



Redox potential as a soil health indicator – how does it compare to microbial activity and soil structure?

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Abstract

Background and aims Redox potential is a promising soil health indicator, which integrates the combined effect of chemical oxidation–reduction reactions into a single measurement. However, this method has been tested only on a few soils. The aim of this study was to test redox potential as a soil health indicator, to see if it responds to management practices and to compare it with existing soil health metrics for microbial activity (“CO₂burst”) and soil structure.

Methods We collected 35 soil samples in agricultural fields from a carbon farming trial, where contrasting management practices have been applied to increase carbon stock. The soil samples were dried, rewetted and analyzed for redox and microbial respiration during rewetting. In addition, soil structure, texture and organic matter content was measured. The data was analyzed for correlations between the indicators and for the differences between management and control fields.

Results Redox was well correlated with microbial activity, structure, and texture. A low redox state was connected to high microbial activity, indicating bioavailable organic matter availability. Soils with good structure had an oxidized redox status, possibly

reflecting high gas-transport. The carbon farming practices resulted in lower oxidation, possibly due to build-up of plant residues.

Conclusions The findings supported the use of redox as a soil health indicator, but highlighted further research needs for identifying the shared mechanisms linking structure, redox and microbial activity. As such, redox can be a low-cost additional measurement to map changes in soil health, but it cannot replace existing structure or microbial activity measurements.

Keywords Oxidation–reduction · Soil management · Soil quality · Soil amendments

Abbreviations

OM	Organic matter
rH ₂	Relative hydrogen score
VESS	Visual evaluation of soil structure
Eh	Redox potential

Introduction

The capability of soil to provide ecosystem services through four soil functions: carbon cycle, nutrient cycle, pest regulation, and soil structure maintenance (Kibblewhite et al. 2008) is defined as “soil health.” It is closely related to soil quality and productivity but is more integrative and focused on soil biological functions (Lal 2016). These can be quantified

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through process indicators (e.g. carbon metabolism: basal respiration, CO₂burst test, aerobic and anaerobic incubations) (Weil and Brady 2016). One of the most commonly used functional indicators is the 24 h CO₂ respiration test for microbial activity (Franzuebbers et al. 1996). In this method, a soil sample is dried and rewetted, which results in a flush of CO₂ (“CO₂burst”). Physical, chemical, and biological soil processes control the flush of CO₂ (Barnard et al. 2020); therefore, it is an integrative indicator for many soil properties, but the exact mechanisms have puzzled research for decades (Birch 1958; Hicks et al. 2022). The CO₂burst has two valuable properties: i) it responds to management and ii) it reflects nitrogen mineralization, making it important for fertilizer recommendations. Despite the unclear mechanisms, CO₂burst has become an important tool for planning fertilizer recommendations (Haney et al. 2018).

While the CO₂burst took 50 years from the description of the process to its application in management, soil redox potential had a faster development cycle. A review by Husson (2013) brought redox from the use in submerged and paddy soils to aerated mineral soils and highlighted its importance for plant and soil health. Subsequent development has focused on improving measurement (Husson et al. 2016), tracking management-induced changes (Husson et al. 2018), and evaluating the role of redox potential for plant health (Husson et al. 2021). Most of the work has been done by a single research group and only on a few soils and locations, but there is increasing interest among agriculturalists in using redox as an integrative measurement of soil and plant condition (Husson et al. 2021). The redox is a relatively easy measurement, a sensor is pressed to a dried and rewetted sample and results are achieved in 1–2 min.

The redox potential of soil measures the general availability of electrons, or the relative difference between oxidation (loss of electrons) and reduction (gain of electrons) (Zhang and Furman 2021). In soils, one of the main reactions is the oxidation of organic matter ((CH₂O)_n), which supplies electrons. In fully aerobic soils, oxygen serves as the electron acceptor, resulting in the production of CO₂ and H₂O. For example, in rewetted agricultural soil, redox is a strong predictor of CO₂ flux (Bartolucci et al. 2021). When oxygen availability is low (inside aggregates) or when organic matter supply is high,

redox may decrease and other electron acceptors can also be used simultaneously with O₂, resulting in manganese, iron, and nitrogen reduction (Marschner 2021) and improved soil nutrient availability for plants (Husson 2013). Based on this, redox could be a promising indicator for the soil carbon cycle (labile carbon pools) and structure (oxygen availability), two key components of soil health (Kibblewhite et al. 2008).

