Soil Organic Matter Functional Group Composition in Relation to Organic Carbon, Nitrogen, and Phosphorus Fractions in Organically Managed Tomato Fields

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The objectives of this study were to examine soil organic matter (SOM) functional group composition and its relationship to labile SOM fractions with diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). We analyzed soils from 13 organically managed tomato (*Solanum lycopersicum*) fields in northern California for labile organic C, N, and P fractions and by DRIFTS for bands representing organic functional groups, including aliphatic C-H (2924, 2850, 1470, 1405, 1390 cm^{-1}), aromatic C=C (1650 cm^{-1}) and C-H (920, 840 cm^{-1}), polysaccharide and phenol C-O (1270, 1110, 1080 cm^{-1}), and amine and amide N-H (3400, 1575 cm^{-1}). Significant differences in relative band intensities occurred among the 13 organic tomato fields, in particular a relative increase in absorbance of bands representing aliphatic C-H positively associated with soil organic carbon (SOC), as well as permanganate-oxidizable carbon (POXC), extractable organic carbon (EOC) and nitrogen (EON), and potentially mineralizable N (PMN). In comparison, organic P fractions like sodium bicarbonate extractable ($NaHCO_3$–P_o) and sodium hydroxide extractable organic P (NaOH-P_o) were poorly associated with SOC and functional groups represented by bands, including aliphatic C-H. This could reflect limitations of DRIFTS, but is consistent with hypotheses of greater decoupling of C and P vs. C and N in soils. This study implicates relative differences in organic functional groups with differences in SOC and labile SOM fractions, and in agreement with previous studies, identifies absorbance of infrared bands representing aliphatic C-H functional groups in these systems as a potential indicator of SOM transformations related to changes in its labile fractions.

Abbreviations: ANCOVA, one-way analysis of covariance; ANOVA, one-way analysis of variance; ATR, attenuated total reflectance; DRIFTS, diffuse reflectance infrared Fourier transform spectroscopy; EOC, potassium sulfate extractable organic carbon; EON, potassium sulfate extractable organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; $NaHCO_3$–P_o, sodium bicarbonate extractable organic phosphorus; NaOH-P_o, sodium hydroxide extractable organic phosphorus; PCA, principal component analysis; PMN, potentially mineralizable nitrogen; POXC, permanganate-oxidizable carbon; RDA, redundancy analysis; SOC, soil organic carbon; SOM, soil organic matter.

Diffuse reflectance infrared Fourier transform spectroscopy measures absorbance of infrared radiation (4000–400 cm^{-1}) by bonds expressing dipole moments (e.g., C-O, C=O, C=C, C-H, N-H) of functional groups that constitute SOM (e.g., aliphatic, aromatic, amide, phenol, carboxylate, polysaccharides) and strongly influence its chemical and biochemical reactivity (Essington, 2004). Transformations of SOM such as decomposition and mineralization entail changes in functional group chemistry, such as the relative increase in aromatic to aliphatic groups during decomposition (Hsu and Lo, 1999). By quantifying relative changes in functional groups, DRIFTS can be used to help explain SOM transformations and stabilization (Chefetz et al., 1998). The sensitivity of DRIFTS to small changes in quality and quantity of labile organic matter (Calderón et al., 2013) and management effects on SOM (Parikh et al., 2014) make it well-suited for assessing SOM quality in on-farm contexts (Aranda et al., 2011; Veum et al., 2014). Overlap of mineral and organic bands is common in soils, but correction of soil spectra by subtraction of a mineral background can be used to improve DRIFTS characterization of SOM (Chefetz et al., 1998; Cheshire et al., 2000; Ellerbrock et al., 1999). Further corroborating its potential utility, subtraction of mineral backgrounds has been used on soils amended with pure organic standards to validate specific absorbances for spectral interpretation (Calderón et al., 2013).

In organically managed systems, mineralization of SOM is crucial for meeting crop nutrient demand. Relating functional group composition of SOM to labile fractions can provide insight to the degree to which the chemistry of SOM can influence its lability (Calderón et al., 2011a). Long-term experiments have shown that organic management can increase labile C in the short-term and total soil C in the longer-term (Gattinger et al., 2012; Marriott and Wander, 2006), though labile SOM responds more rapidly to management than total SOC (Drinkwater et al., 1998; Robertson and Paul, 2000). The degree to which SOM compositional changes are associated with these increases in SOM is largely unknown and may offer insight into observed increases in labile SOM.

Differences in SOM functional groups characterized by DRIFTS have been implicated in stabilization of C fractions under different input treatments (Gillespie et al., 2014; Verchot et al., 2011), though the relationship of such changes with labile C and non-C fractions of SOM is unclear. Additionally, in contrast to long-term experiments, on-farm management commonly combines nutrient management practices (Drinkwater et al., 1995) which can vary annually, and could influence labile SOM through reciprocal feedbacks of input diversity and soil microbial activity (Gude et al., 2012; Wickings et al., 2012). On-farm research across a landscape provides an opportunity to examine fields under different management practices and with variation in SOM quantity, allowing determination of relationships between SOM functional group composition with differences in labile SOM.

The objectives of this study were to characterize relative differences in SOM functional group composition across organically managed fields encompassing a wide range of total and labile SOM, and to determine relationships between spectral data and labile SOM. To address this objective, DRIFTS was used to quantify relative differences in organic functional groups across 13 organically managed tomato fields representing a three-fold range of SOC. This study was part of a larger project examining how plant–soil–microbial interactions affect nutrient cycling and productivity across this set of fields (Bowles et al., 2014). Diffuse reflectance infrared Fourier transform spectroscopy bands representing functional groups were determined for surface soils and related to SOC and labile organic C, N, and P fractions. We hypothesized that across the 13 fields, there would be differences in DRIFTS bands, and that these would strongly associate with differences in SOC. Relative differences in DRIFTS bands among fields were expected to be more dependent on SOC than SOM fractions. Additionally, we hypothesized that associations of bands with C, N, and P fractions would reflect associations of bands with SOC.

