

Relationships between field management, soil health, and microbial community composition[☆]

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ABSTRACT

More meaningful and useful soil health tests are needed to enable better on-farm soil management. Our objective was to assess the relationship between field management, soil health, and soil microbial abundance and composition (phospholipid fatty acid analysis (PLFA)) in soil collected from two fields (farmer-designated ‘good’ versus ‘poor’) across 34 diverse (livestock, grain or vegetable cropping) farms in Maritime Canada. Soil health was measured using soil texture, surface hardness, available water capacity, water stable aggregates, organic matter, soil protein, soil respiration, active carbon, and standard nutrient analysis. All soils were medium to coarse textured (< 8% clay). Mixed models analysis showed that both CSHA and PLFA were able to resolve statistical differences between cropping systems, however conventional soil chemical analysis was the only testing method to resolve statistical differences between farmer designated ‘good’ and ‘poor’ fields. Principle component analyses determined management history (rotation over previous three years), but not ‘good’ or ‘poor’ field designation, to be an important determinant of soil health. Water-stable aggregates and soil respiration were positively correlated with all PLFA microbial groups, and negatively correlated with sand, P, Cu and Al. Lower-intensity management (perennial forage, mixed annual-perennial cropping), manure application and low tillage were linked to higher soil respiration, water-stable aggregates, fungi, mycorrhizae, Gram negative bacteria, and lower soil available P. Correlations between CSHA and PLFA shows promise for integrating these two tests for improved soil health assessment.

1. Introduction

Soil is a dynamic living system whose condition underpins agricultural productivity and ecosystem function (Doran et al., 1996). While healthy soils promote the provision of ecological services, soil degradation can lead to environmental strain and loss of productivity (Bennett et al., 2010; Pepper, 2013). Broadly, soil health is understood to be the combination of physical, chemical and biological properties that promote the ability of a soil to support human, plant and animal needs while maintaining or enhancing environmental quality (Doran et al., 1996; Moebius-Clune et al., 2016).

Assessing soil health requires a more comprehensive approach to

testing than conventional soil quality work that focuses on a few individual parameters; ideally, it must encompass chemical, biological and physical indicators as well as trends and emergent properties (Karlen et al., 1997). Methods for assessing soil health range from in-field observational scorecards, such as the Wisconsin Soil Health Scorecard (Romig et al., 1995) to comprehensive laboratory tests of a minimum set of indicators, such as the Cornell Soil Health Assessment (CSHA) (Moebius-Clune et al., 2016). Other soil health work has explored bio-indicator species, such as presence or abundance of nematodes, earthworms, collembola, or abundance and diversity of microbial indicators (Pankhurst et al., 1995; van Bruggen and Semenov, 2000; Griffiths et al., 2016). Phospholipid fatty acid (PLFA) profiling of soil

Abbreviations: G +ve, Gram positive; G –ve, Gram negative; ACE, Autoclaved-Citrate Extractable; AMF, arbuscular mycorrhizal fungi; AWC, available water capacity; BSA, bovine serum albumin; CEC, cation exchange capacity; CSHA, Cornell Soil Health Assessment; ENVT, environmental and management data; F:B, fungi:bacteria; FID, flame ionization detector; g, farmer-identified good field; KOH, potassium hydroxide; OSHA, Ontario Soil Health Assessment; p, farmer-identified poor field; PCA, principal component analysis; PLFA, Phospholipid fatty acid analysis; REML, restricted maximum likelihood; SOC, soil organic carbon; SOM/OM, soil organic matter; WSA, wet stable aggregates

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microbial groups is another possibility for soil health testing (Bossio et al., 1998; Shestak and Busse, 2005; Zhang et al., 2014). Recent work suggests that many biological indicators differentiate between site-level differences, though most are not sensitive enough to management practices on their own and should be integrated with a suite of other indicators (Griffiths et al., 2016).

The CSHA was made available in 2006 as a cost-effective protocol for assessing soil health in the New York region of the United States. It incorporates key physical, chemical and biological indicators chosen for their relevance, sensitivity, consistency and cost, and has been evaluated in multiple trials (Schindelbeck et al., 2008; Idowu et al., 2008; Moebius-Clune et al., 2016). As part of the analysis, between 9 and 16 indicators are assessed. The standard suite includes soil texture, available water capacity (AWC), surface and subsurface hardness, wet aggregate stability (WSA), organic matter (OM), Autoclaved-Citrate Extractable (ACE) soil protein, soil respiration, active C, and standard nutrient analysis. Indicators are individually scored out of 100 and mean values are used to generate an overall soil health score. These scores are calculated within soil textural classes based on Cornell's soil database, and thus are a comparison to a regional range of parameters. This is a challenge for using the test outside of the New York area, as the scoring is not calibrated to yield data, nor does it reflect real biological or physical thresholds.

Recent research has worked to validate the CSHA for use in Canada. In southwestern Ontario, the CSHA was used to assess soil health differences between tillage management regimes in long-term (20 year) field trials; the CSHA's assessment of soil health matched the assessments of soil organic carbon (SOC) and total N (Van Eerd et al., 2014). Congreves et al. (2015) compared soils from field trials of no-till, diverse rotational systems to conventionally tilled monocultures in the same region. The authors compared the CSHA to an Ontario Soil Health Assessment (OSHA), which assessed the same indicators as the CSHA but used weighted averages based on principal component analysis (PCA) (Congreves et al., 2015). PCA showed that root health, sand content, Mn, and pH were less valuable as soil health indicators in Ontario, and using weighted averages made the OSHA more sensitive to management effects (Congreves et al., 2015).

Phospholipid fatty acids are present in all living cells and are found in the cell membranes of microorganisms (Hill et al., 2000). Unique fatty acids, or groups of fatty acids, have been linked to specific functional groups of microorganisms and can be differentiated based on chain length, branching and saturation (Willers et al., 2015). Biomarker patterns or individual biomarkers can be used to identify microbial groups, such as arbuscular mycorrhizal fungi (AMF) or Gram-negative bacteria (Willers et al., 2015). The PLFA profile can be used to characterise community composition, determine microbial biomass, and provide an indication of the metabolic or functional state of the community (Frostegård et al., 2010).

