

# Comparison of Permanganate-Oxidizable Carbon and Mineralizable Carbon for Assessment of Organic Matter Stabilization and Mineralization

**Tunsisa T. Hurisso\***

**Steve W. Culman\***

School of Environment  
and Natural Resources  
Ohio State Univ.  
OARDC  
Wooster, OH 44691

**William R. Horwath**

**Jordon Wade**

**Deandra Cass**

Dep. of Land, Air and Water Resources  
Univ. of California  
Davis, CA 95616

**Joshua W. Beniston**

Santa Rosa Junior College  
1501 Mendocino Ave.  
Santa Rosa, CA 95401

**Timothy M. Bowles**

Dep. of Land, Air and Water Resources  
Univ. of California  
Davis, CA 95616

**A. Stuart Grandy**

Dep. of Natural Resources  
and the Environment  
Univ. of New Hampshire  
Durham, NH 03824

**Alan J. Franzluebbers**

USDA-ARS  
Plant Science Research Unit  
Raleigh, NC 27607

**Meagan E. Schipanski**

Dep. of Soil and Crop Sciences  
Colorado State Univ.  
Fort Collins, CO 80523

**Shawn T. Lucas**

College of Agriculture, Food Science,  
and Sustainable Systems  
Kentucky State Univ.  
Frankfort, KY 40601

**Carmen M. Ugarte**

Dep. of Natural Resources  
and Environmental Sciences  
Univ. of Illinois at Urbana-Champaign  
Urbana, IL 61801

Permanganate-oxidizable C (POXC) and mineralizable C (as determined by short-term aerobic incubation of rewetted soil) are measures of active organic matter that may provide early indication of soil C stabilization and mineralization processes. To date, the relationship between these two promising active organic matter tests has not been comprehensively evaluated, and little is known about their functional role in the soil ecosystem. Here, we examined the relationship between POXC and mineralizable C across a wide range of soil types, management histories, and geographic locations across the United States (13 studies, 76 total sites;  $n = 1071$ ) and the ability of POXC and mineralizable C to predict crop yield and total aboveground biomass. Results from this comparative analysis showed that POXC and mineralizable C are related ( $r^2 = 0.15\text{--}0.80$ ) but that the relationship was differentially influenced by management practices. Overall, POXC better reflected practices that promote organic matter accumulation or stabilization and therefore can be a useful indicator of long-term soil C sequestration. Conversely, mineralizable C better reflected practices that promote organic matter mineralization and therefore can be a useful indicator of short-term soil nutrient availability. Our results also show that both mineralizable C and POXC were better predictors of corn (*Zea mays* L.) grain yield, aboveground biomass, and tomato (*Solanum lycopersicum* L.) fruit yield than other soil C fractions evaluated here. Thus, the integrated use of POXC and mineralizable C can provide a complementary framework to assess the relative dynamics of soil C stabilization and nutrient mineralization functions in agroecosystems.

Abbreviations: CC, cover crop; KBS-LTER, Kellogg Biological Station–Long-Term Ecological Research; LFL, Living Field Lab; MBC, microbial biomass carbon; POM-C, particulate organic matter carbon; POXC, permanganate-oxidizable carbon; SOM, soil organic matter; WORT, Windsor Organic Research Trial.

Of the three pools that constitute soil organic matter (SOM), the active or labile pool is comprised of rapidly cycling organic material that mostly turns over in a shorter time frame (days to a few years) relative to the intermediate (a few years to decades) and stable (decades to centuries) pools (Cambardella and Elliott, 1992; Gregorich et al., 1994; Parton et al., 1987; Wander, 2004). In particular, the active pool is only a small fraction (5–20%)

## Core Ideas

- POXC and mineralizable C were evaluated across diverse agroecosystems.
- The two are related but differentially influenced by management practices.
- POXC better reflected SOM stabilizing practices.
- Mineralizable C reflected SOM mineralizing practices.
- Both predicted agronomic performance better than other soil C fractions.

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\*Corresponding authors (hurisso.1@osu.edu; culman.2@osu.edu).

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of the soil's total organic matter that greatly influences key soil functions, such as nutrient cycling and availability, soil aggregation, and soil C accumulation (Cambardella and Elliott, 1992; Grandy and Robertson, 2007; Schmidt et al., 2011; Six et al., 1998; Wander, 2004; Weil and Magdoff, 2004). Despite its well-recognized importance as an indicator of overall soil quality (Gregorich et al., 1994; Haynes, 2005; Wander and Drinkwater, 2000), total SOM is not an ideal indicator of nutrient availability because much of the bulk SOM is in forms that turn over slowly (Drinkwater et al., 1998; Robertson et al., 2000). Thus, rather than testing for total SOM, as is often the case in standardized soil testing, testing the active organic matter pool provides better insight into how changes in management affect nutrient cycling and potential soil C accumulation or loss (Haynes, 2005; Lewis et al., 2011; Wander, 2004; Wardle, 1992). For example, accumulation of particulate organic matter is considered to represent an initial stage of C sequestration in soils and, as a consequence, is widely used in research settings (Awale et al., 2013; Haynes, 2005; Ladoni et al., 2015; Mirsky et al., 2008). However, most analytical methods for measuring the active SOM pool are time consuming and expensive, limiting their full use as routine tests for farmers (Franzluebbers, 2016).

Permanganate-oxidizable C and mineralizable C are promising and affordable active organic matter tests that are currently being implemented in soil health frameworks (e.g., Moebius-Clune et al., 2016). The measurement of POXC is based on chemical oxidation of organic matter by a weak potassium permanganate solution (Weil et al., 2003), whereas mineralizable C measures flush of CO<sub>2</sub> from rewetted soils during a short-term aerobic incubation (typically 1–3 d) (Franzluebbers et al., 2000a; Haney et al., 2001, 2008). In a comparison of POXC and other more established measures of active organic matter, Culman et al. (2012b) found that POXC was closely related with smaller and heavier particulate organic C fractions, indicating that POXC reflects a relatively processed or stabilized pool of active soil C. Their work also showed POXC is more sensitive to changes in management than other soil C fractions, which suggests that POXC can be a useful metric for rapidly tracking management-induced changes in SOM. Short-term mineralizable C, on the other hand, correlates well with long-term C mineralization rates, soil microbial biomass, particulate organic matter, and nutrient mineralization (Franzluebbers et al., 2000a; Haney et al., 2001, 2008). Carbon mineralization also has been shown to be sensitive and an early indicator of management-induced changes in total soil C (Franzluebbers and Stuedemann, 2008; Franzluebbers et al., 2000a; Haney et al., 2001; Ladoni et al., 2015; Schomberg et al., 2009; Vahdat et al., 2010). There have been a limited number of studies that examined the relationship between POXC and short-term mineralizable C (Culman et al., 2013; Morrow et al., 2016; Wang et al., 2003), but, to our knowledge, there has been no comprehensive evaluation across a wide range of soil types, management histories, and geographic locations.