MATERIALS AND METHODS
Study sites and soil sampling

The study site encompassed 13 organically managed fields growing Roma-type tomatoes selected to be representative of the local landscape in terms of soil characteristics, landscape context, and organic management via a GIS and cluster analysis approach (Hollander, 2012). Fields are located within the Sacramento Valley in Yolo County, CA, which has a Mediterranean-type climate, with Thermic soil temperature and Xeric soil moisture regimes. Comparing soils of similar mineralogy and parent material facilitates spectral interpretation of soil DRIFTS data. The soils in this study developed from mixed alluvium (e.g., smectite, kaolinite, quartz) and express loam and silt loam textures, with circumneutral pH and no detectable ($<0.2\%$ CaCO$_3$) carbonates (Table 1, Supplementary Table 1).

Table 1. Soil properties (0-15 cm) and field nutrient amendments for 13 organically managed fields in Yolo County, CA. Adapted from Bowles et al. (2014).

Field	Organic C†	Clay	Silt	Sand	Texture class	pH	Amendments‡
		Particle-size fractions			
	g kg^{-1}			
1	6.7	139	471	391	loam	6.7	manure
2	9.6	214	593	193	silt loam	6.8	manure
3	10.7	194	556	251	silt loam	6.7	manure
4	11.1	176	614	210	silt loam	6.6	vetch
5	11.2	181	601	219	silt loam	6.3	manure, vetch
6	12.5	162	635	203	silt loam	6.3	manure, vetch
7	12.8	146	521	333	silt loam	7.2	compost, vetch
8	13.2	97	484	419	loam	6.8	compost
9	13.9	140	581	278	silt loam	6.4	manure, vetch
10	16.5	157	638	206	silt loam	6.6	compost
11	17.1	175	673	153	silt loam	6.9	compost
12	18.1	112	478	410	loam	6.8	compost
13	20.0	164	664	173	silt loam	6.5	compost

† Calculated from total soil C due to the lack of inorganic C as carbonates.

‡ Compost and manure were applied in fall 2010, with the exception of Field 5, in which manure was applied in early spring before tomato (*Solanum lycopersicum*) transplanting. Winter vetch cover crops were incorporated before transplanting. Compost was composted green waste with a C/N ranging from 15 to 18. Manure was poultry manure or poultry litter with a C/N ranging from 9.8 to 15.

The primary organic amendments included composted green waste, manure, and/or vetch cover crops (Table 1). Fields had similar tillage practices.

Surface soils (0–15 cm) were collected over a 2-wk period in June 2011, at the period of peak tomato growth. In each field, soils were randomly sampled from six plots (4 m^2). Cores (15-cm diam.) were taken 15 cm from the centerline of the planting row. Soils were thoroughly mixed and visible plant residues were removed by hand before air-drying and grinding to pass a 2-mm sieve.

Soil Properties

Soil analyses were performed as described by Bowles et al. (2014). Soil particle size was determined by the laser diffraction method according to Eshel et al.(2004). Soil pH was determined on air-dried samples using a 1:2.5 soil/water ratio. Soil carbonate content was determined gravimetrically by loss of CO_2 in presence of excess hydrochloric acid (U.S. Salinity Laboratory Staff, 1954). Total soil C was quantified at the Univ. California Davis Stable Isotope Facility on an Elementar Vario EL Cube elemental analyzer (Elementar, Hessia, Germany). Due to the lack of carbonates, total soil C was considered an accurate measure of SOC.

Organic Carbon, Nitrogen, and Phosphorus Fractions

Labile C was determined as permanganate-oxidizable C (POXC) according to Culman et al. (2012). Microbial biomass C (MBC) and N (MBN) were determined by the chloroform fumigation extraction method as the difference between extractable organic C (EOC) and N (EON) in non-fumigation and fumigation samples (Ros et al., 2009; Vance et al., 1987). Microbial biomass was measured in field-moist samples stored on ice and analyzed within 6 h of sampling. Organic C in non-fumigation and fumigation extracts was quantified using a Dohrmann Phoenix 8000 UV-persulfate oxidation analyzer (Tekmar-Dohrmann, Cincinnati, OH) and organic N was quantified using alkaline persulfate oxidation (Cabrera and Beare, 1993). No correction factors were applied. Potentially mineralizable N (PMN) was determined by anaerobic incubation at 37°C (Waring and Bremner, 1964). Organic P was measured by a modified version of the Hedley fractionation (Tiessen et al., 1983). Briefly, air-dried soil was sequentially extracted (1:40), first by 0.5 M $NaHCO_3$, then by 0.1 M NaOH. Molybdate-reactive P was determined in extracts to estimate inorganic P (Murphy and Riley, 1962), and in acid-persulfate digested extracts to determine total P (Rowland and Haygarth, 1997). Organic P extractable by sodium bicarbonate ($NaHCO_3$–P$_o$) and sodium hydroxide (NaOH-P$_o$) was calculated as the difference between total and inorganic P for the respective extract.