PLFA profiles are detailed enough to demonstrate differences in the microbial community affected by management practices and soil factors. Bossio et al. (1998) found significant differences between PLFA profiles in organic and conventional field plots, and Bardgett et al. (1997) found a clear shift in microbial community as grasslands shifted between grazed and ungrazed management. The effect of pH on PLFA profiles is also marked: PLFA profiles are sensitive to pH both in agricultural soils (Rousk et al., 2010) and in forest soils which have received lime (Frostegård et al., 1993). Compaction has a smaller effect on PLFA profiles (Shestak and Busse, 2005). PLFA is useful for measuring fungal:bacterial biomass ratios (Frostegård and Bååth, 1996; Bardgett et al., 1996; Baath and Anderson, 2003), where a higher fungal:bacterial biomass ratio is linked to increased C storage potential in soils (Malik et al., 2016). Given PLFA's strengths, it would be useful to explore its relationship to more comprehensive soil health assessments.

Linking the CSHA to PLFA would provide valuable insights into the relationship between a comprehensive soil health assessment and the

widely used soil microbial profiling tool. Evaluating and comparing these two tests on a wide range of soil types and under diverse management regimes will contribute to improved understanding of soil management effects on soil health and biological characteristics and contribute valuable insights into the CSHA applicability in more diverse regions. The costs and accessibility of these technologies are key factors for the uptake of these methods with farmers. PLFA analysis is a highly technical and relatively cost prohibitive approach to soil analysis that requires a deeper understanding of the soil functioning and knowledge of the existing literature for contextual interpretation. The CSHA, although significantly more practical than the PLFA approach, is time consuming and is cost prohibitive for farmers interested in characterizing several fields. Therefore, it is important to evaluate the efficacy of these emerging approaches relative to conventional soil analysis which are low cost and accessible to all farmers. Based on these needs, the objectives of this study were to evaluate the use of the CSHA in Canadian Maritime agricultural fields by 1) exploring the relationship between CSHA and PLFA profiles; and 2) relating changes in these soil factors to field management history, conventional soil chemical analysis, and inherent soil characteristics.

2. Materials and methods

2.1. Site selection and soil sampling

Thirty-four farms in the Maritime region of Eastern Canada were sampled between August 11 – September 16, 2016, with 12 farms in Nova Scotia (NS), nine in New Brunswick (NB) and 13 in Prince Edward Island (PEI) (Fig. 1). Farmer participation was initiated through an online survey circulated through Maritime agricultural organisations. An initial pool of 59 farmers provided contact and basic demographic information to participate in the project, and responded to the question “How do you know what is a healthy soil and what is a bad soil?” The final 34 farm types varied widely: 14 farms were organic and 20 conventional, including 17 vegetable, eight dairy, six field crops, and three beef/sheep farms (Table 1).

Farmers were asked to select a ‘good’ (g) and a ‘poor’ (p) soil on their farm based on their own perceptions and knowledge of the productivity of each field. These g and p designations were used as subjective tags only and were not used to drive analyses. In total, 68 fields were sampled on 34 farms. Farmers provided basic background information about the farm, including selected field history and management intensity (current crop and 3-year crop history; intensity and frequency of tillage; type, frequency and amount of inputs used, including manure, compost, synthetic fertilizers and pesticides) (Table 2).

Soil samples were collected from both g and p fields based on the methods described in the Comprehensive Assessment of Soil Health Manual for the CSHA (Moebius-Clune et al., 2016). Samples were randomly collected at 10–15 points per field, depending on field size, in a “W” pattern. At each point, surface debris was removed, and a core was collected 5 cm in diameter and 15 cm deep using a shovel. Penetrometer readings (Dickey-John Corporation, Auburn, IL) were taken and maximum hardness recorded for the depths 0–15 cm and 15–46 cm (surface and subsurface hardness, respectively). Samples were mixed thoroughly in a bucket, and 2–3 kg of soil were bagged and placed in a cooler.

Bulk soil samples were stored at 4 °C until further processing and ~50 g of soil was separated and stored at –20 °C for PLFA analysis. At the end of the sampling period, approximately 1 kg of soil was sieved (8 mm) and air dried to a constant weight. Approximately 250 g was further subsampled for chemical analyses, and 100 g was bagged for soil respiration analysis and Autoclave-Citrate Extractable (ACE) protein test (Cornell Soil Health Laboratory, 2016). The remainder of the air-dried soil was further sieved (2 mm) and divided for the following tests: active C and AWC (~200 g) and textural analysis and soil moisture content (100–150 g). In addition, approximately 80 g of soil

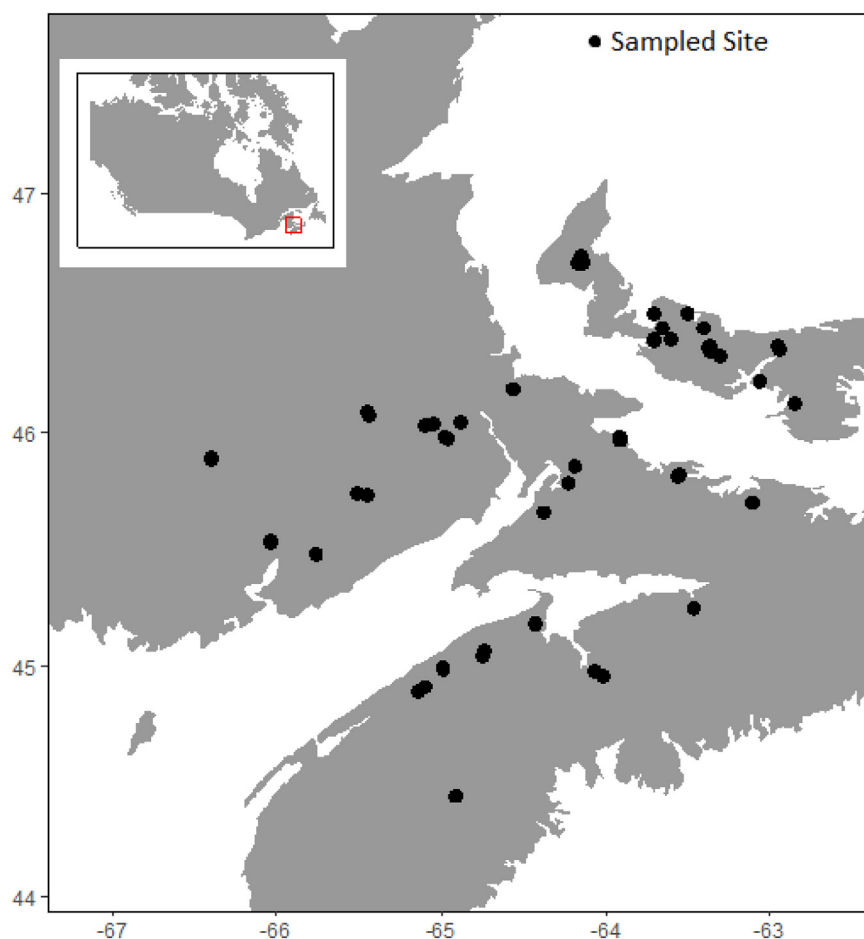


Fig. 1. Points indicate the 34 sampling sites in the Maritime provinces located in Eastern Canada.

macroaggregates (0.25 mm - 2 mm) were separated (W.S. Tyler Ro-Tap, Mentor, OH) for WSA testing.