Redox can also serve as an indicator of nutrient availability and pest regulation (Husson 2013). Redox can be thought of as a parallel to pH which measures proton availability whereas redox (Eh) measures electron availability. Like pH, also redox can influence nutrient availability considerably: redox is managed to avoid toxic As and Cd buildup in rice paddies (Evans et al. 2021) and to improve Mn supply (Husson 2013; Zhang and Furman 2021). However, a key challenge for using redox as an indicator is that it changes rapidly with soil water and oxygen conditions (Zhang and Furman 2021). To standardize the evaluation of a soil, Husson et al. (2016) proposed the use of redox potential as a soil test from dried soil samples. In the proposed test, dried soil was rewetted to field capacity and the redox was measured for 2 min. In theory, the test is similar to the CO₂burst test, but takes only 2 min instead of 24 h. It therefore has great potential as a high-throughput indicator for soil health, but it should be tested in different soils and management and also compared with established soil tests.

To evaluate redox as a soil biological health indicator, we compared it with commonly used indicators, focusing on carbon cycling (CO₂burst) and structure (visual evaluation of soil structure VESS, Ball and Munkholm 2015). It was also compared to soil organic matter, which is a key driver for both biological activity and structure maintenance (Weil and Brady 2016). We used 20 sites from an ongoing carbon sequestration experiment (Carbon Action experiment 2019–2023, described in Mattila et al. 2022), where each site had a carbon farming trial plot and a control plot. The aim was to see if i) redox could be explained by microbial activity and soil structure or if it is a new complementary measure of soil health and ii) if three years of management had an effect on the measured soil health indicators.

Materials and methods

The materials for the study were soils sampled from the Carbon Action experiment (Mattila et al. 2022) intensive observation set of farms (Mattila 2020). The intensive observation farms are located in a 200×500 km area, which covers the main agricultural areas of Finland (ranging from 63.18 N to 60.36 N in latitude). The farms tested five carbon sequestration practices (cover crops, compost, grazing practices, leyfarming, and subsoiling). The experiment started in the spring of 2019 and the samples were collected in July 2021, the third growing season of the experiment. Each farm had a split field, where one side had carbon farming practices implemented and the other was held as a continued normal-practice control. Both sides of each field were sampled using a composite sample of pooled soil cores. Samples for each field side were collected around 3 GPS reference points from a 10 m radius, resulting in 30 cores (16 mm diameter, 0–17 cm sample depth), which were pooled to a composite sample.

The composite samples were used in the experiment monitoring for routine soil analysis (organic matter, clay, pH, nutrients; Mattila and Girz 2021; presented in Table 1), but for this study we also took a 100 ml part of the sample for redox and CO₂burst assessment. This allowed comparing redox with existing indicators as well as with soil properties and management.

The collected samples were dried at room temperature and gently sieved through a 5 mm sieve following the established guidelines for CO₂burst (Franzliebbers and Haney 2018; Woods End Laboratories 2019). (Due to an unfortunate laboratory accident, 5 samples were lost during processing, resulting in an overall sample amount of $n=35$. The lost samples resulted in the loss of two farm replicates in both treatment and control and one treatment sample from one farm. The farm with

only one sample was ignored when calculating the statistical treatment effect, but was included in the correlations with other metrics.) The sampled soils covered a large range of soil texture and organic matter: the median clay content was 35% (4–63%) and the OM 6.8% (2.6–15.5%). The farming systems covered annual cropping, grass in rotation, and perennial pastures.