Diffuse Reflectance Infrared Fourier Transform Spectroscopy

To enhance organic bands, mineral absorbances were subtracted from soil spectra (Chefetz et al., 1998). Removal of OM followed by subtraction of the resulting mineral spectrum from the original bulk soil sample enhances absorbance of the removed organic bands, enabling operational characterization of relative organic functional group composition in soil samples (Calderón et al., 2011a, 2011b; Cheshire et al., 2000; Clark Ehlers et al., 2010; Cox et al., 2000; Ellerbrock and Gerke, 2004; Kaiser et al., 2007; McCarty et al., 2010; Nguyen et al., 1991; Painter et al., 1980; Rumpel et al., 2001; Sarkhot et al., 2007; Schnitzer and Desjardins, 1965; Šimon, 2007). Since the accuracy of ashed subtraction spectra can be compromised by thermal alteration of mineral features, chemical oxidation has been proposed as an alternative (Reeves, 2012). Of soil C removal methods like hydrogen peroxide, sodium hyposulfite, and sodium hypochlorite, hypochlorite is reported to be one of the most efficient and reliable to remove C without dissolving pedogenic oxides (Mikutta et al., 2005; Siregar et al., 2005), von Lützow et al., 2007). In selecting hypochlorite oxidation for subtraction spectra, we compared hypochlorite vs. ashing in their ability to (i) remove SOC and (ii) provide mineral backgrounds for subtraction. Hypochlorite oxidation removed a comparable amount of SOC as ashing (85.8 ± 3.3% vs. 95.6 ± 1.7%, respectively; $n = 78$), and did not produce significant artifacts of subtraction, in contrast to ashing. Unlike ashing, hypochlorite does not appear to alter bands representing mineral bonds (Si-O, Si-OH, Al-O; Supplementary Fig. 1; Farmer, 1974, p. 331-363). In contrast, ashed soil spectra show loss of Si-OH at 3700–3600 cm^{-1} and Al-OH at 909 cm^{-1}. We additionally evaluated hypochlorite oxidation vs. ashing using attenuated total reflectance (ATR) FTIR because this mode of FTIR spectroscopy offers greater sensitivity to mineral Si-O bonds than DRIFTS (Supplementary Fig. 2; Parikh et al., 2014). The results of this comparison confirm Reeves' hypothesis (2012) that chemical oxidation of SOM can produce fewer changes in spectral features of the mineral component compared with ashing, and spectroscopically corroborate the ability of hypochlorite oxidation to remove the majority of soil C with minimal disturbance to mineral structure (Siregar et al., 2005).

Sodium hypochlorite oxidation was therefore performed to provide a mineral-enriched background used to calculate subtraction spectra, for all soils (6 plots × 13 fields; $n = 78$). Hypochlorite oxidation was adapted from Anderson (1961). Briefly, 4 g of air-dried and ground soil was thoroughly mixed with NaOCl (25 mL 6% w/w, pH 9.5) and incubated to allow oxidation (15 min, 80°C). Solutions were centrifuged (15 min, 1081 × g) and the supernatant discarded. This was repeated twice for a total of three treatments. Soils were then washed twice with 20 mL of deionized H_2O, sedimented by centrifugation (15 min, 1081 × g), air-dried, and re-ground.

Soil samples (bulk and oxidized) were air-dried and finely ground by hand with an agate mortar and pestle before DRIFTS analysis. Soils were loaded into an aluminum well and surface smoothed using a razor. Spectra were collected on neat soil samples (no KBr dilution) and corrected against KBr in ambient air using a Digilab FTS 7000 (Varian, Inc., Palo Alto, CA) with a deuterated triglycine sulfate (DTGS) detector and a diffuse reflectance accessory (Pike AutoDIFF, Pike Technologies, Madison, WI). Spectra were calculated as the mean of 64 scans across 4000–400 cm^{-1} at 4 cm^{-1} resolution.

Table 2. Functional group assignments for 13 bands used to evaluate DRIFT spectra of SOM in surface soils (0–15 cm) of 13 organically managed fields in Yolo County, CA. Based on Parikh et al. (2014).

Band, cm^{-1}	Assignment†
3400	ν(N-H), ν(O-H)
2924	aliphatic ν_{as}(C-H)
2850	aliphatic ν_{s}(C-H)
1650	aromatic ν(C = C)
1575	amide δ(N-H) and ν(C = N)
1470	aliphatic δ(C-H)
1405	aliphatic δ(C-H)
1390	aliphatic δ(C-H), potential contributions from carboxylate ν_{s}(C-O)
1270	phenol ν_{as}(C-O), carboxylic acid ν(C-O)
1110	polysaccharide ν_{s}(C-O)
1080	polysaccharide ν_{s}(C-O)
920	aromatic δ(C-H)
840	aromatic δ(C-H), less substituted

† ν, stretching vibration; ν_{as}, asymmetric stretching vibration; ν_{s}, symmetric stretching vibration; δ, bending vibration

Correction of spectra for minerals was performed by spectral subtraction: bulk soil-oxidized soil. Subtractions were performed using OMNIC 7.0 (Thermo Scientific, Waltham, MA) software and a subtraction factor of 1 (Smith, 2011). Spectra were corrected by calculating a linear tangential baseline with zero points representing local absorbance minima (Capriel et al., 1995; Haberhauer et al., 1998; Smidt et al., 2002) using OMNIC 7.0. Based on prominent peaks and shoulders observed in spectra, 13 bands representing organic functional groups (Table 2, Fig. 1) were selected and measured for absorbance intensity. To quantify relative changes in band absorbance, for each spectrum
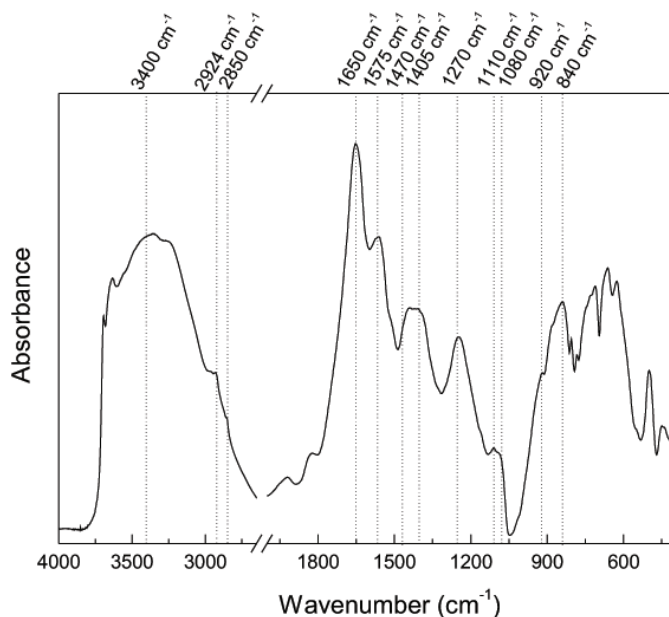

Fig. 1. Mean diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) spectrum of surface soils (0–15 cm, *n* = 78) of 13 organic tomato (*Solanum lycopersicum*) fields in Yolo County, CA. Bands (13 total) used to analyze organic functional groups are indicated, with the band at 1390 cm^{-1} omitted for clarity. Individual spectra were corrected for mineral absorbances by subtraction of a mineral background obtained by sodium hypochlorite oxidation.

the absorbance intensity of each of the 13 bands was normalized as the percent absorbance intensity of the sum of absorbance intensities of the 13 bands (Haberhauer et al., 1998).