2.2. CSHA analyses

CSHA analyses (soil texture, surface and subsurface hardness, AWC, WSA, OM, ACE soil protein, soil respiration, active C, and standard nutrient analysis) were performed according to procedures outlined in Cornell's Standard Operating Procedures (Cornell Soil Health Laboratory, 2016). Nutrient analysis, pH and OM were conducted by the Prince Edward Island Analytical Laboratory (Charlottetown, PE), where OM was measured on a combustion analyzer (Hoogsteen et al. 2015) and nutrient analyses using Mehlich-3 extractions. Soil texture was determined using the Buoycous Hydrometer method (Sheldrick and Wang, 1993). Active C was measured by permanganate oxidation; WSA (%) was measured using 0.25–2 mm aggregates placed under a rainfall simulator. Soil respiration was measured via EC change to a KOH trap caused by CO₂ respiration of re-wetted soil. ACE protein was measured

using sodium citrate as the extractant in conjunction with autoclaving, and quantification was performed using bovine serum albumin (BSA) standards on a microplate reader (Biotek™ PowerWave XS2, Winooski, VT). AWC was measured by calculating the difference in water content between soils at field capacity and at permanent wilting point on a 5 Bar Pressure Plate Extractor and a 15 Bar Ceramic Plate Extractor.

2.3. Phospholipid fatty acid analysis

All samples to be used for PLFA analysis were bulked to form a composite sample and stored at -20°C until processing. Soil moisture content was determined gravimetrically for each sample prior to the extraction of fatty acids using a modified Bligh and Dyer technique (Bardgett et al., 1996). Extracted fatty acids were methylated and quantified using an HP 7890 gas chromatograph equipped with a flame ionization detector (FID) (Agilent, Santa Clara, CA). Peaks were integrated and quantified using MIDI software (MIDI Corp, Newark, DE). PLFA bioindicators included actinomycetes, Gram positive (G + ve) and

Table 1
Participant farms by province and farm type.

	New Brunswick		Nova Scotia		Prince Edward Island		Total
	Organic	Conv.	Organic	Conv.	Organic	Conv.	
Vegetable	2		5	3	4	3	17
Dairy		6		1		1	8
Field crops		1			2	3	6
Beef/sheep			1	2			3
Total	2	7	6	6	6	7	34

Table 2
Description of factors and categories for field history data.

Factor	Categories	Meaning
g,p	g	Farmer-identified “good” field
	p	Farmer-identified “poor” field
Rotation type	Grass	Three years of perennial grass (hay, grass, native grass, triple mix, grass forage, pasture)
	Grain	Three years of majority annual grain (corn, wheat, oat, barley, soybean, canola, potato)
	Veg	Three years of majority vegetables
	Mix	Three years of a combination of annual (veg/grain) and perennial crops
	Fallow	Three years of fallow
Tillage intensity and frequency ^a	1	No-till
	2	Medium intensity/conservation till (conservation till, broadfork/hand till, s-tine, harrow, chisel plow, spading machine)
	3	High intensity (land-forming, sub-soiling, raised bed maker, conventional till, potato till, moldboard, 3-furrow, disc, plow, till, rototill, etc.)
Manure	Total amount applied	Amount (Mg ha ⁻¹) of manure applied to each field over the duration of the previous three years.
Compost	Total amount applied	Amount (Mg ha ⁻¹) of organic inputs applied (including mussel shells, biowaste, Irish moss) over the previous three years.
Lime	Total amount applied	Amount (Mg ha ⁻¹) of dolomitic or calcitic lime applied over the previous three years.
Synthetic fertilizer	# of applications	Total number of applications of synthetic fertilizers (N, P, K, S) was applied over the previous three years.
Pesticides	# of products used	Total number of pesticides applied over the previous three years including fungicides, insecticides and herbicides.
Sand, silt or clay	%	Percentage of each soil component

^a Tillage intensity is determined for each year then added together to give one measurement (i.e. low tillage for three years = 1 × 3 = 3).

Gram negative (G – ve) bacteria, fungi, AMF, and eukaryotes. Ratios of total fungal:bacteria (F:B) biomass, and G – ve stress indicator were also computed automatically in the software. The G – ve stress indicator is based on observations of increased G – ve PLFAs with stress conditions (Frostegård et al., 1993; Willers et al., 2015). Soil quantification of PLFAs are presented as nmol g⁻¹ of soil (Frostegård et al., 1993; Frostegård and Bååth, 1996; Bardgett et al., 1999; Baath and Anderson, 2003; Kelly et al., 2003; Kelly et al., 2007; Wardle and Jonsson, 2014).

2.4. Statistical analysis

Field history data provided by the farmers were used to generate correlations between soil factors and management practices. Management factors were categorised according to three-year field history based on the most common management practice over the past three years, for example, a vegetable (veg) rotation was considered any rotation that consisted of a majority of vegetable crops, whereas a field that had a rotation of hay/hay/vegetable would be considered “mix” (Table 2).

All categories of crop rotation (fallow, grain, grass, mix, veg) were present in both good and poor fields, although only one good field was in fallow (g fallow). Manure was applied more commonly on grass fields under dairy production systems, compared to other field types. Organic farms – which were mainly vegetable farms – tended to apply more compost and other organic matter inputs including crab meal, bone meal, diatomaceous earth, and Irish moss, compared to conventional producers. The factors Tillage, Manure, Compost, Lime, and Synthetic fertilizer were treated as categorical variables and were coded as 1–3 based on the frequency of their use in each field the previous three years before the sampling period. The variable “Pesticides” was treated as a categorical variable based on aggregated annual frequency of each of herbicide, insecticide, and fungicide (0–9 with 0 being no pesticide use and 9 being annual application of each of the three groups over three years).