Soil organic matter plays a key role in controlling soil fertility and thus agroecosystem productivity (Schmidt et al., 2011; Tiessen et al., 1994; Wander, 2004; Weil and Magdoff, 2004). The results of numerous previous studies, spanning many different crop types, indicate that losses of SOM can lead to reductions in yields and that increased SOM levels lead to yield increases. Lal (2006) reported that increasing SOM by 1 Mg ha<sup>-1</sup> via adoption of conservation-oriented management practices led to yield increases of 10 to 50 kg ha<sup>-1</sup> for rice (*Oryza sativa* L.), 20 to 50 kg ha<sup>-1</sup> for soybean [*Glycine max* (L.) Merr.], 20 to 70 kg ha<sup>-1</sup> for wheat (*Triticum aestivum* L.), and 30 to 300 kg ha<sup>-1</sup> for corn. However, much less work has focused on linking the active SOM pool with agronomic performance (e.g., crop yields). To our knowledge, only a few published studies have explicitly examined these relationships (e.g., Culman et al., 2013; de Moraes Sá et al., 2014; Lucas and Weil, 2012; Majumder et al., 2007; Stine and Weil, 2002).

Soil organic matter levels are the balance of C inputs to soil (through crop residues and amendments) and losses via mineralization (i.e., CO<sub>2</sub> respiration). These dynamics (stabilization vs. mineralization) are mediated through the soil food web, which plays a large role in SOM decomposition and supports crop nutrition. Growers have a vested interest in both processes because they rely on mineralization for short-term crop productivity but also strive for stabilization to build soil resilience, tilth, and quality. Culman et al. (2013) found that POXC was influenced more by compost additions and that mineralizable C was influenced more by crop rotations, particularly rotations with leguminous cover crops. Based on this previous work, we hypothesized that POXC would be associated more with management practices that promote organic matter building or stabilization and that mineralizable C would be associated with practices that promote organic matter mineralization.

The objectives of this study were (i) to determine the relationship between POXC and mineralizable C across a wide range of soil types and management histories, (ii) to determine important soil ecosystem processes that each of these active organic matter tests reflect, and (iii) to determine the ability of both POXC and mineralizable C to predict crop yields and total aboveground biomass.

## MATERIALS AND METHODS

### Data Description

For this comparative analysis we compiled a database of soil C fractions and crop yields from a total of 13 studies (76 sites in total;  $n = 1071$ ) conducted across diverse geographic locations in the United States (Table 1). These studies were chosen to represent a wide range of soil types, management histories, and geographic locations. Plant communities were primarily crops (corn, soybean, wheat, tomato, legume, and/or grass cover crops) but also included natural communities, such as native tallgrass prairie. Site characteristics (soil type and climate), experimental design and treatments, and the overall objective of each study are more fully described in the references provided in Table 1.

**Table 1. Description of studies used in this comparative analysis.**

Study name†	State, county (location)	Study characteristics	Sites	References
Russell Ranch	California, Yolo County (38°32' N, 121°52' W)	long-term experiment involving corn–tomato rotation with mineral fertilizer only, composted manure and legume cover crop, or mineral fertilizer and legume cover crops	1 (75)‡	Kong et al. (2005), Wade (2016)
CA Cover Cropping	California, Fresno County (36°20' N, 120°7' W)	annual clover mix under conventional tillage and conservation tillage	1 (16)	Mitchell et al. (2015), Wade (2016)
CA Grower Survey	California, Fresno, Salinas, San Joaquin and Yolo counties (38°37'–36°49' N, 121°51'–119°49' W)	survey of grower fields managed with mineral fertilizer only or with mineral fertilizer and mixed cover crops	21 (66)	Wade (2016)
KBS-LTER	Michigan, Barry County (42°24' N, 85°22' W)	long-term experiment involving corn–soybean–winter wheat rotations differing in management intensity (chemical inputs, tillage, cover crops, etc.)	1 (55)	Grandy and Robertson (2007)
GA Crop–Livestock	Georgia, Oconee County (33°62' N, 83°25' W)	summer grain (sorghum or corn) with winter cover crop (cereal rye) and winter grain (winter wheat) with summer cover crop (pearl millet) under conventional tillage or no-till management	1 (170)	Franzluebbers and Stuedemann (2007, 2008)
LFL	Michigan, Barry County (42°24' N, 85°24' W)	long-term experiment involving continuous corn vs. corn–soybean–wheat rotation under conventional, compost, or integrated management systems	1 (144)	Culman et al. (2013)
Mid-Atlantic	Maryland, Washington and Prince George counties; Pennsylvania, Lancaster County (39°00'–39°51' N, 76°49'–76°48' W)	annual cropland converted from long-term tillage (tilled history) and long-term sod (no-till history) to no-till corn–soybean rotation with and without cereal rye cover crop	3 (48)	Lucas and Weil (2012)
KS Prairie–Crop	Kansas, Ottawa County (38°58' N, 97°28' W)	fertilized annual cropland converted from tallgrass prairie to no-till wheat (“no-tillage cropland”) vs. unfertilized tallgrass prairie harvested annually for >75 yr (“harvested grassland”)	1 (36)	Beniston et al. (2014)
NY Grain Farms	New York, Seneca and Yates counties (42°36'–42°44' N, 76°42'–77°03' W)	grain farms across management-induced gradient of soil fertility	30 (118)	Schipanski et al. (2010), Schipanski and Drinkwater (2011)
OH Urban Garden	Ohio, Mahoning County (41°04'49"N, 80°40'35"W)	urban garden experiment involving tomato production with compost, compost and biochar, or compost and sorghum–sudangrass cover crop	1 (24)	Beniston et al. (2015)
CA Organic Tomato	California, Yolo County (38°33'–38°51' N, 121°48'–122°12' W)	organically managed fields growing Roma-type tomato with compost, manure, or no amendment across a gradient of total soil C	13 (78)	Bowles et al. (2014, 2015)
GA Crop–Pasture	Georgia, Oconee County (33°52' N, 83°25' W)	cropland and pasture under different management regimes and land use histories	1 (112)	Franzluebbers et al. (2000b), Franzluebbers and Stuedemann (2002)
WORT	Illinois, Champaign County (40°06' N, 88°16' W)	organic research trial involving annual cropland converted from perennial ley, row crop, and vegetable production systems with organic inputs from compost, manure, or cover crops	1 (129)	Ugarte and Wander (2013), Ugarte et al. (2013)

† KBS-LTER, Kellogg Biological Station-Long Term Ecological Research; LFL, Living Field Lab; WORT, Windsor Organic Research Trial.

‡ Total *n* in parentheses.

## Soil Analyses

### Permanganate-Oxidizable Carbon

Analysis of POXC was based on the method of Weil et al. (2003). Details of this method can be found in Culman et al. (2012a). Briefly, 20 mL of 0.02 mol L<sup>-1</sup> KMnO<sub>4</sub> were added to 50-mL polypropylene screw-top centrifuge tubes containing 2.5 g air-dried soil. The tubes were shaken for exactly 2 min at 240 oscillations min<sup>-1</sup> and allowed to settle for exactly 10 min, after which 0.5 mL of the supernatant was transferred into a second 50-mL centrifuge tube and mixed with 49.5 mL of deionized water. Sample absorbance was read with a spectrophotometer at 550 nm and POXC (mg kg<sup>-1</sup> soil) was calculated as

$$\text{POXC} = \left[ 0.02 \text{ mol L}^{-1} - (a + b\text{Abs}) \right] \times (9000 \text{ mg C mol}^{-1}) \times (0.02 \text{ L solution Wt}^{-1})$$

where 0.02 mol L<sup>-1</sup> is the initial concentration of the KMnO<sub>4</sub> solution, *a* is the intercept of the standard curve, *b* is the slope of the standard curve, Abs is the absorbance of the unknown soil sample, 9000 mg is the amount of C oxidized by 1 mol of MnO<sub>4</sub> with Mn<sup>7+</sup> getting reduced to Mn<sup>4+</sup>, 0.02 L is the volume of KMnO<sub>4</sub> solution reacted with soil, and Wt is the amount of soil (kg) used in the reaction.

