Received: 24 April 2020 | Revised: 30 November 2020 | Accepted: 2 December 2020 | Published online: 28 April 2021

DOI: 10.1002/saj2.20211

Soil Science Society of America Journal

SOIL BIOLOGY & BIOCHEMISTRY

Comparing biological methods for soil health assessments: EL-FAME, enzyme activities, and qPCR

Lumarie Pérez-Guzmán[1] | **Lori A. Phillips**[2] | **Miguel A. Acevedo**[3] | **Verónica Acosta-Martínez**[1]

[1] USDA-ARS, Cropping Systems Research Lab., Wind Erosion and Water Conservation Unit, Lubbock, TX 79415, USA

[2] Agriculture and Agri-Food Canada, Harrow, ON, N0R 1G0, Canada

[3] Wildlife Ecology and Conservation Dep., Univ. of Florida, Gainesville, FL 32611, USA

Correspondence
Verónica Acosta-Martínez, USDA-ARS, Cropping Systems Research Lab., Wind Erosion and Water Conservation Unit, Lubbock, TX 79415, USA.
Email: veronica.acosta-martinez@usda.gov

Assigned to Associate Editor Jorge Rodrigues.

Abstract

Soil health initiatives have categorized assays for enzyme activities (EAs) that measure *p*-nitrophenol and ester-linked fatty acid methyl ester (EL-FAME) as Tier 2 indicators for biological activity and community structure analysis, respectively. Quantitative polymerase chain reaction (qPCR) assays of functional and taxonomic communities are emerging Tier 3 indicators. To facilitate comparisons of soil biological health between research groups that may employ different methods, we applied these current and emerging indicators to semiarid soils from the Texas High Plains sampled in the growing season and postharvest from 2014 through 2018. Microbial groups via EL-FAME markers and EAs were strongly correlated ($r > .79$) with qPCR assays of equivalent taxonomic and functional genes. To further quantify the predictive power of these relationships, we modeled several genes as a function of EA or EL-FAME markers, combined with other related covariates (e.g., soil texture, pH, irrigation, and soil organic C [SOC]) using a generalized linear model. The latter was trained using data from 2014, which was an average year in terms of temperature and precipitation for the region. Subsequently, the model was tested making predictions for 2015–2018, which represented high variability in climatic conditions, ensuring a thorough assessment of its predictive power. In most cases, soil texture, SOC, and Tier 2 indicators were identified as moderate to strong predictors of the biological responses. Our results suggest that the different approaches for assessing either function or community in these semiarid soils were highly comparable and provided similar information on how microbial communities were responding to both management and climate.

1 | INTRODUCTION

There is increasing interest in identifying soil health indices that can be used to assess how different management practices impact soil functions, including C sequestration, crop productivity, erosion, nutrient cycling, and water storage. To achieve this outcome, soil physical, chemical, and biological properties need to be evaluated using methods that are meaningful in an agro-ecosystems context, and sensitive to

Abbreviations: AMF, arbuscular mycorrhizal fungi; EA, enzyme activity; EL-FAME, ester-linked fatty acid methyl ester; FAME, fatty acid methyl ester; GWC, gravimetric water content; PB, percent bias; PLFA, phospholipid fatty acid; PNP, *p*-nitrophenol; qPCR, quantitative polymerase chain reaction; RSE, relative squared error; SiC, silt and clay; SOC, soil organic carbon; SOM, soil organic matter; ST, soil temperature; TN, total nitrogen.

variation in agricultural management. Soil health initiatives including the Soil Health Institute, the USDA–NRCS, and the scientific community from several institutions have identified a set of Tier 1 indicators, which are a group of properties (primarily physicochemical parameters such as organic matter and pH) that respond to specific management strategies, have defined thresholds (i.e., rankings of poor to good) and have been benchmarked nationally (Stewart et al., 2018). However, several studies have suggested that biological and biochemical indicators might be more useful when assessing soil health, due both to their higher sensitivity and to their relationship with soil microbial communities and soil processes (Frankenberger & Dick, 1983; Stott, Andrews, Liebig, Wienhold, & Karlen, 2009; Trasar-Cepeda, Leirós, Seoane, & Gil-Sotres, 2000).