A residual maximum likelihood model (REML) was done on all variates with a random effect for provinces and a fixed effect for rotation and environment/management factors. The means from the REML were used in all the principle component analysis (PCA) steps. PCA was used for data reduction and data analysis: first, PCA was used as a data reduction technique on the four main data sources: environmental and management data (ENVT), soil chemical data from the Mehlich 3 extractable nutrients (“CHEM”), Cornell Soil Health Assessment data

(“CSHA”) and phospholipid fatty acid data (“PLFA”). The principal components of these four PCAs were then used to further evaluate relationships between factors explaining the greatest amount of variability in the dataset. A final PCA analysis, factors on score 1 from all of the previous analyses which had the greatest relative importance (eigenvalues of ≥2.0) were then used in a subsequent PCA to explore their relationship between each other under the context of the farmer perceived “g” and “p” under each cropping system category. Correlation matrices were generated between factor groups and visualized using package “ggcorrplot” in R. All correlation plots had the factors grouped based on the first principle component.

3. Results

Fields ranged in size from < 0.25 ha to > 40 ha. The soils ranged widely and included Entisols, Inceptisols, Spodosols, Boralfs and Aquisuborders, but all fields were coarse- or medium-textured, with average sand, silt and clay content at 53.6%, 39.1% and 7.3% respectively. Samples were collected at the end of a summer in which most regions had experienced significant drought, which negatively impacted the reliability of penetrometer readings. For this reason, penetrometer readings were not used in the final analyses.

3.1. Mixed models evaluations

Mixed models analyses (REML) showed significant differences between cropping practices (rotation) and environment/management factors with regards to tillage, compost application and pesticides under environmental and management factors. These analyses did not show a significant difference between g vs. p fields, nor the interaction between the two terms (Table 3). Similarly, mixed models analysis of the variables measured under the CSHA showed significant differences between rotations for everything except subsurface hardness and available water holding capacity. Of all of the CSH variates, only pH showed a significant difference between farmer-designated g vs. p fields (6.29 vs. 5.91). Additionally, with the CSHA approach there were no significant interactions between factors rotation and g vs. p. Mixed models analysis performed on PLFA bioindicator data showed significant differences between the rotations for all variables except for total fungi, and F:B biomass (Table 3). No significant interactions were observed of PLFAs between rotation and g vs. p, nor were there any significant interactions between the two terms (Table 3). Mixed models analysis of soil chemistry showed the greatest resolution in detecting some statistical

Table 3
Mixed models analysis output showing mean values and *F*-statistics for each factor tested.

Factor	<i>F</i>	<i>R</i>		<i>F</i>	<i>G</i> vs. <i>P</i>	<i>F</i>	<i>R</i> × <i>G</i> × <i>P</i>
Sand	0.71	54.48		0.21	56.09	1.35	53.27
Silt	0.70	38.35		0.38	36.69	1.56	39.51
Clay	0.38	7.23		0.12	7.28	0.38	7.29
Tillage	20.82	4.99	***	1.92	5.35	0.31	4.92
Manure ^b	0.56	6.49		0	5.05	0.28	6.81
Compost ^b	4.35	7.51	***	0.15	5.15	0.04	7.75
Lime ^b	1.05	0.21		2.33	0.32	1.03	0.23
Synthetic fertilizer	0.58	1.11		1.34	0.22	0.25	1.07
Pesticides	5.48	0.91	***	0.51	1.16	1.91	0.81
Cornell Soil Health Assessment							
Surface hardness	3.13	236.41	*	2.68	244.22	1.73	234.72
Subsurface hardness	0.93	299.82		2.01	299.82	1.07	299.84
AWC	1.32	0.21		0.16	0.23	1.08	0.22
WSA	3.29	59.49	*	0.17	55.72	1.45	60.59
Active carbon	2.73	553.94	*	0.07	526.31	1.07	559.44
Organic matter	3.39	4.04	*	0	3.84	0.95	4.12
ACE soil protein index	2.58	10.34	*	0.43	9.65	1.33	10.41
Soil respiration	4.72	0.89	**	0.21	0.85	0.91	0.91
P	2.82	130.10	*	3.22	135.74	1.18	124.11
K	5.14	105.72	**	0.67	102.21	0.65	106.01
pH	3.62	6.12	*	4.83	6.11	0.63	6.12
PLFA							
AMF	5.92	0.49	***	0.07	0.45	0.17	0.49
G –ve bacteria	5.67	4.12	***	1.26	3.75	0.28	4.01
Eukaryotes	3.08	0.34	*	0	0.34	0.51	0.34
Non-mycorrhizal fungi	2.48	0.21		3.53	0.24	0.47	0.22
G +ve bacteria	5.33	2.32	**	0.45	2.17	0.42	2.29
Anaerobe	3.91	0.11	**	0.21	0.09	0.07	0.12
Actinomycetes	4.80	0.72	**	2.76	0.66	2.23	0.73
F:B	2.25	0.01		1.04	0.12	1.98	0.01
G –ve stress	3.29	203.72	*	0.39	199.71	0.64	204.82
Soil chemistry analysis ^a							
P	3.19	130.12	*	2.77	135.73	1.12	124.12
K	5.23	105.72	**	0.62	102.21	0.58	106.44
Mg	2.35	163.24		4.68	155.23	0.75	164.23
Fe	0.51	237.51		0.03	230.22	0.73	240.1
Mn	1.18	60.52		1.94	56.25	1.12	62.44
Zn	1.67	2.89		3.05	2.85	0.62	2.99
Na	0.94	24.88		0.38	24.29	1.24	24.93
B	1.95	0.72		4.05	0.65	1.31	0.74
Cu	0.81	2.66		1.95	3.02	2.85	2.41
S	5.68	21.66	***	0.02	21.08	1.06	21.66
Ca	5.13	1479.42	***	6.61	1421.12	0.35	1487
Al	2.65	1281.21	*	0.62	1342.09	0.55	1265
pH	3.62	6.12	**	4.83	6.12	0.62	6.12
CEC	2.50	10.96		2.54	10.46	0.43	11.12
K (%)	2.85	2.63	*	0.52	2.67	0.52	2.59
Mg (%)	2.54	13.16		0.13	12.84	0.59	12.99
Ca (%)	1.34	67.33		3.85	67.65	0.64	66.49
H+ (%)	2.94	11.53	*	5.43	11.85	0.51	12.41
Na (%)	1.60	1.11		1.56	1.09	1.01	1.11
Total base cations (%)	1.64	83.14		4.36	83.17	0.9	82.09

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

^a Unless otherwise stated, units for soil nutrient concentration is ppm.