Statistical Analysis

To compare variation in functional groups represented by DRIFTS bands and organic C, N, and P fractions among fields, F-statistics were calculated using one-way analysis of variance (ANOVA) using the PROC GLM feature of SAS v.9.3 (SAS Institute, Cary, NC). Field was considered an explanatory factor (13 levels) in ANOVA models analyzed separately for each response variable (13 bands and 8 fractions). F-statistics, that is, the ratio of variance among fields to variance within fields, were calculated for the response variable of band intensity. The F-statistic from these analyses was used to compare the relative magnitude of field effect for each variable (Culman et al., 2012). To assess SOC vs. non-SOC effects on among-field variation in bands and fractions, F-statistics for bands and C, N, and P fractions were also calculated using one-way analysis of covariance (ANCOVA) with SOC as a covariate. Field was considered an explanatory factor (13 levels) with SOC as the covariable for ANCOVA models analyzed separately for response variables of bands and fractions.

Variation and relationships among intensities of the 13 bands were explored with principal component analysis (PCA) based on a correlation matrix with the *vegan* package in R (Oksanen et al., 2012). To visualize associations of bands and fields, component scores for each of the six plots within a field were used to generate 95% confidence ellipses.

To relate differences in functional group composition to SOM quality, two band ratios were calculated. These two indices use ratios of bands representing functional group types (e.g., aliphatic, O-functional group) which in previous studies have been established as indexes of relative decomposition and recalcitrance of SOM (Veum et al., 2014):

$$\text{Index I} = \frac{1650+920+840}{2924+2850+1470+1405}$$

$$\text{Index II} = \frac{2924+2850+1650+1470+1405+920+840}{3400+1270+1110+1080}$$

Index I is hypothesized to be a metric of decomposition as the ratio of aromatic to aliphatic functional groups (Table 2), and ratios of bands representing these two functional groups have been shown to increase with increasing degree of decomposition (Chefetz et al., 1998; Hsu and Lo, 1999). Index II is a ratio of C- to O-functional groups (Table 2), an increase of which is thought to be associated with greater recalcitrance of SOM (Ding et al., 2002; Veum et al., 2014). Regression analysis was performed between indexes and SOC. Linear, quadratic, exponential, and power models were calculated and the model expressing the greatest coefficient of determination (R^2) was chosen as the curve of best fit.

Relationships among bands and C, N, and P fractions were determined by linear correlation analysis and redundancy analysis (RDA). Pearson's correlation coefficients (R) were calculated for two-directional correlations between absorbance intensities of bands and C, N, and P fractions. Correlation analysis was separately performed to calculate R coefficients for two-direction correlations between SOC and C, N, and P fractions.

Redundancy analysis was used to further evaluate relationships of functional groups represented by bands with C, N, and P fractions. A type of canonical ordination, RDA combines regression and PCA to determine the structure of two related data sets (Borcard et al., 2011). Organic C, N, and P fractions were treated as an explanatory set of variables to constrain ordination of bands as the set of response variables. All variables were standardized before analysis. Including SOC as an explanatory variable did not change ordination of bands, and so was retained in the model to allow visualization of interrelationships of bands, SOC, and fractions. Redundancy analysis was performed with the *rda* function in the *vegan* package in R. The significance of the canonical model and individual explanatory variables were evaluated by permutation tests.

RESULTS
Differences in Diffuse Reflectance Infrared Fourier Transform Spectroscopy Bands among Fields

Bands representing organic functional groups showed significant differences among fields (Table 3). Bands showing strongest field effects represented aliphatic C-H at 1390 cm^{-1} (F = 24.6), 1405 cm^{-1} (F = 20.8), 2850 cm^{-1} (F = 17.6), and 2924 cm^{-1} (F = 14.4). Weakest field effects were shown by bands representing amine functional groups at 3400 cm^{-1} (F = 2.5, p = 0.011) and 1575 cm^{-1} (F = 1.84, p = 0.059). In contrast to bands, SOM fractions showed a greater range in F-statistics, indicating a more variable influence of field in comparison with functional groups. Relative to functional groups, SOM fractions showed higher and lower field effects, which were greatest for EON (F = 48.1), EOC (F = 42.5), and POXC (F = 32.3), and least for PMN (F = 8.1) and NaHCO$_3$–P$_o$ (F = 10.3), which did not necessarily reflect coefficient of variation across fields (Supplementary Table 2).

Exclusion of SOC from field effect by its inclusion as a covariate in analysis of covariance (ANCOVA) models slightly and consistently decreased the magnitude of field effects for bands,

Table 3. *F*-statistic of diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) bands and organic C, N, and P fractions in surface soils (0–15 cm) of 13 organically managed fields in Yolo County, CA. The *F*-statistic measures the magnitude of the effect field on band intensities or fraction size. *F*-statistics were calculated separately to include soil organic C (SOC) in field effects one-way analysis of variance (ANOVA) and to exclude SOC from field effects one-way analysis of covariance (ANCOVA) by inclusion of SOC as a covariate.†

ANOVA				ANCOVA			
Band, cm^{-1}	F-statistic	SOM fraction	F-statistic	Band, cm^{-1}	F-statistic	SOM fraction	F-statistic
3400	2.5*	POXC	32.3****	3400	2.3*	POXC	50.4****
2924	14.4****	EOC	42.5****	2924	13.6****	EOC	55.3****
2850	17.6****	MBC	12.1****	2850	16.7****	MBC	17.7****
1650	4.9****	PMN	8.1****	1650	4.5****	PMN	8.1****
1575	1.8	EON	48.1****	1575	1.7	EON	55.5****
1470	9.0****	MBN	12.8****	1470	8.4****	MBN	12.5****
1405	20.8****	NaHCO$_3$–P$_o$	10.3****	1405	19.2****	NaHCO$_3$–Po	10.0****
1390	24.6****	NaOH-P$_o$	14.7****	1390	22.5****	NaOH-Po	13.5****
1270	7.4****			1270	6.8****		
1110	4.9****			1110	4.6****		
1080	4.8****			1080	4.6****		
920	4.7****			920	4.4****		
840	3.2****			840	3.2**		