### Short-Term Mineralizable Carbon

Short-term mineralizable C (i.e., the flush of CO<sub>2</sub> during a 1- to 3-d incubation) was determined using air-dried soils that had been rewetted. High correlation exists between 1-d versus 3-d incubations across a range of soil types (Franzluebbers et al., 2000a). In both GA crop–pasture and GA crop–livestock studies, mineralizable C was measured on rewetted soils after 3 d of incubation with 1 mol L<sup>-1</sup> NaOH base trap as outlined

in Franzluebbers (2016) (Table 2). In the Mid-Atlantic study, air-dried soils were rewetted to 60% water-filled pore space, and CO<sub>2</sub>-C was measured after 2 d of incubation (Table 2) as described in Drinkwater et al. (1996). In the remainder of the studies, flush of CO<sub>2</sub> during a 1 d incubation was determined on rewetted soils following Franzluebbers et al. (2000a) and Haney et al. (2001). Briefly, air-dried soils were moistened to 50% water-filled pore space and incubated at 25°C in 1-L canning jars for 24 h. The CO<sub>2</sub> concentration was measured by sampling 0.5 mL of air from the headspace and injecting it into a Li-Cor LI-820 infrared gas analyzer. One-day CO<sub>2</sub>-C was determined as the difference between the zero-time and 1 d CO<sub>2</sub> concentration. A close relationship ( $r^2 \geq 0.93$ ) has been reported between CO<sub>2</sub>-C measured by NaOH base trap method and infrared gas analysis (Culman et al., unpublished data, 2016; Haney et al., 2008; Sherrod et al., 2012).

### Other Soil Carbon Fractions

Other soil C fractions (soil organic C [SOC], particulate organic matter C [POM-C], and microbial biomass C [MBC]) along with soil texture were measured in the laboratories of the respective principal investigators (Table 1). In all studies, total SOC was determined with a direct combustion analyzer. Particulate organic matter C was measured in a total of six studies, of which four used size-based fractionation (GA crop–livestock, KS prairie–crop, GA crop–pasture, and Windsor Organic Research Trial [WORT]) and two used a combination of size- and density-based fractionation (Kellogg Biological Station-Long Term Ecological Research [KBS-LTER] and NY grain farms) (Table 2). Microbial biomass C was determined either

with the chloroform fumigation incubation method (Jenkinson and Powlson, 1976; GA crop–livestock and GA crop–pasture studies) or with the chloroform fumigation–extraction method (Vance et al., 1987; KS prairie–crop, NY grain farms, OH urban garden, and CA organic tomato studies) (Table 2).

### Agronomic Performance

Agronomic performance is defined here as crop productivity as determined by grain or vegetable fruit yield and total aboveground biomass (grain + stover). A total of 6 of the 13 studies considered in this comparative analysis had crop productivity data. Corn grain yield and total aboveground biomass were measured in four studies (Russell Ranch, Living Field Lab [LFL], Mid-Atlantic, and KBS-LTER). Wheat grain yield was measured in the Russell Ranch, GA crop–livestock, and KBS-LTER studies. Marketable fresh tomato fruit yield was measured in four studies (Russell Ranch, CA organic tomato, OH urban garden, and WORT). All of the studies measured crop productivity across multiple years ( $\geq 2$  yr) or across multiple sites, with the exception of the LFL and WORT studies.

### Statistical Analyses

To assess the relationship between POXC and mineralizable C as well as the relationship of these two active SOM measures with other soil C fractions (MBC, POM-C, and SOC), a linear model was fit for each study individually using the *lm()* function in R (R Development Core Team, 2015). Soil texture, sampling depth, and site were entered into the linear regression model using the *lm()* function to test for

**Table 2. Soil depth sampled, soil texture (clay and sand), and analytical methods by which mineralizable C, microbial biomass C (MBC), and particulate organic matter C (POMC) were measured for each study.**

Study†	Soil depth	Soil texture (clay, sand)‡	C fraction measurement method		
			Mineralizable C	MBC§	POM-C
	cm	g kg <sup>-1</sup> soil			
Russell Ranch	0–25	291 ± 72, ¶ 125 ± 11	IRGA, # 1-d incubation		
CA Cover Cropping	0–25		IRGA, 1-d incubation		
CA Grower Survey	0–25	301 ± 119, 310 ± 178	IRGA, 1-d incubation		
KBS-LTER	0–5		IRGA, 1-d incubation		density (1.6 g cm <sup>-3</sup> )
GA Crop–Livestock	0–3, 3–6, 6–12, 12–20, 20–30	N/A, †† 663 ± 92	NaOH base trap, 3-d incubation	CFI	size (>53 µm)
LFL	0–25	77 ± 29, 458 ± 67	IRGA, 1-d incubation		
Mid-Atlantic	0–7.5	258 ± 69, 342 ± 273	IRGA, 2-d incubation		
KS Prairie–Crop	0–10, 10–20, 20–40, 40–60, 60–80, 80–100	355 ± 57, 164 ± 57	IRGA, 1-d incubation	CFE	size (2000–250; 250–53 µm)
NY Grain Farms	0–20	273 ± 59, 428 ± 69	IRGA, 1-d incubation	CFE	size (>53 µm) and density (1.6 g cm <sup>-3</sup> )
OH Urban Garden	0–10		IRGA, 1-d incubation	CFE	
CA Organic Tomato	0–15	158 ± 32, 264 ± 94	IRGA, 1-d incubation	CFE	
GA Crop–Pasture	0–5, 5–12.5	197 ± 67, 643 ± 94	NaOH base trap, 3-d incubation	CFI	size (>53 µm)
WORT	0–15 cm		IRGA, 1-d incubation		size (>53 µm)

† KBS-LTER, Kellogg Biological Station-Long Term Ecological Research; LFL, Living Field Lab; WORT, Windsor Organic Research Trial.

‡ For studies with multiple soil depths, mean clay and sand values were obtained by averaging across depth.

§ CFE, chloroform fumigation extraction; CFI, chloroform fumigation incubation.

¶ Values are mean ± SE.

# Infrared gas analyzer.

†† Not available.

the effects of covariates. To determine the relative influence of a specific treatment (tillage, amendment, or cover crop) on POXC and mineralizable C, we used average residuals of the linear model for each treatment within each study. With the exception of POXC, data for all the other soil C fractions were slightly skewed and, as a result, log transformed before analysis. To assess the ability of soil C fractions to predict agronomic performance, stepwise multiple regression was run using the *regsubsets()* function in the R package *leaps*. Grain or fruit yield and total aboveground biomass data averaged across the years or sites were used as the response variables with soil C fractions as the predictor variables. All subsets regression uses an exhaustive search algorithm (i.e., all possible combinations included) and ranks model variables in order of importance from highest to lowest based on Mallows'  $C_p$ , an indicator of model parsimony (Mallows, 1973).