^b Mg ha⁻¹.

differences between rotations, *g* vs. *p* fields, and the interaction of both terms. Significant ($p < 0.05$) differences between rotations were observed for P (83.7–205.3 ppm for grass and fallow), K (71.79–151.3 ppm for grass and veg), Al (1215–1714 ppm for grass and fallow), pH (5.92–6.41 for grass and veg), K (1.8–3.2% in grass and veg), H+ (5.7–17.2% in grain and fallow); highly significant ($p < 0.001$) differences were observed in S (18.6–27.3 ppm in grass and veg) and Ca (1075–1876 ppm in fallow and veg). Significant differences between *g* vs. *p* fields were observed with Mg (172.1 vs. 138.2 ppm), B (0.73 vs. 0.56), Ca (1641 vs. 1201 ppm), pH (6.29 vs. 5.91), H+ (4.93 vs. 18.76%), and total base cations (89.2 vs. 77.1%). There was a significant interaction between rotation and *g* vs. *p* fields observed with Cu (ranging from 8.81 ppm in *g* fallow to 1.04 ppm in *p*

grass (Table 3).

Differences were observed between rotations and farmer designated *g* and *p* fields regarding the various environmental and management factors (Fig. 2). The use of tillage (frequency and intensity), compost, fertilizer, and lime appeared to be higher in the *g* fields relative to the *p* fields for most rotations. Manure application was higher in *p* fields for all rotations except for grain and veg. The 'icides' category representing pesticide application was higher in the fallow, grain and grass rotations, but the opposite was observed for the mix and veg rotations (Fig. 2).

3.2. Multivariate analysis

Principal component analysis was used as a data reduction strategy

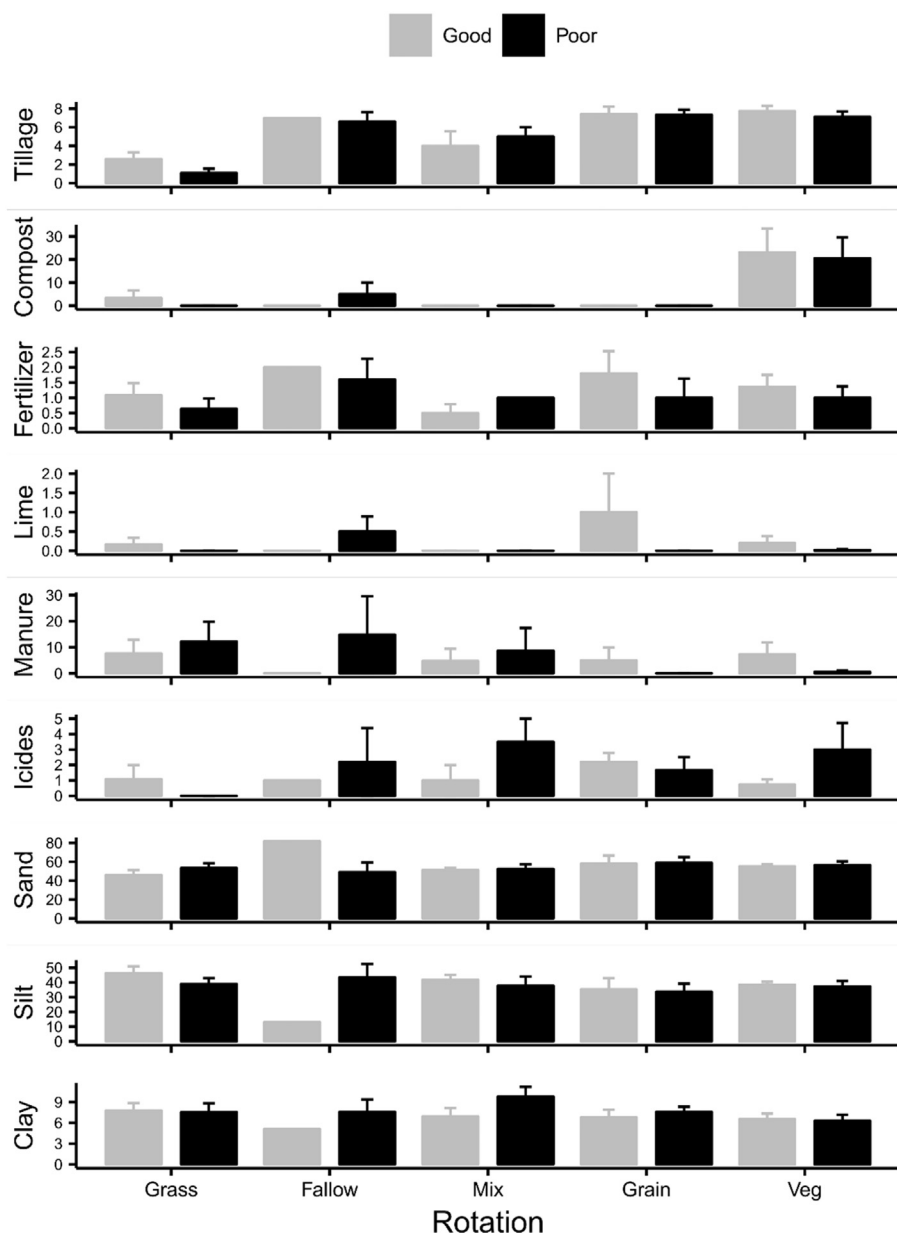


Fig. 2. Mean values of *g* vs. *p* farmer selected fields. Mean values for tillage, fertilizer and icides are based on categorical designations for frequency and intensity; lime, manure and compost means are based on actual values of estimated amount applied in Mg ha^{-1} ; sand, silt and clay mean values are based on percentage of each component in the soil sample.

to infer correlations between the tested factors (Fig. 3a–d). For the evaluation of environmental factors, 43% of variability in the data was explained by loadings on the first score with tillage, synthetic fertilizer and sand loading positively, and manure, silt and clay loading negatively. Environmental factors on the second score loading positively included lime and compost to a greater extent, and the loads on the negative side were explained largely by pesticides. A PCA biplot of environmental factors on rotation (Fig. 3a), shows associations between *g* grain rotations and increased use of synthetic fertilizer and tillage; correlation between *g* veg rotations and higher levels of compost and lime; both *g* fallow and *g* and *p* grass rotations correlated with soil textural components including sand, silt and clay. *P* grain rotations correlated with increased pesticide use; *p* fallow was associated with lower levels of pesticide and higher levels of manure application.