Soil health assessments require different methods to measure changes in microbial communities in relation to the complex interactions of management practices and climate variability. Soil microorganisms regulate biogeochemical transformations and improve soil structure by their role in aggregate formation. Thus, their abundance, diversity, and activities are essential to many functions provided by soils (Bardgett & van der Putten, 2014; Lehman et al., 2015). Many of the current soil biological indicators are considered Tier 2 and, although recommended for soil health assessments, require further research before users can have the same level of confidence in their use and interpretation as those obtained from Tier 1. Currently, EAs and ester-linked fatty acid methyl ester (EL-FAME) assays are accepted as Tier 2 indicators of biological activity and community structure, respectively. Several EAs have been used for soil health assessments; however, β-glucosidase, β-glucosaminidase, alkaline or acid phosphatase, and arylsulfatase have been identified as core EAs due to their important roles in C, N, P, and S cycling, respectively (Acosta-Martínez, Pérez-Guzmán, Veum, Núnes, & Dick, 2021; Stott, 2019). An enzyme assay measures the rate of reaction according to its released product (e.g., *p*-nitrophenol [PNP]), and reflects the amount of the enzyme present (Tabatabai & Dick, 2002). Enzyme assays, however, are only valid and reproducible across investigations if the reaction occurs under optimal conditions, which include excess substrate concentrations, optimum temperature and pH, and other possible co-factors. Consequently, since EA assays are performed under controlled conditions and cannot distinguish between activities associated with viable cells (intracellular) or extracellular enzymes, they measure "potential activity." Nonetheless, EAs are sensitive in detecting changes associated with cropping systems (Bandick & Dick, 1999; Cotton, Acosta-Martínez, Moore-Kucera, & Burow, 2013; Schutter & Dick, 2002; Veum, Goyne, Kremer, Miles, & Sudduth, 2014), tillage (Balota, Kanashiro, Colozzi Filho, Andrade, & Dick, 2004; Cotton & Acosta-Martínez, 2018; Dick, 1984), and climate variability (Acosta-Martínez, Moore-Kucera, Cotton, Gardner, & Wester, 2014; Pérez-Guzmán, Acosta-Martínez, Phillips, & Mauget, 2020).

Core Ideas

- FAME markers, EAs, and genes via qPCR provided a similar response to climate variability.
- β-glucosidase activity had a strong correlation with genes of equivalent function.
- FAME markers and soil physicochemical properties were accurate predictors of genes.
- Tier 2 and 3 indicators of soil health provided a comprehensive overview of soil biology.

Fatty acid profiling methods such as phospholipid fatty acid (PLFA) and EL-FAME have been commonly used for characterizing soil microbial community structure by extracting fatty acids from the microbial cell membrane, and converting them into fatty acid methyl esters (FAMEs) using an alkaline reagent (e.g., Acosta-Martínez, Mikha, & Vigil, 2007; Frostegård & Bååth, 1996; Moore-Kucera & Dick, 2008; Schutter & Dick, 2002; Zelles, 1999). Additionally, these methods allow for the identification of broad groups such as Gram-positive, Gram-negative, Actinobacteria, and saprophytic fungi (Willers, Jansen van Rensburg, & Claassens, 2015; Zelles, 1999). Recently, Li, Cano, Acosta-Martínez, Veum, and Moore-Kucera (2020) compared PLFA and EL-FAME using 172 soil samples representing a wide range of physicochemical properties, and provided detailed information regarding equipment, costs, practical advantages, and results. For example, although certain FAME markers may be of plant origin, leading to slight overestimation of some groups, total FAMEs have been used as a proxy of microbial biomass (e.g., Li et al., 2018; Li et al., 2020). Also, since phospholipids are easily degraded upon cell death, fatty acids derived from PLFA analysis are thought to represent the viable microbial community (Zelles, 1999). However, EL-FAME has become popular due to its simplicity and because it is less time consuming than PLFA analysis (Miura, Makoto, Niwa, Kaneko, & Sakamoto, 2017; Schutter and Dick, 2000). Additionally, the extraction in EL-FAME is directly from soil samples, and thus, may contain other lipid fractions including neutral lipids (Zelles, 1999; Miura et al., 2017), which give the advantage of evaluating the arbuscular mycorrhizal fungi (AMF) marker 16:1ω5c (Cotton & Acosta-Martínez, 2018; Li et al., 2020). The EL-FAME method has been shown to be sensitive at detecting changes in microbial communities under different management practices and regions (e.g., Mbuthia et al., 2015; Li et al., 2018), and after natural climatic disturbances such as drought, hurricanes, and extreme temperatures (Bérard, Bouchet, Sévenier, Pablo, & Gros, 2011;