## RESULTS AND DISCUSSION

### Soil Carbon Fraction Descriptive Statistics

Across all studies there was a wide range of POXC, MBC, mineralizable C, and SOC values measured (Table 3). Permanganate-oxidizable C values ranged from 87 to 1450 mg kg<sup>-1</sup> soil, and mineralizable C values ranged from 2 to 2259 mg kg<sup>-1</sup>. The active fractions of SOM constituted only a small portion of the total SOC: POXC was 1 to 4%, mineralizable C was 1 to 3%, and MBC was 1 to 4% (Table 3).

### Relationship between Permanganate-Oxidizable and Mineralizable Carbon

Significant correlations between POXC and the mineralizable C were found in all but four studies (CA cover cropping, NY grain farms, CA organic tomato, KBS-LTER), with the Russell Ranch and CA grower survey studies demonstrat-

ing the weakest relationship and the OH urban garden study demonstrating the strongest relationship (Fig. 1; Table 4). The CA grower survey, NY grain farms, and CA organic tomato studies were all multisite studies in which soils were sampled from more than a single site (Table 1). The weak relationship ( $r^2 \leq 0.17$ ) between POXC and the mineralizable C noted in these three studies was therefore likely due to spatial variability and low SOM content. Adding site into the linear model as a covariate contributed significantly to improving the relationship, with  $r^2$  values increasing by 0.87, 0.19, and 0.68 in the CA organic tomato, NY grain farms, and CA grower survey studies, respectively (data not shown). The CA cover cropping and KBS-LTER studies were from single sites but did not show a significant relationship between POXC and mineralizable C. Both of these studies were on coarse-textured soils, which may have contributed to the lack of relationship. A recent study by Morrow et al. (2016) found a significant relationship between POXC and mineralizable C ( $r^2 = 0.42$  and  $0.45$  for 1- and 3-d incubations, respectively), a finding consistent with results presented here and in other studies (Rudrappa et al., 2006; Wang et al., 2003). Nonsignificant relationships between mineralizable C (30-d incubation) and POXC have also been reported (e.g., Awale et al., 2013).

In general, POXC shared a stronger relationship to the other measured soil C fractions than mineralizable C (Table 4). Relative to mineralizable C, POXC was more closely related to MBC in five out of six studies, POM-C in four out of six studies, and SOC in 9 out of 11 studies. Permanganate-oxidizable C is a chemically extracted fraction of soil C, whereas mineralizable C is a measurement that integrates soil C lability and the size and metabolic potential of the microbial community. These findings underscore that, although related, POXC and mineralizable C reflect unique C pools in the soil.

**Table 3. Descriptive statistics for soil C fractions by individual study.**

Study†	POXC‡		MBC§		Mineralizable C		SOC¶	
	Mean (SD)	Range (median)	Mean (SD)	Range (median)	Mean (SD)	Range (median)	Mean (SD)	Range (median)
	mg C kg <sup>-1</sup> soil							
Russell Ranch	444 (204)	160–967 (383)			76 (30)	3–153 (73)	11 (2)	6–15 (11)
CA Cover Cropping	495 (187)	277–877 (432)			58 (36)	12–126 (52)	6 (1)	5–8 (6)
CA Grower Survey	310 (245)	24–1010 (238)			46 (32)	8–163 (43)	9 (3)	4–20 (9)
KBS-LTER	609 (110)	369–881 (614)			66 (28)	12–137 (69)	13 (2)	8–17 (13)
GA Crop-Livestock	528 (323)	87–1450 (446)	524 (409)	58–2558 (371)	156 (137)	18–771 (113)	14 (10)	4–51 (11)
LFL	292 (90)	108–543 (295)			54 (12)	33–96 (53)	9 (2)	6–15 (9)
Mid-Atlantic	661 (147)	418–931 (656)			234 (114)	32–490 (231)	18 (6)	6–31 (18)
KS Prairie-Crop	465 (331)	124–1160 (359)	151 (110)	37–386 (116)	494 (413)	73–1553 (324)	16 (9)	5–33 (14)
NY Grain Farms	631 (126)	355–955 (640)	130 (88)	9–394 (103)	48 (18)	8–83 (46)	18 (5)	11–34 (18)
OH Urban Garden	803 (375)	142–1257 (928)	167 (117)	5–408 (187)	1029 (511)	274–2259 (1132)	56 (31)	10–113 (63)
CA Organic Tomato	557 (111)	321–863 (544)	117 (30)	55–218 (117)	443 (157)	60–904 (457)	13 (4)	6–22 (13)
GA Crop-Pasture	813 (411)	201–1469 (720)	645 (333)	220–1490 (551)	318 (325)	2–1098 (221)	19 (12)	5–49 (15)
WORT	558 (99)	364–800 (543)			758 (265)	122–1588 (746)	23 (5)	13–34 (22)
% of SOC	1–4		1–4		1–3			

† KBS-LTER, Kellogg Biological Station-Long Term Ecological Research; LFL, Living Field Lab; WORT, Windsor Organic Research Trial.

‡ Permanganate-oxidizable C.

§ Microbial biomass C.

¶ Soil organic C.