CSHA evaluation of soil health indicators through PCA explained 52% of the variation in the dataset under score one (positive loading: AWC, WSA, active C, OM, ACE, soil respiration; negative loading: P).

Score 2 explained 26% of the variability in the data; it loaded positively with K and pH, and negatively with surface and subsurface compaction (Fig. 3b). Both *g* veg and *g* grain were associated with increasing K and pH values. Additionally *p* mix and fallow correlated with increasing values of surface and sub-surface compaction. Both *g* and *p* grass-based rotations were associated with increasing soil respiration values. The *g* mixed rotation correlated with increasing values of AWC and WSA.

Variation in the PLFA microbial community dataset (Fig. 3c) was mainly explained in the first score (80%) which loaded positively with all factors. Only 9% of variation was explained by the second score which loaded positively with the eukaryote biomarker, and loaded negatively with the fungi biomarker and F:B. Both *g* and *p* grass rotations as well as *g* and *p* mixed rotations correlated with increasing concentrations of the G –ve bacteria biomarker. Finally, *g* fallow rotation correlated with decreasing concentrations of actinomycete and fungi biomarkers, as well as decreased G –ve stress indicator.

Principal components analysis of the soil chemistry parameters

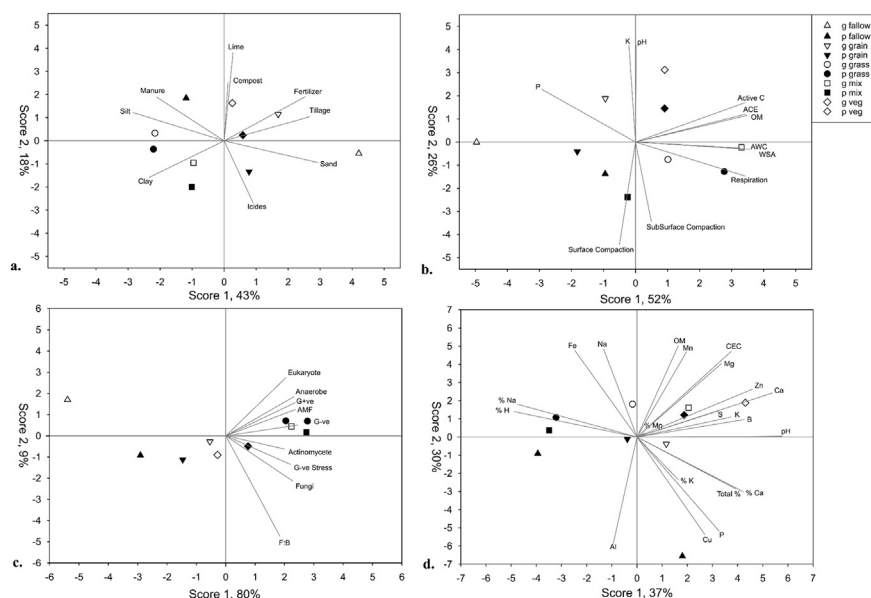


Fig. 3. a–d: PCA biplot based on individual correlation matrices to evaluate a. management and environmental conditions; b. Cornell soil health assessments, c. phospholipid fatty acid (PLFA) analysis; d. traditional soil chemistry analysis under different rotations in good vs. poor farmer selected fields.

(Fig. 3d) explained 37% of the variation on score 1 (loading positively with most factors except OM, % K, % Mg and negatively with % H and % Na); score 2 accounted for 30% of the variability in the dataset (positive loading: OM, Na, Zn, Mg, Fe, Ca, Mn and CEC; negative loading: P, Cu, and Al). PCA analysis of soil chemistry resulted in the best resolution between *g* and *p* fields of any of the chosen analysis methods. The majority of the *p* rotations were associated with increasing values for % H and % Na as well as measured Fe and Na concentrations in the soil *g* mix, *g* veg and *p* veg correlated positively with increasing values of Mg, Zn, Ca, S, K, and B. *G* fallow correlated with increasing soil concentrations of Cu, P and Al.

As a visualization exercise, Pearson correlation coefficients for the PLFA and CSHA were plotted and grouped based on the first principle component (Fig. 4). These graphs show significant positive correlations ($p < 0.05$) between most PLFA groups and soil respiration, water stable aggregates, ACE, active C, percent organic matter and AWC. There are also significant ($p < 0.05$) negative correlations between P and *G* –ve bacteria, AMF, and actinomycetes biomarkers; pH is significantly negatively correlated with WSA, AWC and surface compaction. Management factors showing significant positive correlations between elements associated with intensive agriculture including increased tillage, fertility, pesticides, lime application, P and K; significant negative correlations between these elements and biologically associated indicators including ACE, organic matter, WSA, and soil respiration. Although compost addition showed significant negative correlations with surface compaction, and positive correlations with active C, ACE and percent organic matter, manure application did not show a correlation with any of these factors (Fig. 4).

In order to interpret those factors which explained the majority of the variation observed in the previous PCA analyses under the context of both management factors and farmers perceived *g* vs. *p* fields, the factors with eigenvalues on score 1 greater than or equal to 2.5 were then used to evaluate all factor groups simultaneously (Fig. 5). There was a clear separation between *g* and *p* fields and for the most part cropping systems were grouped together on the biplot. For the first score, positive loadings with longer eigenvectors (≥ 2.0) included soil respiration, *G* –ve bacteria, fungi, WSA, AMF, AWC, silt, *G* +ve bacteria, anaerobic bacteria and *G* –ve stress ratio; negative loadings included sand, synthetic fertilizer and tillage. Positive and negative loadings combined accounted for 51% of the variability in the dataset. On the second axis, positive loadings of similar eigenvector length

included Ca, pH, Mg, B, active C, % organic matter, % Ca (base cation), total percent base cations; negative loadings included % H, % Na and % clay. Positive and negative loadings combined accounted for 25% of the variability (Fig. 5). The resulting biplot shows correlation between both *g* and *p* grass rotations and several PLFA biomarkers (*G* –ve bacteria, fungi, *G* +ve bacteria, anaerobic bacteria) as well as increasing % silt and soil respiration. The *g* mix rotation correlates with increasing values of organic matter, ACE, AMF biomarker and the *G* –ve stress indicator, whereas both the *p* mix and *p* fallow rotations correlate with increasing values for manure application, % silt as well as % Na and % H. The *g* veg rotation correlates with increasing values for K, B, and pH; *p* veg was in relatively close proximity with correlation with Mg. Finally, *g* grain correlates with increased % sand, tillage and P whereas *p* grain correlates with increasing values for synthetic fertilizer use. For the fallow rotations, *g* fallow was closely associated with the negative side of the first axis, and *p* fallow was closely associated with the negative side of the second axis (Fig. 5).