For CO₂burst analysis, the dry samples were rewetted to approximately 50% pore space (i.e. 30 ml of soil and 9 ml of water; Woods End Laboratories 2019). The sample was placed in a 475 ml container and sealed with a CO₂ respirometer cap (Woodsend IRTM). The CO₂ concentration was measured for 24 h at 5 min intervals with the integrated infrared cell-datalogger and the increase in CO₂ level (ppm) over 24 h was converted to mg CO₂-C/kg³ soil by multiplying with container air space and dividing by sample mass (26–39 g/30 ml sample, average 31 g).

For the redox analysis, 30 ml of soil was also rewetted to 50% pore space by adding 9 ml water. This followed the 30% humidity recommendation for measuring redox from rewetted soils in Husson et al. (2016) and used in Husson et al. (2018). The redox was measured using an Extech RE300 Exstik Platinum oxidation–reduction-potential (ORP) sensor (platinum electrode, silver/chloride reference electrode). The flat end ORP sensor was pressed to the moist soil sample and allowed to stabilize 1–3 min, until the ORP reading changed only slowly. Three repeated measurements were made of the same sample and the average value was used. The sample pH was measured with a Horiba LAqua Twin pH meter using a 1:1 ratio of distilled water:soil. The ORP reading was converted to Eh (mV) by adding the reference electrode voltage (200 mV) and pH corrected to a relative hydrogen score by the equation $rH_2 = 33.83 \text{ Eh} + 2 \text{ pH}$ (where Eh is in V; Husson et al. 2016). For interpretation, the readings were compared to suggested norms for “healthy” soils (Husson 2013). The Eh value was used for soil classification in the Eh/pH parameter space (Husson 2013). Otherwise the analysis was based on the rH₂ metric which includes both pH and Eh (i.e. a pH corrected redox).

For additional interpretation, the soil samples were classified according to organic matter (OM) content, soil clay content, soil structure (VESS (Ball and Munkholm 2015)), and type of crop (perennial, annual). These were collected and published as

Table 1 Basic soil properties of the Carbon Action experimental fields sampled for this study

	Clay (%)	Organic matter (%)	pH
Median	35	6.8	6.5
Min	4	2.6	5.8
Max	63	15.5	7.6

ongoing monitoring in the Carbon Action experiment (Mattila and Girz 2021). Soil OM and clay content were determined in a commercial lab (Eurofins) by loss-on-ignition and dry sieving, respectively. The VESS analysis was performed in the field, it is a visual soil assessment (VSA) method developed in 2007 from existing methods and used subsequently in hundreds of studies (Franco et al. 2019). Although the method is visual, it gives numerical scores that are repeatable and correlate well with traditional measurements such as bulk density (Johannes et al. 2017). In the VESS, the soil is divided into layers and each layer is scored in a range of 1–5 based on the severity of structural damage. The layer scores are then multiplied by the layer depth and divided by the total depth of the sample to give a depth-weighted average score.

The statistical analysis was done in R programming language (R Core Team 2022). In order to compare redox measurements with other soil health metrics, Spearman's correlation coefficients were calculated between redox, CO₂burst, OM, soil structure, and clay content. These soil indicators range from highly labile (structure) to permanently stable (clay content). CO₂burst is known to respond to OM quality more than to OM amount (Haney et al. 2012). To test if redox could represent organic matter quality in forecasting CO₂burst, a least squares linear regression model was used to explain CO₂burst based on redox (Eh) and OM. If this provided a strong fit, the 24-h CO₂burst could be estimated with a 2-min redox test. To test the effect of carbon farming practices on redox and CO₂burst, a Wilcoxon test was used to test if the difference between control and treatment was larger (CO₂burst) or smaller (redox) than zero.