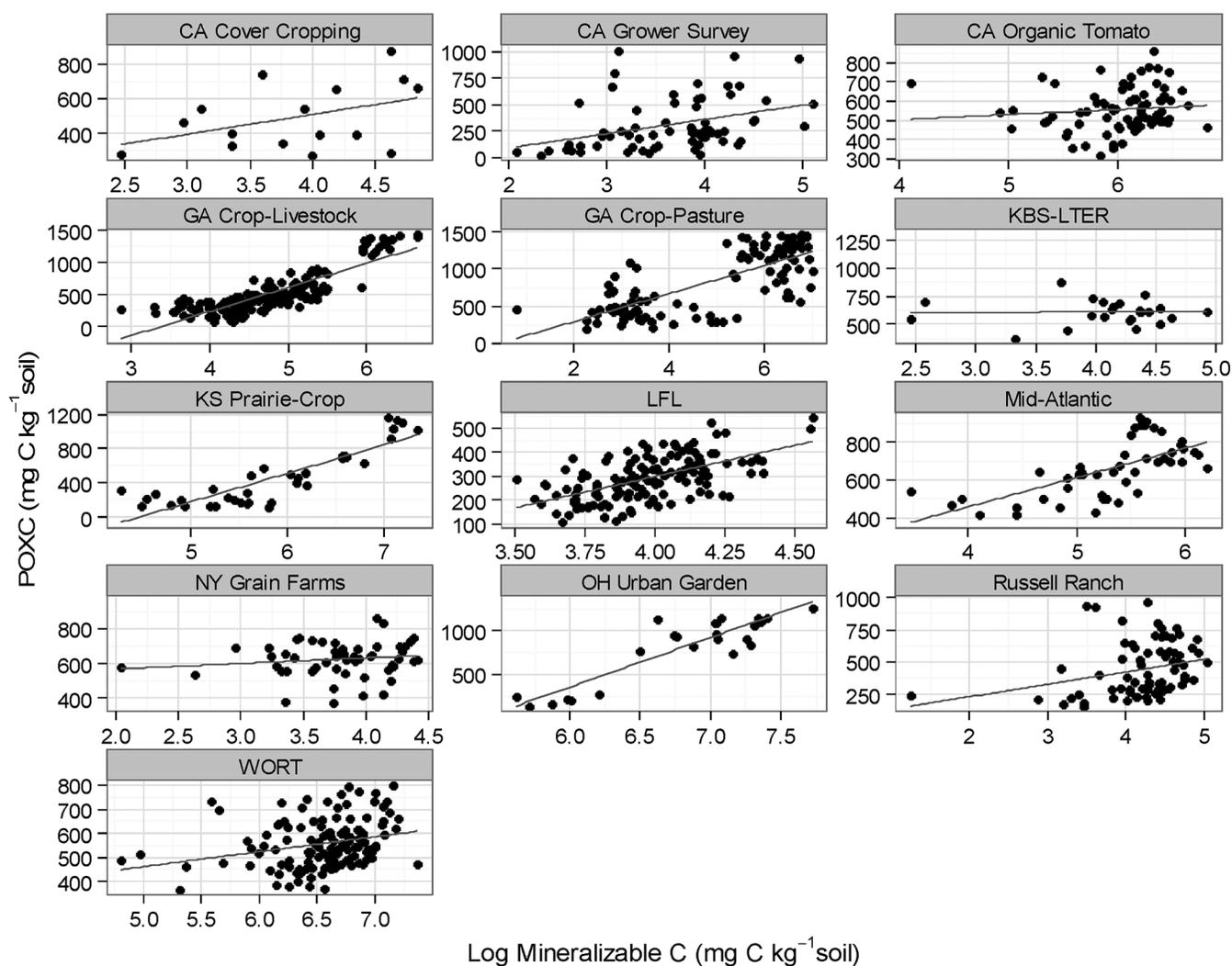


Fig. 1. Relationship between permanganate-oxidizable C (POXC) and mineralizable C by individual studies. The  $r^2$  values for the above shown relationships are given in Table 4. KBS-LTER, Kellogg Biological Station–Long Term Ecological Research; LFL, Living Field Lab; WORT, Windsor Organic Research Trial.

Table 4. Coefficients of determination between mineralizable C, permanganate-oxidizable C (POXC), and other soil C fractions (microbial biomass C [MBC], particulate organic matter C [POM-C], and soil organic C [SOC]) by individual study.

Study†	Mineralizable C vs.				POXC vs.		
	POXC	MBC	POM-C	SOC	MBC	POM-C	SOC
Russell Ranch	0.07*			0.13*			0.01 NS‡
CA Cover Cropping	0.17*			0.07 NS			0.04 NS
CA Grower Survey	0.14 NS			0.01 NS			0.01 NS
GA Crop-Livestock	0.73***	0.77***	0.79***	0.85***	0.65***	0.71***	0.85***
KBS-LTER	0.02 NS		0.05 NS	0.03 NS		0.33***	0.61***
LFL	0.35***			0.39***			0.46***
Mid-Atlantic	0.44***			0.64***			0.87***
KS Prairie-Crop	0.75***	0.69***	0.58***	0.71***	0.89***	0.79***	0.91***
NY Grain Farms	0.02 NS	0.01 NS	0.13*	0.04 NS	0.11***	0.21***	0.74***
OH Urban Garden	0.81***	0.30**		0.69***	0.53***		0.87***
CA Organic Tomato	0.01 NS	0.07‡		0.07‡	0.18*		0.51***
GA Crop-Pasture	0.56***	0.60***	0.66***	0.63***	0.77***	0.91***	0.84***
WORT	0.43***		0.35**	0.43***		0.33**	0.69***

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

† KBS-LTER, Kellogg Biological Station-Long Term Ecological Research; LFL, Living Field Lab; WORT, Windsor Organic Research Trial.

‡ Significant at the 0.10 probability level; NS, not significant.

## Variation from Edaphic Covariates

To determine whether some of the unexplained variation between POXC and mineralizable C could be accounted for by major edaphic covariates, we added soil texture (clay and sand) and depth of sampling into the linear model in the subset of studies with these measurements. Model outputs with edaphic covariates were then compared with those without edaphic covariates. Accounting for differences in soil sampling depth considerably improved the relationship between POXC and mineralizable C, including the relationship of each of these active organic matter measures with the other soil C fractions considered in this study (Table 5). In contrast, the addition of clay or sand into the linear model as covariates contributed little to improving the relationship of POXC with mineralizable C relative to soil depth (Table 5). This was despite some significant relationship between POXC and clay ( $r^2 = 0.12-67$ ; data not shown) in six out of nine studies that had texture data and between mineralizable C and clay ( $r^2 = 0.10-0.66$ ; data not shown) in four out of nine studies with texture data. This suggests that depth has a greater influence on these active fractions than soil texture. The development of these tests for farmer use, therefore, will have to pay close attention to sampling depth to standardize measurements across sites and laboratories (Franzluebbers, 2016).

## Soil Processes Inferred from Permanganate-Oxidizable and Mineralizable Carbon Relationship

To better understand functional differences associated with each of these active SOM fractions, we examined the relative influence of imposed treatments or grower practices (e.g., tillage, amendments, cover crops) on POXC and mineralizable C within each study. Specifically, we used linear regression models with mineralizable C as the predictor variable and POXC as the response variable and examined the residuals of the model for each experimental factor. Positive residuals (i.e., residuals above the linear least squares line) reflected observations with POXC values greater than those predicted by the least squares line, whereas negative residuals (i.e., residuals below the least squares line) reflected observations with greater-than-predicted mineralizable C values (Fig. 2, right panel). For each study, we averaged the residuals of all observations within a given treatment and used these average residual values to make inferences about how treatments differentially influenced POXC and mineralizable C values. Positive average residuals indicated treatments that exerted greater influence on POXC values relative to mineralizable C values, whereas negative

average residuals indicated the converse. The designation of positive or negative is arbitrary, but here we have chosen to orient the axes so that a positive residual would be more related with POXC and a negative residual would be associated more with the mineralizable C. It should also be emphasized that POXC and mineralizable C are often related (Table 4), so increases in a value of one measurement will likely be reciprocated in the increase of the other measurement. Overall, our approach attempts to assess the relative impact of management on POXC versus mineralizable C rather than focusing on the absolute values of either.

Average residuals from linear regression models revealed that imposed treatments had consistent patterns of influence on POXC and mineralizable C across the majority of the studies (Fig. 3; Table 6). Treatments expected to stabilize organic matter had positive average residuals, which indicates that those treatments had greater influence on POXC relative to mineralizable C. Conversely, treatments expected to promote organic matter mineralization had negative average residuals, indicating relative enrichment of mineralizable C in soils under those treatments relative to POXC, as illustrated in subsequent sections.

## Soil Organic Matter Building Conservation Tillage

The no-till treatment in both GA crop–livestock and CA cover cropping studies had positive average residuals (Fig. 3; Table 6), indicating that reduced soil disturbance influenced POXC more than mineralizable C relative to the conventionally tilled treatments. Similarly in the Mid-Atlantic study, the no-tilled history treatment, which was converted from long-term sod to a no-till annual cropland, was associated more with POXC (positive average residual) than with mineralizable C relative to the cropped history treatment that had long-term conventional tillage history (Fig. 3; Table 6). In the WORT study, the treatment with a history of reduced tillage had a positive average residual (Table 6), indicating that the combination of a perennial ley system (alfalfa, red clover, timothy, orchardgrass, and alsike clover) and low-intensity tillage had greater influence on POXC than mineralizable C relative to treatments with a history of annual tillage (vegetable and row cropping systems) (Fig. 3; Table 6). Altogether, these findings generally demonstrate relative enrichment of POXC in soils under conservation-oriented practices that have been shown to promote organic matter accumulation or stabilization (e.g., Awale et al., 2013; Chen et al., 2009; Eichler Inwood et al., 2015; Grandy et al., 2006; Lewis et al., 2011; Weil et al., 2003).