TABLE 1 Quantitative polymerase chain reaction assays for genes associated with taxonomic groups and functional processes

Target genes	Function	EC number	Nutrient pool	Microbial group; main targets	Reference
Bacterial 16S	taxonomic classification	N/A	N/A	bacteria	Watanabe, Kodama, & Harayama, 2001
Fungal 18S	taxonomic classification	N/A	N/A	fungi	Vanio & Hantula, 2000
Laccase multicopper	polyphenolic C breakdown	1.10.3.2	C	bacteria and fungi	Kellner et al., 2007
Bglu (β-glucosidase)	breakdown of oligosaccharides	3.2.1.21	C	bacteria	Cañizares, Benítez, & Ogunseitan, 2011
apr (alkaline metallopeptidase)	SON mineralization	3.4.24	N	bacteria, primarily Gammaproteobacteria	Bach et al., 2001
npr (neutral metallopeptidase)	SON mineralization	3.4.25	N	bacteria, primarily Bacilli	Bach et al., 2001
narG (nitrate reductase)	ammonification/denitrification	1.7.5.1	N	bacteria, Proteobacteria	Gregory, Karakas-Sen, Richardson, & Spiro, 2000
nrfA (nitrite reductase)	dissimilatory nitrate reduction	1.7.2.2	N	bacteria	Welsh, Chee-Sanford, Connor, Löffler, & Sanford, 2014
nxrA (nitrite oxidoreductase)	nitrite oxidation	1.7.99.4	N	bacteria, Nitrobacter	Poly, Wertz, Brothier, & Degrange, 2008
phoD (alkaline phosphatase)	P mineralization	3.1.3.1	P	primarily bacteria, Alphaproteobacteria	Ragot et al., 2015

Note. EC, enzyme commission; N/A, not applicable; SON, soil organic N.

Cantrell et al., 2014; Pérez-Guzmán et al., 2020). Moreover, combining fatty acid profiling with EAs allows for more comprehensive soil biological health assessments, as changes in microbial community detected via FAME markers have been positively correlated with changes in several EA indicators of biogeochemical cycling and soil organic matter (SOM) dynamics (Acosta-Martínez et al., 2007; Cotton et al., 2013).

Quantitative polymerase chain reaction (qPCR) assays targeting either functional or taxonomic components of soil microbial communities are promising Tier 3 indicators that may allow for rapid quantitative assessment of both biogeochemical cycling processes and phylogenetic groups (Fierer, Jackson, Vilgalys, & Jackson, 2005; Stone et al., 2015; Thiele-Bruhn et al., 2020). Current qPCR indicators typically use taxa-specific primers to target phylogenetically discrete processes performed by organisms with very defined niche preferences (Wessén & Hallin, 2011). For example, genes associated with nitrification, including archaeal and bacterial ammonium monooxygenase genes, have been used as indicators of soil health shifts in response to xenobiotic contamination (Brandt et al., 2015; Mundepi, Cabrera, & Norton, 2019), soil reclamation (Dose et al., 2015), and agricultural management (Bhowmik, Fortuna, Cihacek, Bary, & Cogger, 2016; Ouyang, Reeve, & Norton, 2018). Over the past decade, the increasing use of more degenerate primers in qPCR has allowed researchers to analyze phylogenetically diverse nutrient cycling processes and broader microbial groups (e.g., Table 1 and references therein). Many of these qPCR assays evaluate functions and groups that are being measured in current Tier 2 soil health assays (i.e., via EL-FAME and EA), but there is limited information on whether the different approaches provide similar information on soil health (Ouyang et al. (2018), Thiele-Bruhn et al., 2020). One of the few such studies performed to date evaluated chitinolytic, proteolytic, and ureolytic EA, and related gene abundance in an organic farming system. The researchers found that direct correlative linkages generally decreased as functional redundancy associated with the biogeochemical cycling process increased, and that gene abundance itself did not improve predictive models of EA.