Results

The redox potential (Eh) of the 35 studied soils ranged from reduced (+340 mV) to oxidized (+560 mV) and the pH ranged from acidic (pH 5.8) to slightly alkaline (pH 7.6) (Fig. 1). Most of the soils were in an Eh/pH space considered favorable for plant growth (pH 5.5–8; Eh +350–500 mV; Husson 2013). One soil was more reduced than the favorable area, it was a seasonally waterlogged silty clay loam with horse manure applied previously. Ten soils were classified as more oxidized than the plant-favorable region: the alkaline and oxidized soil was a low OM

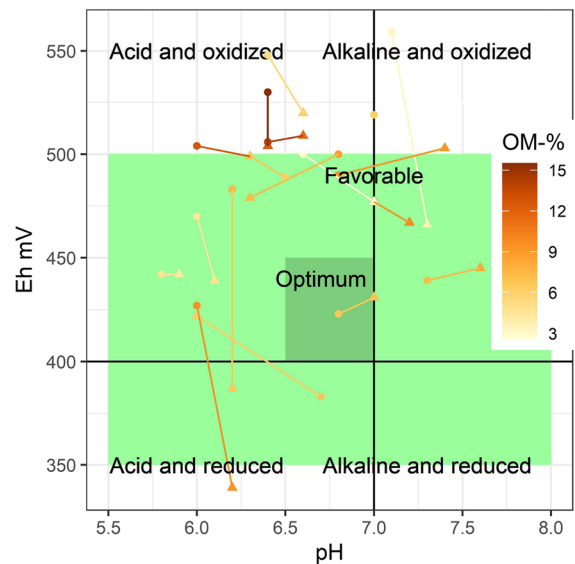


Fig. 1 Tested soils in a pH/Eh Pourbaix plot. Triangles = carbon farming plots, rounds = control plots. Color = OM content. Favorable and optimum regions for plant growth drawn based on Husson 2013

loamy sand, but the acidic and oxidized soils were a diverse group with high OM (>12%) or high clay (>60%).

The carbon farming plots had lower Eh than the corresponding control plots (Figs. 1 and 2): the management-induced reduction was on average -20 mV (Wilcoxon $p=0.039$; 95% confidence interval -90..+40 mV) and the largest reductions (-90 mV) were on fields where compost had been applied.

Soil redox potential correlated with soil properties as well as with two other soil health metrics (soil structure VESS and microbial activity CO₂burst) (Table 2). The correlation with CO₂burst was statistically significant and strong ($p<0.01$; $r=0.44$). Eh explained variability of CO₂burst better than pH ($p=0.17$; $r=0.23$), soil texture ($p=0.04$; $r=0.46$) or OM content ($p>0.10$; $r=0.43$). A linear model of Eh and OM could explain 46% of the variability in CO₂burst, with both input variables being significant ($p=0.01$, $\text{CO}_2\text{burst}=211 \cdot \text{OM} - 220 \cdot \text{Eh}$). The highest microbial activity was found in reduced soil conditions, but some soils with reduced soil conditions had only moderate CO₂burst. Oxidized soils did not have high CO₂burst values, but the microbial activity ranged between moderate and low, suggesting other controls than redox. The carbon farming treatment

Fig. 2 The effect of five carbon farming practices on soil redox potential. (Light shade = control plot; dark shade = carbon farming plot. Dot color = OM content (range 3–16%, legend in Fig. 1). Samples taken in July 2021, on the third growing season of the Carbon Action experiment.)