* $p < 0.05$

** $p < 0.01$

**** $p < 0.0001$

† SOC, soil organic carbon; POXC, permanganate-oxidizable carbon; EOC, potassium sulfate extractable organic carbon; MBC, microbial biomass carbon; PMN, potentially mineralizable nitrogen; EON, potassium sulfate extractable organic nitrogen; MBN, microbial biomass nitrogen; NaHCO$_3$–P$_o$, sodium bicarbonate extractable organic phosphorus; NaOH-P$_o$, sodium hydroxide extractable organic phosphorus.

but led to larger increases as well as decreases in the magnitude of field effect for C, N, and P fractions. Changes in F-statistic ranged from −9.1 to −2.2% for bands, with greatest decrease for 1650 cm^{-1} and least for 840 cm^{-1}. In contrast to bands, excluding SOC from field effects caused a range of changes in the magnitude of field effect on fractions. Generally, the magnitude of field effects on SOM fractions increased, with greatest changes for C fractions like POXC (+55.8%) and MBC (+45.8%), and least for P fractions NaHCO$_3$–P$_o$ (-2.8%) and NaOH-P$_o$ (−7.9%).

Principal component analysis showed separation of bands along Axis 1 by representation of aliphatic C-H vs. non-aliphatic functional groups (Fig. 2a), which clustered soils by field of increasing SOC (Table 1) when visualized on the ordination (Fig. 2b). Bands representing aliphatic C-H (2924, 2850, 1470, 1405, 1390 cm^{-1}) were most positively associated with the first axis (44.7% of the total variation in intensity of bands), whereas bands representing aromatic C-H (920, 840 cm^{-1}), C-O of polysaccharide (1110, 1080 cm^{-1}), and C-O of phenol and carboxylic acids (1270 cm^{-1}) were negatively associated with this axis. The band at 1650 cm^{-1} assigned to aromatic C=C was weakly associated with this axis. Fields with greater SOC separated from fields with lower SOC, by greater intensity for bands representing aliphatic C-H. Fields did not show separation by SOC along the second axis (19.2% of the total variation in band intensities), which showed strong positive loadings for bands representing polysaccharide C-O at 1110 and 1080 cm^{-1} and strong negative loadings for the 3400 cm^{-1} band representing amine N-H and hydroxyl O-H.

The DRIFTS band indices of SOM decomposition and recalcitrance related differently with SOC (Fig. 3). An exponential
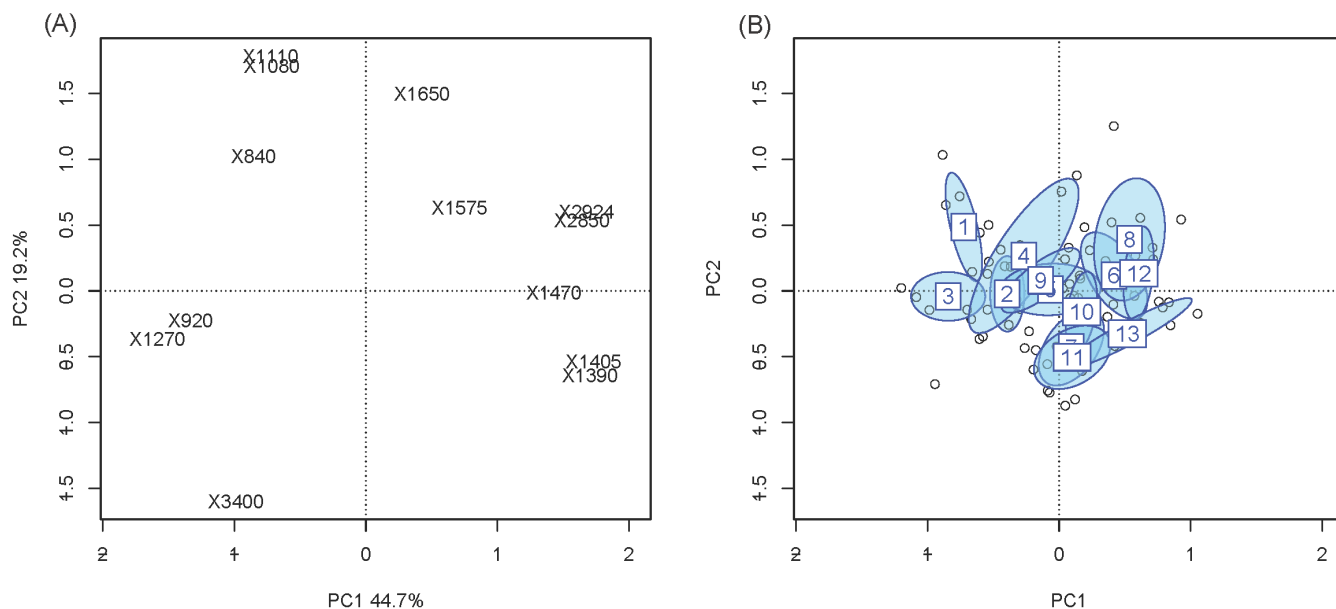
Fig. 2. Principle component analysis (PCA) ordination plot of (a) intensities of diffuse reflectance **infrared** Fourier transform spectroscopy (DRIFTS) bands representing organic functional groups in surface soils (0–15 cm, *n* = 78) of 13 organic tomato (*Solanum lycopersicum*) fields in Yolo County, CA. Axis 1 and 2 explain 44.7 and 19.2% of variation in band intensities. (b) Principal component scores for field plots (6 plots per field) visualized by ellipses and numbered for each field (*n* = 13). Bands represent amine and hydroxyl O-H and N-H (3400 cm^{-1}), aliphatic C-H (2924, 2850, 1470, 1405, 1390 cm^{-1}), aromatic C=C (1650 cm^{-1}) and C-H (920, 840 cm^{-1}), phenol and carboxylic acid C-O (1270 cm^{-1}), polysaccharide C-O (1110, 1080 cm^{-1}), and amide N-H and C=N (1575 cm^{-1}).