4. Discussion

Soil texture is a determinant for many other soil health indicators such as OM, aggregation, nutrient available, water-holding capacity, and compaction, which is why the CSHA accounts for texture in its scoring functions. Clay content can play a large role in determining CEC and the capacity of the soil to retain nutrients and OM, as well as serving as a component for the formation of aggregates, although the relatively low clay content overall for the soils in this study suggests aggregate dynamics would be much more influenced by management. Due to the inherently sandy nature of the soils in the region, cropping systems tend to consist of a more intensive management approach – undiversified vegetable and grains – with grass-based and mixed cropping system categories tending to be found on soils slightly less sandy in texture. Soil texture was an important factor in the CSHA and PLFA analysis results: sand content had a high negative load on PCA Score 1, and clay had a high negative load on PCA Score 2. Sand was inversely related to soil respiration, AMF, *G* –ve, *G* –ve stress, non-mycorrhizal fungi and WSA, but positively correlated with P, Cu and Al. Along PCA Score 2, clay was inversely correlated with CEC, active C, ACE protein and OM. However, because clay had a lower load on PCA Score 1, it explained less variability through PCA. Congreves et al. (2015) also found that sand was positively correlated with P and

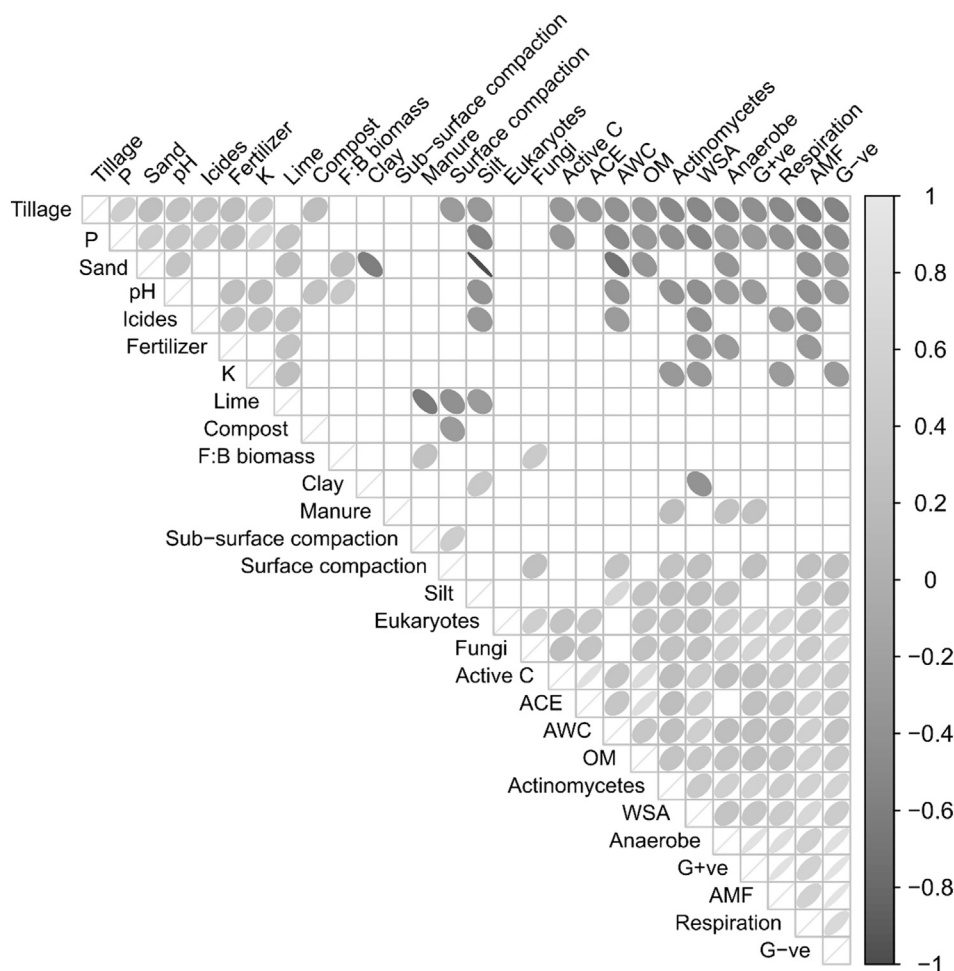


Fig. 4. Visualization of a correlation matrix showing coefficients between CSHA, PLFA, and management and environmental factors. Circles indicate significant ($p < 0.05$) correlations with positive relationships as oriented from the bottom left to the top right, and negative relationships oriented from the top left to the bottom right. The degree of shading indicates the strength of the correlations. Factors are ordered according to the first principle component.

inversely related to biological indicators like OM and Active C, although they deemed sand less important for future OSHA work because it occurred primarily on PC2.

Although all three analytical approaches were able to detect differences in cropping systems and management, traditional soil chemical

analysis was the only approach which was able to resolve differences between farmer perceived ‘good’ and ‘poor’ fields. Soils in the region tend to be lighter, sandier soils with lower organic matter and pH (Nyiraneza et al., 2017). There has been a trend, particularly in Prince Edward Island, of increasing soil P and decreasing Mg levels through