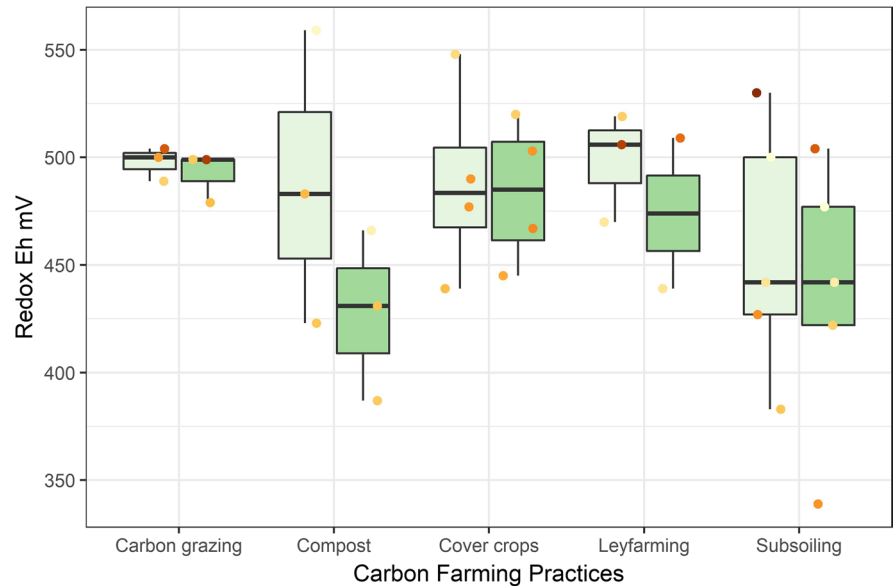


Table 2 Spearman's rank correlation coefficients (r) between soil redox status (Eh and pH corrected rH2) and commonly used soil health indicators (Visual evaluation of soil struc-

ture VESS; CO₂burst; Organic matter; and Clay content). The indicators were arranged from rapidly changing to very slowly changing (Weil and Brady 2016)

		Eh	rH2	CO ₂ -burst	VESS	OM	Clay
Redox	Eh	1.00					
Hydrogen score	rH2	0.81***	1.00				
Microbial activity	CO ₂ burst	-0.44**	-0.43**	1.00			
Structure	VESS	-0.38*	-0.43*	-0.07	1.00		
Organic matter	OM	0.16	0.06	0.42*	-0.47*	1.00	
Texture	Clay	-0.27	-0.24*	0.46**	0.44**	-0.03	1.00
Rate of change		Rapid	Rapid	Rapid	Instant	Slow	Very slow

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

resulted in slightly increased CO₂burst (8 mg CO₂-C/kg, 95% confidence interval -16.68 mg CO₂-C/kg, Wilcoxon test p -value 0.044). The highest increases were in farms that tested improved grazing practices (average 17 mg CO₂-C/kg).

Soil structure had a noticeable effect on the redox potential (Fig. 4) (VESS vs. rH2, $r = 0.43$, $p = 0.04^*$), but almost no effect on CO₂burst ($r = 0.007$). In soils with good structure (VESS < 2.75), the redox status was more oxidized (rH2 30.5) compared to soils with poor structure (VESS > 3.25; rH2 28.6) (Fig. 4). The redox in poorly structured soils was highly variable, with some soils having increased oxidation status. The structure score also correlated with soil texture ($r = 0.44$, $p = 0.008$) and OM

($r = -0.47$, $p = 0.005$) with poorer structure in higher clay and better structure with higher OM levels.

Discussion

Redox potential describes soil conditions and responds to soil management

In this study, redox could be used to evaluate plant growing conditions (Fig. 1), and it also followed management-induced changes (Fig. 2). Redox correlated with microbial activity (CO₂burst, Fig. 3) and soil structure (Fig. 4), making it a promising indicator of soil health. Redox potential is rapid to measure (c.a. 2

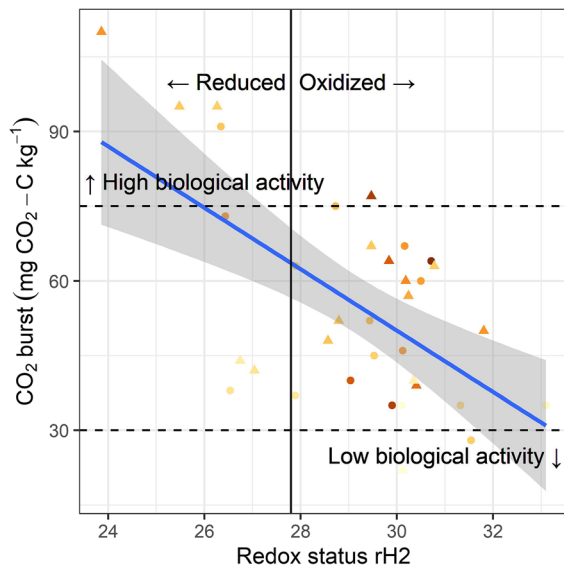


Fig. 3 Soil microbial activity vs. redox status (rH2). The colors represent OM concentration (range 3–16%, legend in Fig. 1) and the shape is the treatment (triangle) vs. control group (round)