Previously, our team evaluated the effect of climate variability on microbial community structure via the EL-FAME

TABLE 2 Soil properties of five agricultural sites (0–10 cm) for all sampling times 2014–2018

Property	Sites				
	1	2	3	4	5
	Physicochemical properties				
Soil series	Amarillo	Amarillo	Olton	Portales	Spur
pH	7.6	7.0	8.3	8.4	8.2
Sand (%)	69	58	52	38	32
Silt (%)	14	21	21	30	32
Clay (%)	17	21	27	32	52
SOC (g kg^{-1} soil)	3.5	4.4	5.4	11	19.4
TN (g kg^{-1} soil)	0.4	0.5	0.6	1.1	2.2
	Microbial groups via FAME				
	nmol g^{-1} soil				
Actinobacteria	4.8b	5.5b	5.5b	13.9a	19.6a
Gram negative	8.9b	7.9b	8.1b	21.8a	27.8a
Gram positive	10.2b	12.1b	10.9b	26.1a	34.4a
AMF	2.1c	3.2b	2.2c	5.0a	5.0a
Saprophytic fungi	14.6b	13.8b	13.4b	31.1a	31.0a
	Genes				
	copies g^{-1} soil				
apr	1.4×10^5b	1.4×10^5b	1.6×10^5b	6.7×10^5b	1.1×10^6a
npr	2.2×10^5a	1.8×10^5a	1.4×10^5ab	1.2×10^5b	1.0×10^5b
laccase	6.9×10^6b	6.2×10^6b	8.1×10^6b	2.2×10^7b	3.5×10^7a

Note. AMF, arbuscular mycorrhizal fungi; SOC, soil organic C; TN, total soil N. Amarillo (fine-loamy, mixed, superactive, thermic Aridic Paleustalf); Olton (fine, mixed superactive thermic Aridic Paleustolls); Portales (fine-loamy, mixed superactive thermic Aridic Calciustolls); Spur (fine loamy, mixed, superactive thermic Fluventic Haplustoll). Values represent the mean of $n = 30$. Numbers within a row that do not share a letter are significantly different at $p < .001$.

method, and overall activity via the geometric mean of four EAs in five semiarid soils under cotton (*Gossypium hirsutum* L.) production. Although sites varied greatly in soil textural class (e.g., from sandy loam to clay), and differed in management (irrigation vs. dryland), the evaluated parameters responded similarly to extreme temperatures in 2016 after a year of record precipitation (Pérez-Guzmán et al., 2020). We found significant decreases in microbial community structure and activities under low soil moisture associated with low precipitation and high soil temperatures. Our current study builds upon this previous work by comparing Tier 2 indicators and emerging biological indicators such as qPCR genes for assessing soil health from these sites. Along with this comparative analysis, the objective was to test the predictive accuracy of microbial groups via EL-FAME and EAs (β-glucosidase, β-glucosaminidase, and alkaline phosphatase) with genes that provide similar taxonomic and functional information (e.g., C, N, and P cycling) using qPCR. To achieve this, we developed and tested a predictive model of soil physicochemical properties, abiotic factors, and biological responses. The primary goal was to facilitate comparisons among research laboratories that may employ different biological methods while contributing to the expansion of databases focused on soil health.

2 | MATERIALS AND METHODS

2.1 | Study sites, soil sampling, and analyses

Soil samples were taken from 2014 to 2018 in five producer fields located in the Southern High Plains in Texas. This warm, semiarid region is characterized by mild winters and low precipitation (<500 mm annually). However, the five years evaluated in this study were among the 10 warmest years on the planet since 1880 (NOAA, 2020), and 2015 brought record precipitation (693 mm) to the region. The fields have been under tilled cotton and represent a wide range of soil textural classes characteristic of the region (Table 2). Sites 1 and 2 are Amarillo (fine-loamy, mixed, superactive, thermic Aridic Paleustalf). Sites 3, 4, and 5 are Olton (fine, mixed, superactive, thermic Aridic Paleustolls), Portales (fine-loamy, mixed, superactive, thermic Aridic Calciustolls), and Spur (fine-loamy, mixed, superactive, thermic Fluventic Haplustolls), respectively. The sites were sampled on the same days each year, once during the growing season and once postharvest (Supplemental Table S1). Samples were collected from three randomly selected locations along a 100-m transect. At each sampling location, three soil samples (0–10 cm) were

collected and composited. Soils were stored in a cooler on ice for transport to the laboratory, where they were sieved (<5 mm), and subsampled for chemical (air dried) and biological (4 ˚C and −20 ˚C) analyses. Soil organic C and total N were analyzed on air-dried soils by automated dry combustion using a Leco TruSpec CN in a commercial lab (Ward Laboratories). Soil pH was measured in air-dried soils using 1:2.5 soil/water ratio using a combination glass electrode (Denver Instrument), and soil texture was determined using the pipette method (Miller & Miller, 1987).