**Table 5. Coefficients of determination between mineralizable C, permanganate-oxidizable C (POXC), and other soil C fractions (microbial biomass C [MBC] and soil organic C [SOC]) with and without edaphic covariates.**

Soil C fraction	Mineralizable C				POXC alone	POXC +		
	alone	Clay	Sand	Depth		Clay	Sand	Depth
POXC	0.28**	0.31***	0.37***	0.80***	–	–	–	–
Mineralizable C	–	–	–	–	0.28**	0.31***	0.39***	0.65***
MBC	0.04*	0.05*	0.41***	0.85***	0.25**	0.39***	0.33***	0.71***
SOC	0.47***	0.32***	0.37***	0.85***	0.59***	0.60***	0.67***	0.74**

\* Significant at the 0.05 probability level.

\*\* Significant at the 0.01 probability level.

\*\*\* Significant at the 0.001 probability level.

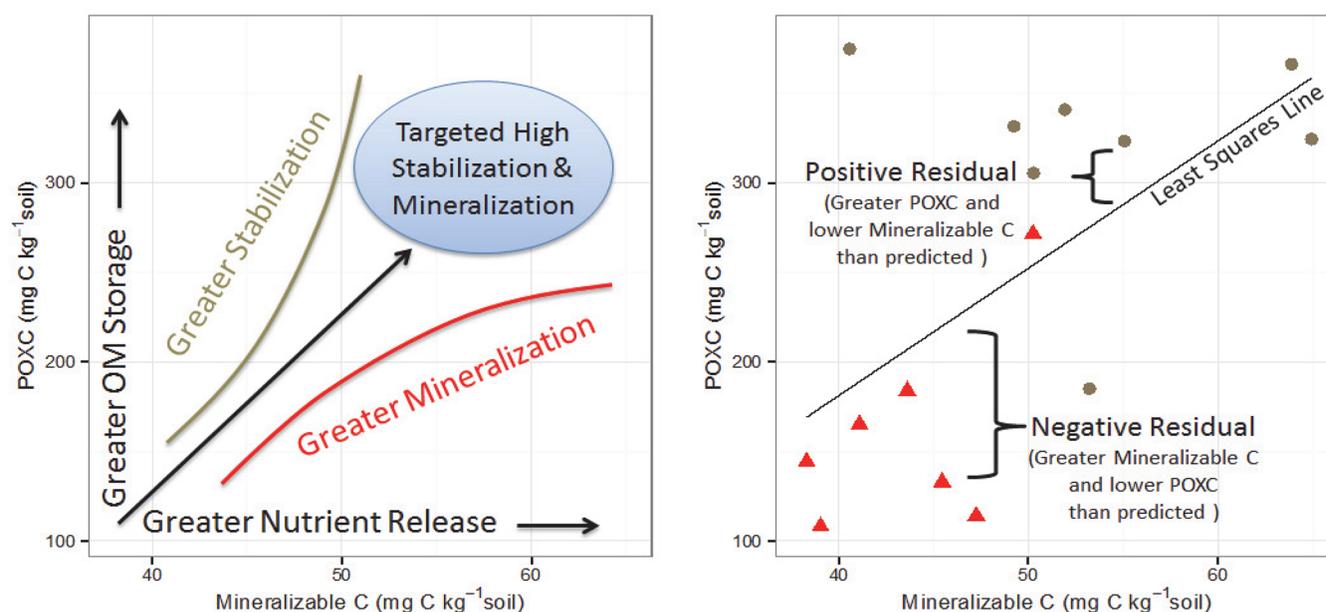


Fig. 2. Conceptual diagram of permanganate-oxidizable C (POXC) vs. mineralizable C. In the left panel, soils with high potential for organic matter stabilization would fall in the upper left quadrant, whereas soils with high potential for nutrient mineralization would fall in the lower right quadrant. Ideal soils would have high values of both measures, ensuring long-term organic matter building with short-term nutrient availability. The right panel illustrates field data from a long-term systems trial in Michigan with brown circles representing soils amended annually with compost and the red triangles from soils without compost (“conventional” treatment from Culman et al. [2013]). A linear least squares regression line illustrates that the treatments differentially influenced the POXC and mineralizable C values, with compost plots returning an average positive residual and conventional plots returning an average negative residual. This approach provides a possible framework for evaluating the relative dynamics of soil organic matter stabilization and mineralization. OM, organic matter.

### Compost

In the LFL study, the compost treatment with a positive average residual indicates that compost additions had greater influence on POXC than mineralizable C relative to the no compost addition (conventional and integrated management systems) treatment (LFL-Compost in Fig. 3; Table 6). In the OH urban garden study, addition of compost (C/N ratio  $\approx 14$ ) in combination with sorghum–sudangrass (*Sorghum bicolor*  $\times$  *S. bicolor* var. *sudanese*, var. BMR) cover crop treatment (i.e., Compost + CC) as well as addition of compost alone and in combination with biochar had positive average residuals, once again demonstrating that addition of amendments with processed C had greater influence on POXC than mineralizable C relative to the control (Fig. 3; Table 6). Similarly in the Russell Ranch study, the positive average residual for the organic system (i.e., Compost + CC) also indicates that the combination of compost addition and inclusion of a cover crop (vetch [*Vicia dasycarpa* Ten.] and pea [*Pisum sativum* L.]) had greater influence on POXC than mineralizable C relative to the cover crop alone system (Fig. 3; Table 6). Finally, the positive average residuals for the fields with compost (C/N ratio  $\approx 15$ – $18$ ) applied in the CA organic tomato study and for the compost treatment in the WORT study (Fig. 3; Table 6) further indicate that organic input from compost addition was associated more with POXC than mineralizable C relative to manure addition. Collectively, these findings demonstrate relative enrichment of POXC in soils amended with stable or processed C, such as compost and/or biochar, that can lead to organic matter building (e.g., Culman et al., 2013; Fortuna et al., 2003; Reeve et al., 2012; Ros et al., 2006).

### Organic Matter Mineralization Conventional Tillage

In both the GA crop–livestock and the CA cover cropping studies, the conventional tillage treatments had negative average residuals, indicating that tillage had a stronger influence on mineralizable C than POXC relative to the no-till treatments (Fig. 3; Table 6). In the Mid-Atlantic study, the tilled history treatment, which had a long-term history of conventional tillage before being converted to a no-till annual cropland, also had a negative average residual, further indicating that long-term tillage had greater influence on mineralizable C than POXC relative to the no-till history treatment. In the WORT study, the annual tillage history treatment, involving annual row crop and vegetable production systems with high tillage intensity, also had a negative average residual, demonstrating the greater influence of tillage on mineralizable C than POXC relative to the perennial ley system with reduced tillage history (Fig. 3; Table 6). These findings demonstrate that higher tillage intensity (e.g., Balesdent et al., 2000; Grandy and Robertson, 2007; Lewis et al., 2011; Six et al., 2002) promotes relative enrichment of mineralizable C.