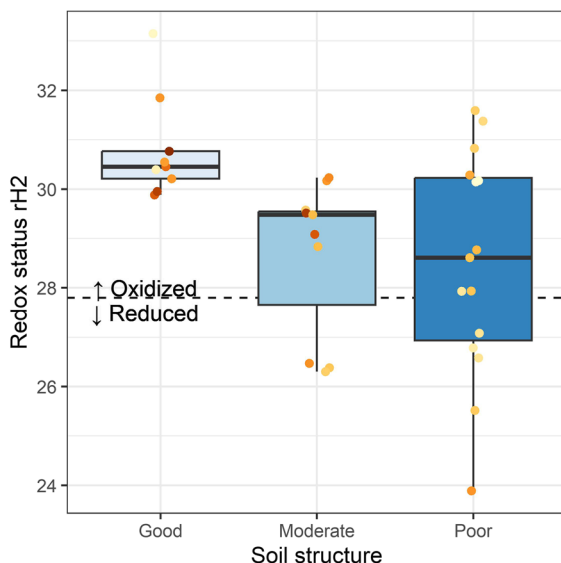


Fig. 4 The redox status (rH2) of soils with a good structure (VESS 1–2.75) was more oxidized than soils with a poor structure (VESS 3.25–5). The colors on the points describe the soil OM concentration (Fig. 1 legend)

min per measurement) and integrates many biological and physical processes. It can serve as an additional

measure for quick screening or used in parallel with existing measures to help interpretation.

Finding a large variation in redox (Fig. 1) in an experiment where dried and sieved soil was rewetted was somewhat unintuitive. One would assume that in an artificial setting, the gas transport would be sufficient to keep the conditions oxidized, especially as the rewetting aimed only to saturate 50% of the pore space (Franzluebbers and Haney 2018). However, the strong correlation with microbial activity (CO₂burst) and the response to management, which added carbon compounds to soil (manures and compost), suggested that the intense microbial activity and high availability of bioavailable carbon compounds can also reduce redox conditions in a well-aerated experimental setting. This confirmed earlier findings, where plant environments could be classified on the basis of redox and pH (Husson 2013). In our study, those soils that fell clearly outside the recommended “favourable” range (pH 5.5–8.0, Eh +350–500 mV) had distinct soil health problems (waterlogging or low OM, weak structure sands). This supported the use of redox as a universal soil health indicator, although the oxidized state of high OM or clay soils in the experiment (upper left corner in Fig. 1) warrants further investigation on the sample pre-treatment and rewetting conditions. Currently, the redox potential is an interesting indicator, but it is still under development, especially when applied to dried and rewetted soil samples. With the currently widely used CO₂burst method, standardizing sample pretreatment has been critical to reduce variability (Franzluebbers and Haney 2018). Similar work needs to be done for redox to ensure application to different textures and OM categories.

The management-induced changes were very similar in our study compared with earlier findings (Husson et al. 2016). Compared to the four soils evaluated in Husson et al. (2016), the soils in this study were more reduced (average +530 vs. +470 mV), which reflects differences in climate (warm vs. cool temperate) and organic matter level (2% vs. 8% average). However, carbon farming management practices resulted in similar redox potential reductions to those found in Husson et al. (2016) for conservation agriculture. In our study, the reduction was on average -20 mV ($p < 0.05$; 95% range -90.. +40 mV). The largest reductions (-90 mV) were on sites that had compost soil amendments applied. This supports the findings of Husson et al. (2016) where conservation

agriculture (residue retention, cover crops and minimum tillage) reduced Eh by -10–25 mV. This suggests that soil redox potential reacts rapidly to the addition of readily decomposable OM. It may take decades before the total soil OM changes enough to detect management effects; therefore, rapid indicators are valuable in guiding management. Microbial activity (CO₂burst) has been used as a rapid indicator that shows change even after a few seasons of different management (Haney et al. 2018; Weil and Brady 2016). In this study, redox showed similar changes, making it a promising indicator for soil carbon cycling.