2.2 | EL-FAME

Soil microbial community composition was characterized by the EL-FAME method described by Schutter and Dick (2000). Three samples per site were analyzed using 3 g of field-moist soil. First, samples were incubated at 37 ˚C with 0.2 M methanolic KOH for 1 h. Then, 1.0 M acetic acid was added to neutralize the solution, and FAMEs were partitioned into an organic phase by adding hexane followed by centrifugation. Subsequently, the hexane layer was evaporated under N_2, and FAMEs were dissolved in 1:1 hexane/methyl-*tert* butyl ether. Lastly, samples were analyzed in a 6890N GC (Agilent Technologies) equipped with a flame ionization detector and a fused silica capillary column (25 m × 0.32 mm × 0.25 µm) using ultra high purity H_2 as the carrier gas. The temperature program was ramped from 170 to 250 ˚C at 5 ˚C min^{-1}. The FAMEs were identified, and their relative peak areas determined with the Microbial ID, Inc., PLFA naming software (Microbial ID).

The FAMEs nomenclature includes the number of C atoms, followed by a colon, the number of double bonds, and the position of the first double bond from the methyl (ω) end of the molecule. *Cis* isomers are indicated by c, while methyl groups are indicated by Me. Branched fatty acids are indicated by the prefixes *i* (iso) and *a* (anteiso). The sum of all FAME biomarkers present in a sample was used as proxy of microbial abundance. Additionally, FAMEs were also summed into broad microbial groups such as Actinobacteria (10Me16:0, 10Me17:0, 10Me18:0), Gram-positive (Gram+; *i*15:0, *a*15:0, *i*16:0, *i*17:0, *a*17:0), Gram-negative (Gram−; cy 17:0, cy 19:0ω8c, 18:1 ω7c), saprophytic fungi (18:1 ω9c, 18:2 ω6c) and arbuscular mycorrhizal fungi (AMF; 16:1 ω5c) as described in the literature (Frostegård & Bååth, 1996; Moore-Kucera & Dick, 2008; Olsson, Bååth, Jakobsen, & Söderström, 1995; Zelles, 1999).

2.3 | Potential enzyme activity

The EAs of β-glucosidase, β-glucosaminidase, and alkaline phosphatase involved in C, C and N, and P cycling respectively, were analyzed using *p*-nitrophenyl derivative substrate concentrations and assay conditions as previously reported (Dick, 2011; Eivazi & Tabatabai, 1977; Parham & Deng, 2000; Tabatabai, 1994). The protocols were modified to use 0.5 g (sieved <5 mm) air-dried soil instead of 1 g, and solutions were reduced by half maintaining the soil/solution proportions of the original assays (Acosta-Martínez & Cotton, 2017). Toluene was omitted to reduce environmental concerns, and because its elimination does not affect EAs during 1 h incubation (Acosta-Martínez & Tabatabai, 2011; Tabatabai, 1994). All EAs were analyzed in duplicates with one control, to which substrate was added after the incubation step. The product PNP was determined colorimetrically at 400 nm in a visible spectrophotometer (Thermo Scientific Evolution 60S). Activity values from the control samples were subtracted from the experimental sample value.