decrease in aromatic C=C and C-H relative to aliphatic C-H functional groups (Index I) with increasing SOC (R^2 = 0.55, *p* < 0.0001) indicated that a lower degree of decomposition associated positively with SOC increases. A relative increase in C- vs. O-functional groups (Index II) was weakly associated with increasing SOC (R^2 = 0.16, *p* < 0.001), indicating an increase in SOM recalcitrance that was less explained by SOC as its degree of decomposition.
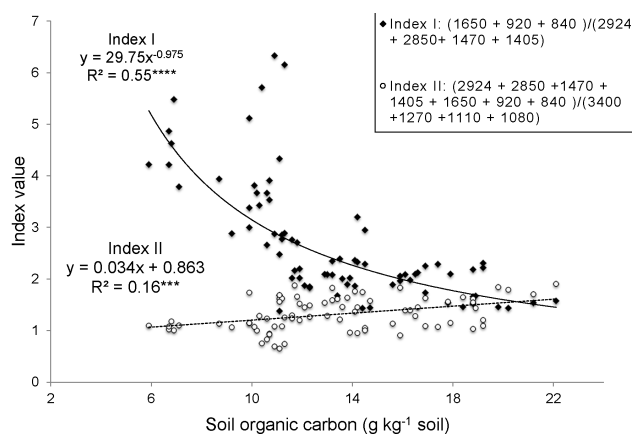

Fig. 3. Diffuse reflectance **infrared** Fourier transform spectroscopy (DRIFTS) bands indices indicating differences in SOM decomposability (Index I) and recalcitrance (Index II) across 13 organic tomato (*Solanum lycopersicum*) fields in Yolo County, CA. Indices are based on intensities of DRIFTS bands representing functional groups in surface soils (0–15 cm). Index I indicates the relative degree of decomposition of SOM as the ratio of aromatic C=C and C-H to aliphatic C-H functional groups. Index II indicates relative recalcitrance as the ratio of C- to O-functional groups.

Relationships of DRIFTS Bands with Organic Carbon, Nitrogen, and Phosphorus Fractions

Soil organic C and POXC overall expressed the strongest correlations with the 13 bands (Table 4). SOC had positive and strongest correlations with bands representing aliphatic C-H (e.g., R_{1405} = 0.70) and negative correlations with bands representing other functional groups such as aromatic C=C (1650 cm^{-1}) and C-H (920, 840 cm^{-1}), phenol and carboxylic acid C-O (1270 cm^{-1}), polysaccharide C-O (1110, 1080 cm^{-1}), and amine N-H and hydroxyl O-H (3400 cm^{-1}). The band at 1575 cm^{-1} representing amide N-H and C=N did not correlate with SOC.

The selected bands generally showed stronger correlations with organic C and N fractions as compared with organic P fractions (Table 4). Bands at 2924 and 2850 cm^{-1}, showing the strongest correlations with SOC, also had a strong correlation with POXC, and with EOC and EON. Correlations of bands with fractions differed from correlations of bands with SOC. Potassium sulfate extractable organic C and EON showed stronger relationships with SOC than POXC (Table 5). In contrast with bands representing aliphatic C-H, the aromatic C=C band at 1650 cm^{-1} showed a significantly negative correlation for EON, NaHCO$_3$–P$_o$, and NaOH-P$_o$. Bands with fewest correlations with fractions represented amine N-H and hydroxyl O-H at 3400 cm^{-1} and amide N-H and C=N at 1575 cm^{-1}. The strongest correlation of a specific fraction with a band varied from weaker to stronger than the correlation of the fractions with SOC (Table 5). Equal or stronger correlations for bands as compared with SOC were observed for POXC, PMN, MBN, NaHCO$_3$–P$_o$, and NaOH-P$_o$, whereas EOC, EON, and MBC showed stronger correlations with SOC. The relative increase in

Table 4. Pearson coefficients (R) for two-directional correlations between absorbance intensity of 13 diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) bands representing organic functional groups with soil organic C (SOC) and organic C, N, and P fractions in surface soils (0–15 cm) of 13 organically managed fields in Yolo County, CA. Band intensities are normalized to the total absorbance intensity of the 13 bands.†

Band, cm^{-1}	SOC	POXC	EOC	MBC	PMN	EON	MBN	NaHCO$_3$–P$_o$	NaOH-P$_o$
3400	-0.23 *	-0.42****	-0.28*						
2924	0.51****	0.70****	0.49****		0.25*	0.31**	0.28*		
2850	0.54****	0.73****	0.53****	0.22*	0.27*	0.35**	0.31**		
1650	-0.37***		-0.41****	-0.29*		-0.54****		-0.27*	-0.30**
1575		0.29*				0.28*			
1470	0.55****	0.45****	0.47****		0.45****	0.35**			
1405	0.70****	0.56****	0.49****	0.23*	0.49****	0.39***	0.25*		
1390	0.71****	0.57****	0.47****	0.30**	0.54****	0.39***	0.31**		
1270	-0.35**	-0.38***			-0.27*				
1110	-0.59****	-0.29*	-0.46****	-0.27*	-0.37**	-0.49****			
1080	-0.34**		-0.23*		-0.30**	-0.26*			-0.26*
920	-0.39**	-0.48****					-0.23*		
840	-0.43****	-0.34**	-0.30**			-0.26*			

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

**** $p < 0.0001$

†SOC, soil organic carbon; POXC, permanganate-oxidizable carbon; EOC, potassium sulfate extractable organic carbon; MBC, microbial biomass carbon; PMN, potentially mineralizable nitrogen; EON, potassium sulfate extractable organic nitrogen; MBN, microbial biomass nitrogen; NaHCO$_3$–P$_o$, sodium bicarbonate extractable organic phosphorus; NaOH-P$_o$, sodium hydroxide extractable organic phosphorus.

band intensities of aliphatic C-H most strongly associated with labile C (POXC, EOC) and N (EON), reflecting associations of these fractions with SOC (Table 5).