Interactions between redox, structure, and microbial activity

The significant correlations of redox with microbial activity, structure and texture (Table 2) make interpretation challenging. Further research into the reaction mechanisms of redox decrease during rewetting and the effect of sample pretreatment may be necessary. The group of oxidized and acidic soils in Fig. 1 (upper left corner) also had high clay or OM concentrations. This oxidized reading may therefore be a result of high water holding capacity, where the water applied for rewetting (9 ml water per 30 ml soil) did not sufficiently saturate the pores. On the other hand, the structure has a strong effect on redox (Table 2 and Fig. 4). Some poor-structure soils had high oxidation status, perhaps due to sharp and angular aggregates, which had air gaps during the experiment. Refining the sample pretreatment (milling and sieving) may reduce the variability in the redox of poor structured soils, but it may result in more artificial settings by exposing protected organic matter, as has been found for CO₂burst (Franzluebbers and Haney 2018).

Although structure was reflected in the redox, it can not be used to replace the field assessment of soil structure. Good structure scores were generally found together with oxidized soil redox, but most poor structure soils were classified as oxidized. Only strong oxidation or reduction could be used as an indicator for good or poor soil structure as these extreme values were only found in those structural classes (Fig. 4).

Redox explained variability in microbial CO₂burst better than OM. When redox was combined in a linear model with OM, the two variables could explain

almost half of the variability in CO₂burst. Redox was not significantly correlated with OM as such, suggesting that it may measure a separate pool of bioavailable OM, as has been found for water-soluble OM (Haney et al. 2012). This supports earlier findings that CO₂burst is controlled more by the availability and quality of OM than by the quantity (Haney et al. 2012). Redox has been found to be an important indicator for soil respiration in field rewetting studies (Bartolucci et al. 2021). However, compared to the laboratory, the effect of redox in the field is the opposite: highest respiration is found with oxidized redox status (Bartolucci et al. 2021). In the field with saturated soils, bioavailable OM accumulates and subsequent drying results in both increased respiration and redox. The rewetting study starts from the opposite end of the moisture spectrum, with highly oxygenated and dry soils, where respiration is limited by water. In that setting, oxygen availability is high and the redox is more likely controlled by the availability of readily degradable organic compounds and microbial biomass (Barnard et al. 2020). The CO₂burst test has been able to predict nitrogen mineralization in field conditions (Haney et al. 2018) but scaling the redox potential to field conditions may be challenging because it depends on texture and structure. Introducing textural classes to the interpretation of “favorable” and “optimum” ranges for plant growth (Husson 2013) would make the indicator more broadly applicable.

Overall, these results confirm the earlier results on the use of soil redox potential to measure soil health and track management-induced changes (Husson 2013; Husson et al. 2016). Previous work on redox and soil management was done in a very different climate and soil texture (warm vs. cool temperate climate, low vs. high OM, low clay content). The replication of those findings in cool, high clay and OM environment supports further investigation into the use of redox as a soil health metric. Strong correlations with microbial CO₂burst and soil structure suggest common mechanisms. Further studies into the chemical reactions causing redox reduction and CO₂burst during rewetting could help interpret the results across structural and textural soil categories. At the same time, redox measurement with an oxidation–reduction potential meter can be a low-cost and simple integrative measurement of both structure and microbial decomposition, which has practical

implications for crop advisory and soil management zone mapping.

Conclusions

In conclusion, soil redox potential was found to correlate with soil texture, structure, and biological activity but not with soil organic matter. It also responded to management practice changes with carbon additions lowering the redox. These findings support the use of redox potential as an integrative measurement of soil function. In general, oxidized soil conditions were found in soils with good structure, and low redox corresponded to a high biological activity. Further research on the measurement methods (sample treatment) and the shared mechanisms between redox and microbial activity is necessary to facilitate a deeper interpretation in different soil textures. In spite of these limitations, redox can serve as a low-cost complementary indicator of carbon cycling and soil structure.

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Data availability The datasets generated during the current study are available in the Zenodo repository “Carbon Action” community.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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