### Manure

The negative average residual for fields with poultry manure or poultry litter (C/N ratio  $\approx 9.8$ – $15$ ) applied in the CA organic tomato study indicates that manure addition influenced mineralizable C more than POXC relative to fields with no amendment and those with compost applied (Fig. 3; Table 6). Similarly in the WORT study, the manure addition treatment had a negative average residual, further indicating the greater influence of manure

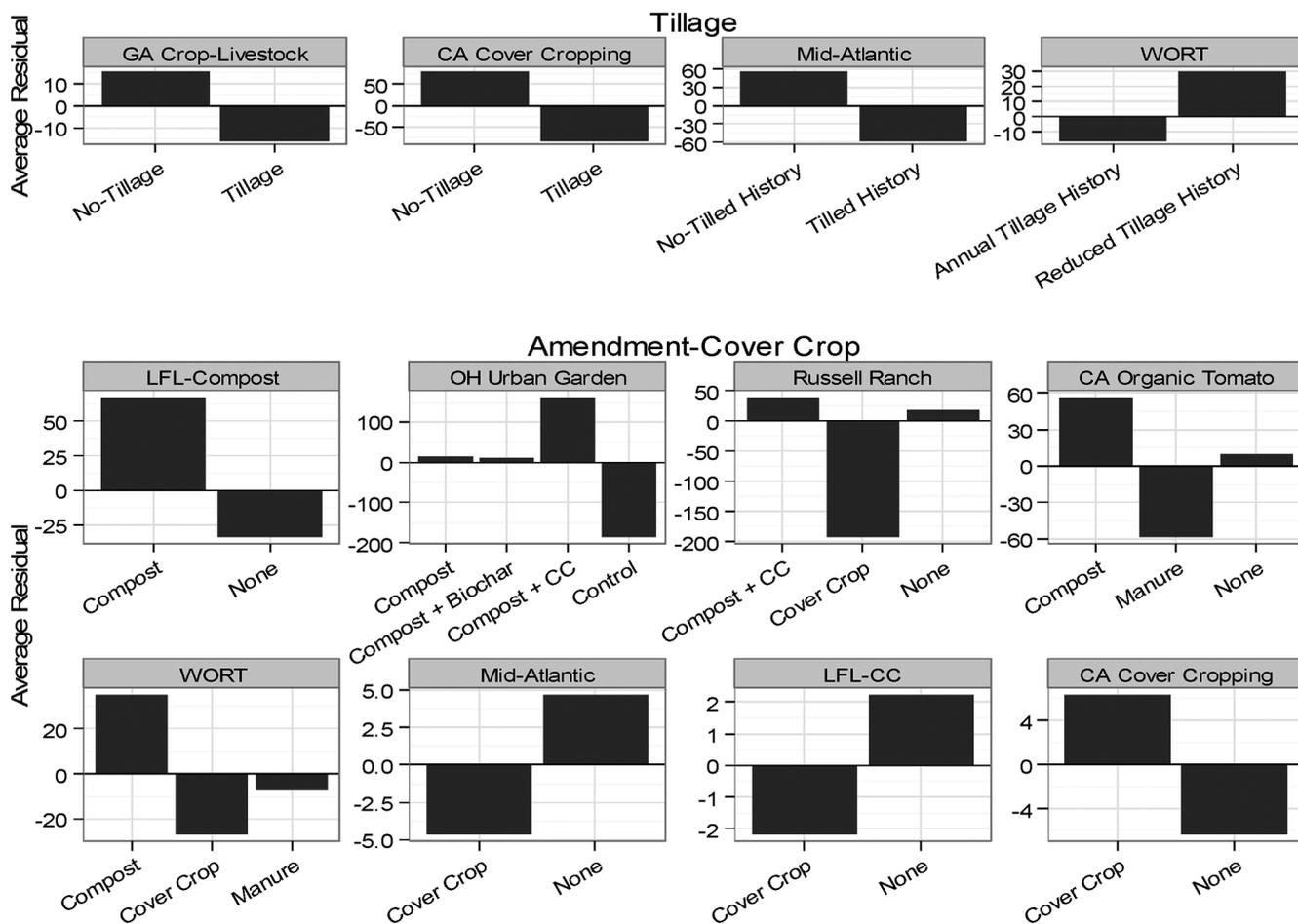


Fig. 3. Average residuals from linear regression models for individual treatments within a given study. A treatment with positive average residual indicates the greater influence of that particular treatment on permanganate-oxidizable C (POXC) relative to mineralizable C, whereas a treatment with negative average residual indicates the greater influence that particular treatment had on mineralizable C relative to POXC. CC, cover crop; LFL, Living Field Lab; WORT, Windsor Organic Research Trial.

addition on mineralizable C than POXC relative to compost addition treatment (Fig. 3; Table 6). The findings from both CA organic tomato and WORT studies demonstrate relative enrichment of mineralizable C in soils amended with manure that can potentially lead to nutrient mineralization and availability, as has been reported previously (Min et al., 2003).

### Cover Crops

The cover crop (*Vicia dasycarpa* Ten. and *Pisum sativum* L.) treatment in the Russell Ranch study had a negative average residual, which indicates that this mixed cover crop treatment was associated more with mineralizable C than POXC relative to the no cover crop and compost plus cover crop treatments (Fig. 3; Table 6). The negative average residual for the cover crop treatment in the Mid-Atlantic study also indicates that the cereal rye cover crop was also associated more with mineralizable C than POXC relative to the no cover crop treatment. Similarly in the LFL study, the cover crop treatment comprised of crimson clover (*Trifolium incarnatum* L.) and annual ryegrass (*Lolium multiflorum* Lam.) from 1993 to 2005 and cereal rye (*Secale cereal* L.) and red clover (*Trifolium pretense*, L.) since 2006 had a negative average residual, indicating that this cover crop treatment was associated more with mineralizable C

than POXC relative to no cover crop treatment (LFL-CC in Fig. 3; Table 6). In the WORT study, the cover crop treatment (hairy vetch [*Vicia villosa* Roth] and cereal rye) had a negative average residual, further indicating that this legume-cereal cover crop mixture had stronger influence on mineralizable C than POXC relative to the compost treatment. Consistent with our findings, previous studies have also shown that cover crop mixtures, especially those containing leguminous cover crops, decompose rapidly and provide greater nutrient mineralization relative to no cover crops (Mendes et al., 1999; Poffenbarger et al., 2015). The cover crop treatment (annual clover mix) in the CA cover cropping study had a positive average residual (Fig. 3; Table 6). When the analysis was rerun separately for each tillage treatment, however, the cover crop by tillage treatment in which cover crops were incorporated had an average negative residual (data not shown). In contrast, the cover crop by no-till treatment in which cover crops had not been incorporated for 15 yr (1999–2014) had an average positive residual (data not shown), indicating relative enrichment of POXC in soils under cover crops and no-till management.