When organic C, N, and P fractions were used to constrain the ordination of DRIFTS bands, the overall RDA model accounted for 35% of the variation in band intensities ($p < 0.001$; Fig. 4). The constrained ordination showed similar patterns of bands and fields as the PCA. Significant effects on band ordination were observed for SOC and POXC ($p < 0.001$), as well as EOC, PMN, and EON ($p < 0.05$). Additional fractions did not significantly constrain ordination of bands.

Bands representing aliphatic C-H were most strongly associated with Axis 1, which accounted for 67.0% of the variation in the fitted model and 28.7% of the overall variation, and distinguished aliphatic C-H from non-aliphatic C-H functional groups (Fig. 4a). When visualized by field (Fig. 4b) a similar trend as in PCA was evident, in which fields separated by SOC content along the first component (Axis 1) with increasing intensity of bands representing aliphatic C-H. The second component (Axis 2) explained a lower proportion of fitted variation in band intensities (18.3%, 7.8% of overall variation) and did not show associations of bands by functional group. Fields with higher SOC and receiving composted green waste separated from plots with lower SOC receiving manure, by greater band intensities for aliphatic C-H.

DISCUSSION
Functional Group Composition across Thirteen Fields in Relation to Soil Organic Carbon

DRIFTS analysis identified relative enrichment in aliphatic C-H functional groups with increasing SOC as the most salient change in functional group composition of SOM across the 13 organic tomato fields. The greater differentiation of fields by absorbance intensity of aliphatic C-H relative to other functional groups reflects the SOC gradient among fields, since aliphatic C-H bands were most associated with SOC compared with other bands. Previous work using laboratory incubations has shown that decomposition of the light fraction (LF) of SOM occurs with a decrease in absorbance at 2930–2850 cm^{-1}, suggesting that these aliphatic C-H bands mark the presence of labile C (Calderón et al., 2011b). Yet aliphatic C-H functionalities include methylene (-CH$_2$) and methyl (-CH$_3$) groups that occur with functional groups like amide N-H and aromatic C=C in organic macromolecules like polypeptides, and are present in compounds considered labile (e.g., amino sugars) and recalcitrant (e.g., lignin). Until more soil decomposition data is available, caution should be taken in interpreting DRIFTS characterizations of functional group composition as causing or resulting from changes in SOM lability (Kleber, 2010).

Previous studies suggest that the observed increase in aliphatics vs. aromatic functional groups with increasing SOC

Table 5. Pearson coefficients (R) for two-directional correlations between soil organic C (SOC) and organic C, N, and P fractions in surface soils (0–15 cm) of 13 organically managed fields in Yolo County, CA.†

POXC	EOC	MBC	PMN	EON	MBN	NaHCO$_3$–P$_o$	NaOH-P$_o$
0.69****	0.84****	0.56****	0.52****	0.78****	0.31**		0.23*

* $p < 0.05$

** $p < 0.01$

**** p < 0.0001

† POXC, permanganate-oxidizable carbon; EOC, potassium sulfate extractable organic carbon; MBC, microbial biomass carbon; PMN, potentially mineralizable nitrogen; EON, potassium sulfate extractable organic nitrogen; MBN, microbial biomass nitrogen; NaHCO$_3$–P$_o$, sodium bicarbonate extractable organic phosphorus; NaOH-P$_o$, sodium hydroxide extractable organic phosphorus.
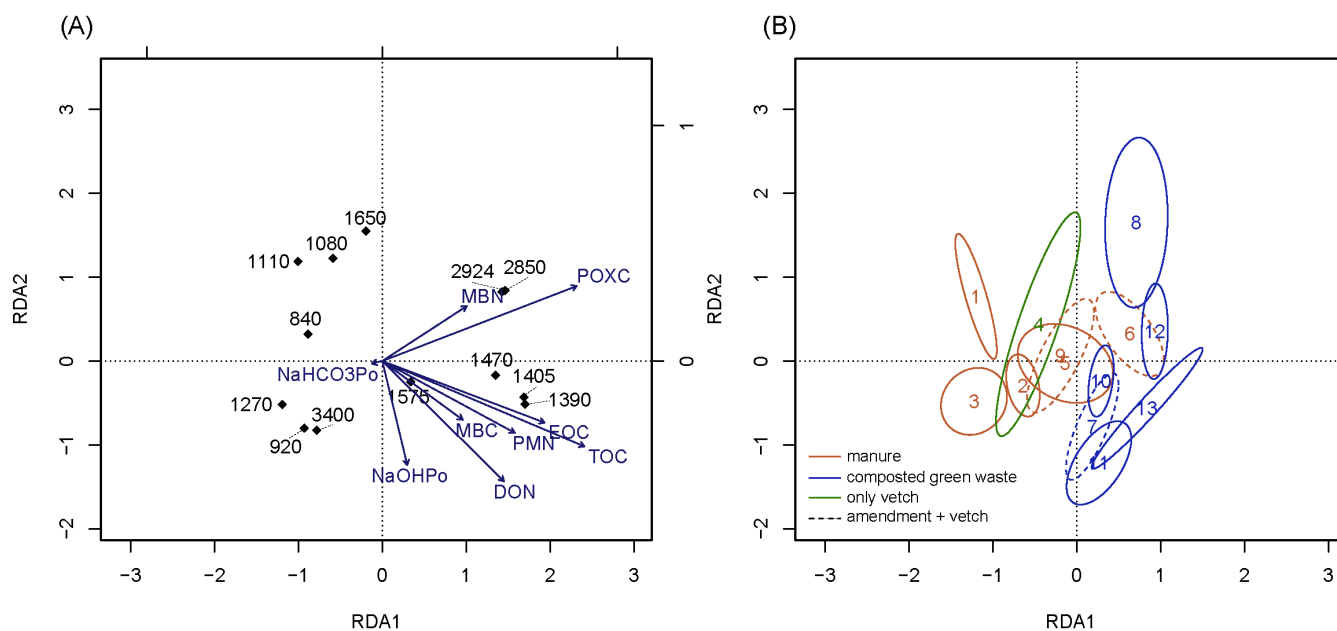
Fig. 4. Redundancy analysis (RDA) ordination plots of intensities of DRIFTS bands representing organic functional groups, constrained by organic C, N, and P fractions in surface soils (0–15 cm, *n* = 78) of 13 organic tomato (*Solanum lycopersicum*) fields in Yolo County, CA. (a) Vectors represent soil organic matter (SOM) fractions. Axes 1 and 2 represent 28.6 and 8.0% of the total variation in band intensities, ordination of which was significantly constrained by organic C, N, and P fractions (*p* < 0.0001). (b) 95% confidence ellipses for RDA field scores (*n* = 6), numbered by field (*n* = 13) and colored by organic amendment used in 2010–2011. Dotted ellipses indicate fields in which vetch cover crop was grown in addition to an amendment (manure or composted green waste).