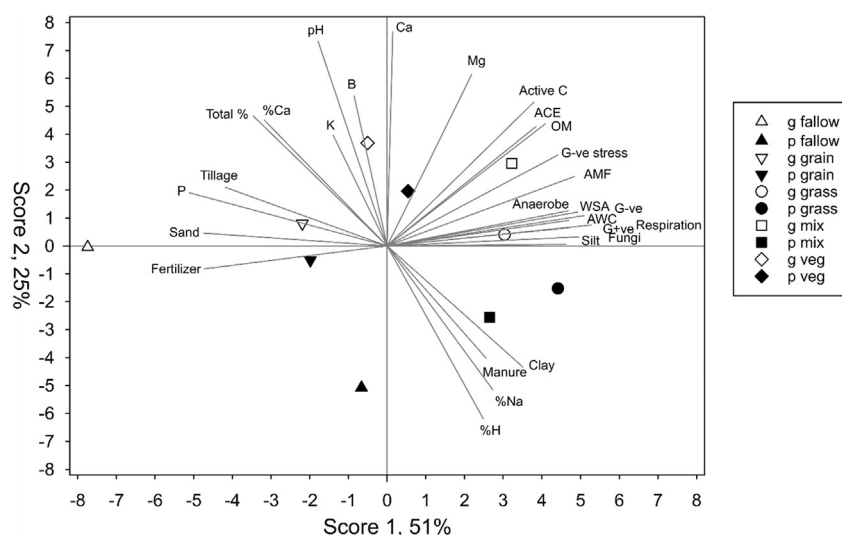


Fig. 5. Final principle component analysis biplot showing eigenvectors selected from previous analyses of a length > 2.5 on the first principle component score.

time (PEIDAF, 2012). In differentiating between good and poor fields, conventional chemical analysis indicated significantly lower levels of boron, calcium, magnesium and overall pH in the farmer-identified poor fields. Boron is present in a pH-dependent equilibrium and is highly mobile and easily leached from the soil under conditions of high precipitation. It is essential for plant cell division and is a necessary component of the cell (Gupta, 2016). Soil B levels are also quite low in the region with typical concentrations below 2 ppm.

Both Ca and Mg are essential plant macronutrients. Ca is an essential component of plant cell walls and is a key component in plant cell signalling (White, 2015); Mg is a key nutrient for the control of photosynthesis and nutrient partitioning among plant parts, and is essential for N transport within the plant (Grzebisz, 2013). The range of field types illustrated that differences in both selected CSHA parameters and PLFA could be linked to management; most notably, perennial grass fields had higher soil respiration, WSA, non-mycorrhizal fungi, AMF, and G –ve bacteria, and lower P. Mixed perennial-annual cropped fields were also higher in these measures than other field types, though not as high as grass. Fields which were high in P were lower in soil respiration, WSA, non-mycorrhizal fungi and AMF. These high P fields generally reflected those that had been more intensively managed, namely grain and veg fields. However, with the exception of pH, there were no clear differences between farmer-identified good and poor fields within any of the rotation types except for g fallow, which contained only one field. Although pH is known to affect PLFA profiles in agricultural soils (Rousk et al., 2010), PCA did not show clear shifts in PLFA profiles between good and poor fields. The PLFA technique has previously been shown to be a powerful tool to discern between different cropping systems (Duncan et al., 2016) when compared to molecular approaches.

Tillage and manure application were inversely related along PCA Score 1, with tillage correlated with P, Cu, Al, sand and manure correlated with biological measures like soil respiration, AMF, G –ve bacteria, G –ve stress indicator, non-mycorrhizal fungi and WSA. In this study, manure was applied most often on dairy farm fields that were in hay or pasture, which explains the inverse relationship between tillage and manure application. Tillage is known to break down macroaggregates and release SOC, thereby affecting biological soil health indicators (Moebius et al., 2007; Acin-Carrera et al., 2013; Sağlam et al., 2015). OM-inputs are known to improve a variety of soil physical and biological indicators, and manure application in particular has been shown to increase SOM, active C, AWC and WSA (Iqbal et al., 2014). Gram negative stress indicator values are automatically included as part of the Sherlock MIDI output and have been included in the analyses. However, the interpretation of these values under the context of environmental studies may not be relevant, as the initial work was based on pure cultures in isolation (Willers et al., 2015).

The CSHA indicators WSA and soil respiration positively correlated with all PLFA microbial groups, most notably AMF, G –ve bacteria, G –ve stress indicator, and non-mycorrhizal fungi; these correlated negatively with P, Cu, and Al. It has been well established that AMF are typically negatively correlated with soil-P (Hijri et al., 2006; Gosling et al., 2013; Bainard et al., 2014; Bainard et al., 2015; Schneider et al., 2015). AMF are also known to stabilize soil structure through physical enmeshing of soil particles by mycorrhizal hyphae and the exudate glomalin, by affecting plant root exudates, and by increasing overall soil carbon (Rillig and Mummey, 2006; Daynes et al., 2013), thereby confirming the positive relationship between WSA and AMF. Soil respiration, an indicator of soil biological activity, was positively associated with increases in all microbial groups. Heavy metals such as Cu and Al have a negative effect on soil microbial biomass and activity, as highlighted here by the inverse relationship between these metals and all biological soil indicators from the CSHA and PLFA (Illmer et al., 1995; Gillera et al., 1998; Vogeler et al., 2008).

Overall, the link between CSHA and PLFA indicators is a valuable finding for the possibility of integrating these two measures. It may be

possible to use PLFA to explore more in-depth aspects of soil biology, both structural and functional, in concert with the biological, physical and chemical components measured in the CSHA. For example, PLFA is efficient for rapid screening of the microbial community and is useful for describing movement of substrates through the soil food web, for determining bacterial:fungal ratios, for measuring microbial biomass (both total, and by group) and for providing some indication of cell activity and cell stress. Linking PLFA with the CSHA may develop a deeper understanding of how microbial structure and function are affected by a wider range of soil health factors.

5. Conclusions

PCA successfully related shifts in the PLFA profile to CSHA indicators: in particular, WSA and soil respiration were positively correlated with all PLFA microbial groups, and negatively correlated with P, Cu, and Al. Management and environmental factors were linked to PLFA and CSHA indicators. Notably, grass and mixed perennial-annual cropped fields were higher in soil respiration, WSA, non-mycorrhizal fungi, AMF and G –ve bacteria, and lower in soil available P. More intensively managed fields like undiversified grain and vegetable rotations showed the reverse trend, with higher P, sand content, and lower levels of biological indicators (soil respiration, WSA, non-mycorrhizal fungi, AMF and G –ve bacteria). Manure application was linked to positive physical and biological indicators like respiration, AMF, non-mycorrhizal fungi and WSA, and tillage and sand content correlated with P, Cu and Al. Overall, the correlations between changes in CSHA indicators and PLFA profiles shows promise for integrating these two tests for stronger soil health assessment. With further research into the possibilities for more in-depth integration with PLFA – for example, the power to explore trophic cascades of C in conjunction with CSHA soil health indicators – these two tests could provide powerful new insights into soil health.

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