The data presented in Fig. 3 and Table 6 support our hypothesized framework that POXC reflects management practices, such as conservation tillage and addition of compost, that

**Table 6. Average residuals from linear regression models and mean values of permanganate-oxidizable C (POXC), mineralizable C, soil organic C (SOC), and total soil N (TSN) by treatment within each study.**

Study†	Factor‡	Treatment	Avg.residual	—mg kg <sup>-1</sup> soil—		—g kg <sup>-1</sup> soil —	
				POXC	Mineralizable C	SOC	TSN
GA Crop–Livestock	1	no-till	15.81	576	173	16	1.35
	1	conventional tillage	-15.81	464	131	11	0.92
	2	summer grain with winter cover crop (cereal rye)	17.61	517	160	14	1.15
	2	winter grain with summer cover crop (pearl millet)	-17.61	523	144	13	1.122
CA Cover Cropping	1	no-till	82.98	728	101	6	1.33
	1	conventional tillage	-82.98	405	72	6	1.22
	2	cover crop	6.32	373	26	7	1.25
Mid-Atlantic	2	none (mineral fertilizer only)	-6.32	474	35	6	1.29
	1	no-till history	57.38	753	276	22	2.32
	1	tilled history	-57.38	569	192	15	1.30
	2	no cover crop	4.66	673	255	18	1.78
WORT	2	cereal rye cover crop	-4.66	648	213	19	1.85
	1	reduced tillage history (ley system)	30.21	589	763	24	1.94
	1	annual tillage history (vegetable system)	1.74	563	788	24	1.95
	1	annual tillage history (row crop system)	-32.61	521	722	22	1.78
	2	compost	35.03	596	787	24	1.96
	2	manure	-7.09	554	786	23	1.89
LFL	2	legume–grass cover crop	-27.01	525	704	23	1.82
	1	compost system	67.16	380	59	12	1.03
	1	none (conventional system with no compost)	-28.16	232	47	7	0.72
	1	none (integrated system with no compost)	-38.99	265	57	9	0.92
	2	no cover crop	2.22	268	48	9	0.87
OH Urban Garden	2	legume–grass cover crop	-2.19	317	60	10	0.91
	1	compost + cover crop	152.93	913	958	65	4.85
	1	compost + biochar	25.01	1041	1381	62	4.49
	1	compost	16.41	913	1184	63	4.71
Russell Ranch	1	control	-223.69	172	354	15	1.18
	1	compost + cover crop	39.44	507	89	13	1.23
	1	none (mineral fertilizer only)	18.02	450	69	9	1.18
CA Organic Tomato	1	cover crop	-191.61	246	72	11	1.27
	1	compost	56.38	615	462	16	1.73
	1	no amendment	9.89	557	359	11	1.38
	1	manure	-58.03	498	439	11	1.26

† LFL, Living Field Lab; WORT, Windsor Organic Research Trial.

‡ The number of factors in the study in which treatments are nested.

are expected to promote SOM accumulation or stabilization, whereas mineralizable C reflects practices that are expected to promote nutrient mineralization, such as addition of manure, conventional tillage, and cover crops (Fig. 2, left panel). As reported by Culman et al. (2012b) and Tirol-Padre and Ladha (2004), POXC represents a more processed organic matter than mineralizable C, indicating that the two tests represent functionally different but complementary pools as discussed above.

### Ability of Soil Carbon Fractions to Predict Agronomic Performance

To assess the ability of active organic matter tests to predict agronomic performance, we ran all subset regressions with crop yield (grain/fruit and total aboveground biomass) as the response variable and soil C fractions (POXC, mineralizable C, total SOC, MBC, POM-C) as predictor variables. Within a study, predictor variables were ranked in order of their predictive

capacity, and only those soil C fractions ranked 1 to 3 are shown in Table 7. Mineralizable C was the best predictor of crop productivity across all studies (six times), followed by POXC (two times) and other soil C fractions (MBC, POM-C and SOC; six times altogether) (Table 7). Permanganate-oxidizable C was the second best predictor eight times across all studies, with mineralizable C five times and other soil C fractions only one time (Table 7). Overall, POXC and mineralizable C were either the first or second best predictor in 21 out of 28 comparisons, indicating that both of these measurements are capable of predicting agronomic performance relative to other soil C fractions. Consistent with the results presented here, Stine and Weil (2002) reported significant relationships between POXC and crop productivity ( $r^2 = 0.58$  and  $0.74$  for corn aboveground biomass and grain yield, respectively). Likewise, Majumder et al. (2007) found a significant relationship ( $r^2 = 0.89$ ) between mineralizable C and crop productivity (in particular rice and wheat). Furthermore, de

**Table 7. All subset regression results where soil C fractions are ranked based on their predictive capacity of crop productivity, as indicated by crop yields.**

Study†	Soil C fraction‡ rank		
	First	Second	Third
		<u>Corn grain yield</u>	
Russell Ranch	mineralizable C (0.25)§	POXC (0.26)	SOC (0.29)
LFL	mineralizable C (0.38)	POXC (0.39)	SOC (0.52)
Mid-Atlantic	POXC (0.30)	mineralizable C (0.31)	SOC (0.55)
KBS-LTER	mineralizable C (0.08)	SOC (0.39)	POXC (0.47)
		<u>Corn total biomass yield (grain + stover)</u>	
Russell Ranch	mineralizable C (0.19)	POXC (0.20)	SOC (0.20)
LFL	SOC (0.38)	mineralizable C (0.73)	POXC (0.45)
Mid-Atlantic	mineralizable C (0.09)	POXC (0.13)	SOC (0.12)
		<u>Wheat grain yield</u>	
Russell Ranch	SOC (0.23)	POXC (0.24)	mineralizable C (0.24)
GA Crop-Livestock	POM-C (0.02)	mineralizable C (0.03)	POXC (0.03)
KBS-LTER	SOC (0.03)	mineralizable C (0.11)	POXC (0.11)
		<u>Tomato marketable fruit yield</u>	
Russell Ranch	mineralizable C (0.15)	POXC (0.25)	SOC (0.16)
OH Urban Garden	POXC (0.57)	mineralizable C (0.84)	SOC (0.84)
CA Organic Tomato	MBC (0.27)	POXC (0.43)	mineralizable C (0.43)
WORT	POM-C (0.02)	POXC (0.03)	mineralizable C (0.03)
Total no. of comparisons	14	14	14
No. of times POXC was best predictor	2	8	4
No. of times mineralizable C was best predictor	6	5	3
No. of times other soil C fractions were best predictors	6	1	7

† KBS-LTER, Kellogg Biological Station-Long Term Ecological Research; LFL, Living Field Lab; WORT, Windsor Organic Research Trial.

‡ MBC, microbial biomass C; POM-C, particulate organic matter C; POXC, permanganate-oxidizable C; SOC, soil organic C.

§ Numbers in parentheses are cumulative  $r^2$  values between crop yields and soil C fractions.

Moraes Sá et al. (2014) reported a significant relationship ( $r^2 = 0.94$ ) between POXC and wheat grain yield.

## CONCLUSION

This study is the first to examine the relationship between POXC and mineralizable C across a broad spectrum of soil types, management histories, and geographic locations. The results presented here demonstrate that POXC and mineralizable C were related but that the relationship was differentially influenced by management practices; that is, POXC better reflected conservation-oriented practices that are expected to promote accumulation or stabilization of organic matter (long-term C sequestration), whereas mineralizable C better reflected practices that are expected to promote organic matter mineralization (short-term nutrient availability). Consistent with findings by others, both mineralizable C and POXC in our study also appeared to predict crop productivity better than other soil C fractions such as total SOC. If used in combination, POXC and mineralizable C can play complementary roles by providing a framework for evaluating the relative dynamics of organic matter stabilization and mineralization functions in agroecosystems.

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