could reflect several mechanisms. Aliphatic enrichment has been observed during residue decomposition (Ait Baddi et al., 2004; Hsu and Lo, 1999), provides a measure of SOM biological quality (Veum et al., 2014) and is associated with increases in labile and total soil C (Demyan et al., 2012; Gerzabek et al., 2006; Giacometti et al., 2013). This has been proposed to result from higher inputs of aliphatic-rich OM (e.g., plant residues from cover crops) and deposition of aliphatic compounds from increased microbial biomass (Aranda et al., 2011). However, for the fields examined in this study, neither aliphatic C-H nor other functional groups associated with fields based on cover crops, and aliphatic C-H band intensity correlated relatively poorly with MBC and MBN, which correlated weakly with SOC across fields (Bowles et al., 2014). One possibility for the weak relationships may be that microbial biomass responds rapidly to management and environmental changes (Kallenbach and Grandy, 2011) whereas SOM composition may integrate changes over a longer period (Solomon et al., 2007). Thus, band intensity of aliphatic C-H may serve as a more stable indicator of labile C. The inverse association of the degree of SOM decomposition (Index I) and SOC may reflect accumulation of less decomposed organic matter that occurs with high C loading of soil (Six et al., 2002; Stewart et al., 2008). Relative changes in bands representing functional groups suggest that SOM quality may change with SOM content. Index I and Index II show a conflicting decrease in decomposition maturity and increase in recalcitrance, though the former shows a stronger relationship with increasing SOC. This may reflect chemistry-centric definitions of decomposition and recalcitrance (Schmidt et al., 2011), or the bulk characterization of SOM. Indices may be sensitive to specific fractions of

SOM, and so both may increase with increasing SOC as a matter of stoichiometry. This illustrates the potential of DRIFTS to provide measures of functional group composition implicated in labile SOM, but also demonstrates limitations on causal conclusions from solely spectroscopic approaches.

Functional Group Composition and Soil Organic Matter Fractions

The weaker influence of field effects on bands as compared with C, N, and P fractions (Table 3, ANOVA) and its lower sensitivity to non-SOC field effects (Table 3, ANCOVA) indicates functional group composition is more strongly associated with SOC among different fields than C, N, and P fractions. This may reflect the more direct relationship of functional groups with SOM as its molecular scaffold. Additionally, functional group composition of SOM could be constrained by similar stoichiometric limitations in living OM (e.g., membrane lipids, proteins), and by converging pathways of decomposition and stabilization in the soil environment (Wickings et al., 2012). Measured fractions reflect processes such as mineralization (e.g., POXC, PMN) or are biomass-based measures (e.g., MBC, MBN), which is likely why these fractions are sensitive to field-specific differences like nutrient management (Culman et al., 2012; Kallenbach and Grandy, 2011). Fractions like POXC and MBC are considered labile and are strongly associated (Culman et al., 2012; DuPont et al., 2010; Melero et al., 2009; Weil et al., 2003), yet showed different associations with bands, suggesting differences in functional group chemistry may underlie similarly labile fractions.

Organic Carbon and Nitrogen vs. Phosphorus Fractions Relate Differently to Functional Group Composition

Positive associations of aliphatic C-H bands with SOC as well as labile organic C fractions, in particular POXC, EOC, PMN, and EON, suggest changes in aliphatic C-H are implicated in increases in labile organic C and N fractions. This may explain previous findings of proportionally greater increases in labile SOM fractions under organic management that increase SOM content (Drinkwater et al., 1998; Graham et al., 2002; Marriott and Wander, 2006; Robertson and Paul, 2000). In contrast, organic P fractions weakly correlated with aliphatic C-H. Though NaOH-P_o appeared to associate with aliphatic C-H (Fig. 4, RDA), it did not correlate with bands representing this functional group but did relate to SOC, suggesting an association of this organic P fraction with SOC unrelated to the observed trend of increasing aliphatic C-H with SOC.

Different relationships of organic C and N vs. organic P fractions may reflect greater coupling of C and N relative to C and P in soils (Condron and Tiessen, 2005) and/or the strong effects on soil P of P-rich inputs like manure common in organic systems (Lehmann et al., 2005). On the other hand, this may reflect the lower proportion of P relative to C and N in soils and SOM (Essington, 2004), which decreases DRIFTS sensitivity to P-O bonds (He et al., 2006). No significant correlations were found for organic P fractions for bands at 1110 and 1080 cm^{-1}, despite absorbance of organic P functional groups in this region, chiefly ν(C-O-P) at 1115 cm^{-1} and ν(P-O) at 1085 cm^{-1} (Parikh et al., 2014).

CONCLUSIONS

This study demonstrates that there are significant differences in organic functional group composition detectable by DRIFTS across 13 organic tomato fields, and that these differences are strongly related to SOC and specific labile organic C and N fractions. The on-farm approach provided the wide range of variation necessary to evaluate relationships between functional group composition and SOM in real world settings, thus complementing experimental research focusing on effects of specific amendments or long-term management. Organic P fractions were in comparison poorly associated with bands, as well as SOC, potentially reflecting biochemical decoupling of C and P relative to C and N in soils. Increases in bands representing aliphatic C-H functional groups associated positively and most strongly with increases in SOC and fractions. Soil organic matter transformations influencing specific labile fractions may therefore involve changes in relative aliphatic C-H abundance. Diffuse reflectance infrared Fourier transform spectroscopy characterization of functional group composition offers potential for understanding relationships of SOM content and lability.

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