2.4 | Microbial community and function via qPCR

DNA was extracted in duplicate from field moist soils using Qiagen DNeasy PowerSoil HTP Kit (Qiagen Inc.) according to the manufacturer's instructions with the following modification: samples were heated at 65 ˚C for 10 min prior to bead-beating. DNA quality was visualized on a 1.4% agarose gel, quality and quantity were verified by spectrophotometer (NanoDrop One), and then duplicate extractions were pooled. Pooled DNA was then quantified using both broad range and high sensitivity fluorescence-based assays (Quant-iT dsDNA BR kits, Life Technologies), normalized to a working concentration of 3 ng $µl^{-1}$, and then requantified using high sensitivity assays (Quant-iT dsDNA HS kits, Life Technologies) to verify the final concentration in each qPCR assay. Microbial community function was assessed by quantitative PCR (qPCR) of key genes involved in C, N, and P transformation, overall bacterial and fungal abundance was proxied by qPCR of 16S rRNA and 18S gene regions (Table 1). Assays were run in triplicate on a Bio-Rad CFX384 Real-Time PCR Detection System (Bio-Rad) in 5 µl reaction volumes with 3.0 ng of DNA (functional genes) or 0.3 ng (taxonomic genes) of DNA and 0.12 µg of UltraPure™ BSA (Life Technologies). Absolute quantification was determined using an appropriate plasmid standard with a concentration range of 2–2 × 10^7 gene copies $µl^{-1}$ DNA. Efficiency ranged from 85 to 98%. Full amplification conditions and standard information are available in Supplemental Table S2.

2.5 | Statistical analysis

Statistical analyses were performed using RStudio (RSTudio Team, 2018). For exploratory data analysis, linear

model interactions and normality were assessed visually using *ggplot2* (Wickham, 2016) and sjPlot (Lüdecke, 2019). Exploratory analysis of the response variables showed that the data were not normally distributed; the relations between soil physicochemical properties and biological parameters (e.g., FAMEs, functional genes, and EAs), as well as among the biological methods were determined by Spearman's correlation (ρ). The Kruskal–Wallis test was used to compare the means of the different biological variables based on site, followed by pairwise comparisons with Dunn's post hoc test using Benjamini–Hochberg error correction. For each sample, the geometric mean of the three EAs measured was calculated by multiplying the values of the three EAs, then taking the third root value as described by García-Ruíz, Ochoa, Hinojosa, and Carreira (2008).

2.6 | Predictive model training and testing

Functional and taxonomic (i.e., bacterial and fungal) genes were predicted as a function of soil enzymes or FAME markers, and controlling covariates (e.g., season, soil texture, pH, irrigation, organic C, and soil moisture via gravimetric water content [GWC]). Models were trained with data from 2014, which was an average year in terms of rainfall and temperature for the region. Subsequently, the model was tested making out-of-sample predictions for the years 2015–2018. These four years were optimal to test the predictive power of the model because they represented a variety of climatic conditions including record precipitation (2015), extreme high temperatures (2016), and low rainfall (2018) years; testing the model against the most challenging predictive conditions ensured a thorough assessment of its predictive power. Each biological indicator of soil health was modeled using a generalized linear model with a gamma distributed response variable and a log-link function. This modeling approach was the most appropriate because the response variables were always positive, and the log-link function was applied due to the wide range of values in the response variables. To select the best combination of covariates for each biological indicator, models were compared with all potential combinations of covariates using 10-fold cross-validations for the year 2014. Cross-validation was preferred to Akaike information criterion model selection because the goal was to increase predictive power, and the maximum number of parameters possible was eight.

Parameters estimated from the generalized linear model-fitted data from 2014 were used to make out-of-sample predictions of functional and taxonomic genes in 2015–2018. Predictive accuracy was assessed by quantifying percent bias (PB), relative squared error (RSE) and R^2. Percent bias describes the average tendency of the simulated values to be greater or smaller than the prediction, as a percentage. Therefore, a PB of zero means that the predictions and the validation data are the same. A positive PB means the model under-predicts the data, while a negative percent bias is the result of the model over-predicting the data. The RSE compares the predictions of the model to a model that predicts the mean for every data point. Thus, model accuracy increases as the PB and RSE are minimized. The R^2 from partial correlations ($R^2_{\text{covariates}}$) and the model (R^2_{model}) are given for each response variable. All analyses were conducted in R v4.0.0 using the packages boot (Canty & Ripley, 2020), rsq, caret, and metrics.

