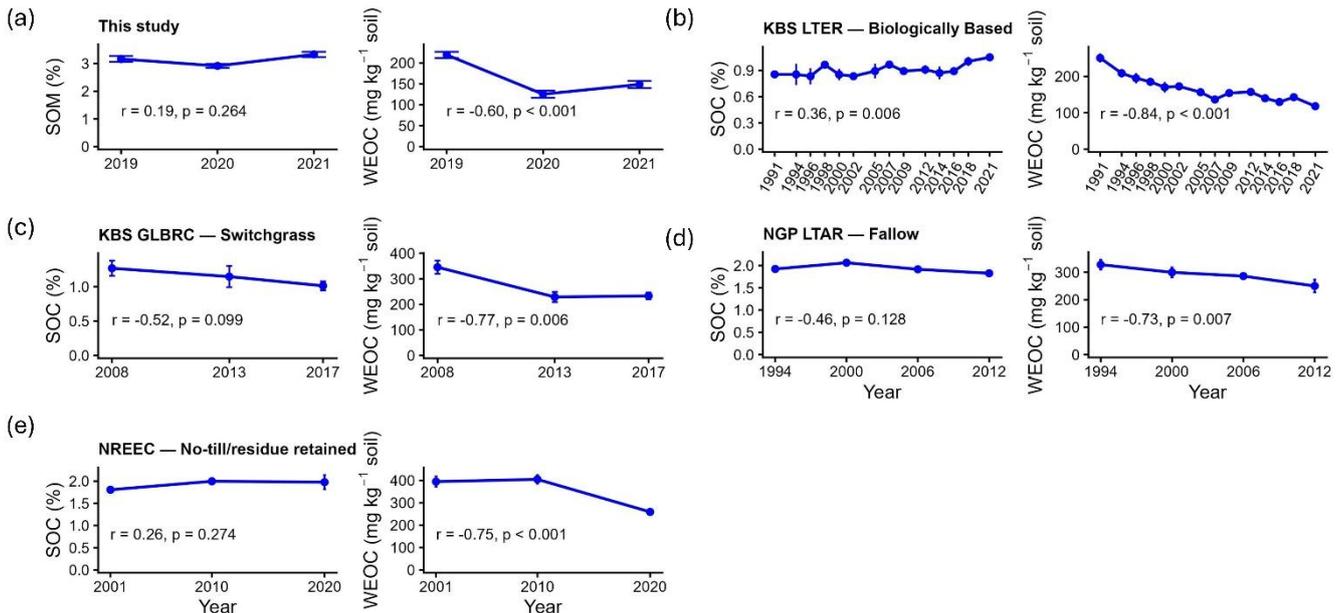

Figure 2: The WEOC trends showed decreasing trends in all the studies, while the SOC trend shows similar decreasing trends for the KBS GLBRC, and significant increase for the KBS LTER, other SOC or SOM trends were stable. The Pearson correlation coefficients (r) with the corresponding p-values are shown.

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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). Drying may also alter mineral equilibria and SOM solubility (Bartlett and James, 1980), increasing WEOC when the soil was rewetted. In contrast, WEN declined during storage (Fig. 1 and Supplementary Fig. 2), 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 (Fig. 1 and Supplementary Fig. 2) 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 (Fig. 1 and Supplementary Fig. 2). 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 is due to what POX-C measures, which is an indirect, chemical measure of a readily oxidizable C pool that is primarily particulate and light-fraction organic matter, including slower decomposing, complex molecules, such as lignin, rather than sugars and starches that decompose quickly by microbial decomposition (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 (Supplementary 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 (Fig. 2 and Supplementary Fig. 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 Sample storage duration confounded WEOC concentrations

The temporal trends of WEOC inferred from archived soils did not necessarily indicate those of bulk C (Fig. 2). We observed that the bulk SOC/SOM temporal trends varied across sites. For example, the KBS LTER showed increase over time, and KBS GLBRC showed decrease over time, while the other sites showed no significant effect of time. The consistent

165 decline in WEOC over time across all sites, regardless of their corresponding trends in total SOC or SOM, suggests that WEOC pools are more sensitive to soil storage: the longer the storage time, the higher the WEOC concentration. A similar proportional increase of WEOC in air-dried archived soils from freshly sampled soils was reported by Sun et al. (2015). Paired air-dried archived vs. fresh soils from the same plots also resulted in similar patterns in the Northern Great Plains and Texas High Plains soils in the USA (Halvorson et al., 2025; Waldrip et al., 2022), highlighting the storage effects on WEOC.

170 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.

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).

175 However, storage-induced changes in these indicators can obscure the interpretation of temporal trends associated with management. The consistent increase of WEOC concentrations after multi-year storage compromises its use as a soil health indicator in archived soils. We also caution against using WEOC to infer long-term trends 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.

180 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.

185 ## 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

190 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

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Supplement link

195 The link to the supplement will be included by Copernicus.

Author contributions

SM: investigation, data curation, formal analysis, visualization, writing – original draft, writing – review & editing, FC, DAHDSL: investigation, data curation, REC, XW, JBD, AP: investigation, SCC, CDS: funding acquisition, writing – review & editing, GML: funding acquisition, investigation, writing – review & editing, YL: conceptualization, funding acquisition, 200 supervision, writing – review & editing.

Competing interests

The authors declare no conflict of interest.

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