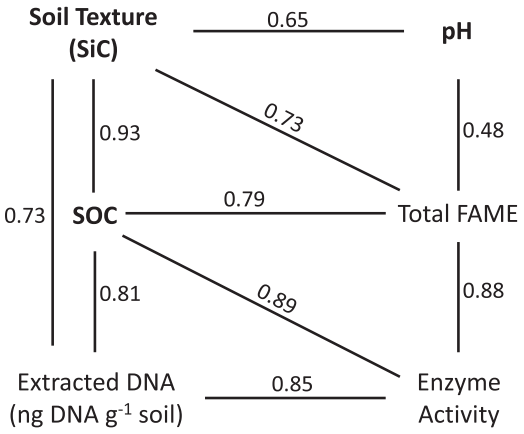

FIGURE 1 Correlation between soil physicochemical properties (bold) and biological indicators. Enzyme activity calculated as the geometric mean of β-glucosidase, β-glucosaminidase and alkaline phosphatase activities, and extracted DNA is given in ng DNA g^{-1} soil. All correlations were significant at $p < .001$. SOC, soil organic C; FAME, fatty acid methyl ester

3 | RESULTS

3.1 | Soil physicochemical properties

The five sites studied varied greatly in their physicochemical properties (Table 2). Soil pH ranged from neutral to moderately alkaline with the lowest and highest pH measured in Sites 2 (pH 7.0) and 4 (pH 8.4), respectively. Soil organic carbon (SOC) and total nitrogen (TN) ranged from 3.5 to 19.4 g kg^{-1}, and 0.4 to 2.2 g kg^{-1} soil, respectively. Both SOC and TN were higher in sites with higher clay content and decreased with increasing sand content (Site 5 > 4 > 3 > 2 > 1). Soil texture was a strong predictor of and was positively correlated ($p < .001$) to physicochemical properties and biological indicators (Figure 1; Supplemental Figures S1 and S2). For example, the percentage of silt and clay (SiC) correlated with pH ($r = .65$), SOC ($r = .93$), and microbial abundance via total FAME ($r = .73$). Similarly, SOC was highly correlated with total FAME ($r = .79$) and EA ($r = .89$). When

TABLE 3 Correlations between soil physicochemical properties and biological indicators

Property	Texture	SOC	TN	pH	NAG	*narG*	*nrfA*
SOC	.93**	1					
TN	.90**	.98**	1				
pH	.65**	.60**	.58**	1			
NAG	.52**	.65**	.65**	.23*	1		
narG	.64**	.73**	.73**	.41**	.63**	1	
nrfA	.67**	.76**	.75**	.47**	.62**	.98**	1
nxrA	.58**	.64**	.62**	.26*	.59**	.88**	.86**

Note. SOC, soil organic C; NAG, β-glucosaminidase; TN, total soil N.
*Significant at the .05 probability level.
**Significant at the .001 probability level.

analyzing TN, there were positive correlations ($r > .58$) with β-glucosaminidase and three genes involved in inorganic N cycling (Table 3).

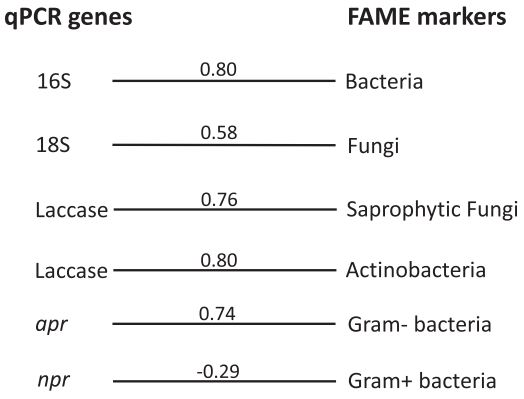

FIGURE 2 Correlations between quantitative polymerase chain reaction (qPCR) taxonomical and functional genes and microbial groups via ester-linked fatty acid methyl ester (EL-FAME). *apr*, alkaline metallopeptidases, *npr*, neutral metallopeptidases. All correlations were significant at $p < .001$

3.2 | Relationship between microbial markers quantified by EL-FAME and qPCR

The abundance of all microbial groups evaluated via FAME markers was significantly ($p < .001$) higher in sites with high clay content (Sites 4 and 5) when compared to the three sandy soils (Sites 1, 2, and 3; Table 2). In the sandy soils, AMF markers were significantly lower in Sites 1 and 3 when compared to Site 2. The number of copies of laccase-like multicopper oxidase (hereafter, laccase) and alkaline metalloprotease (*apr*) genes were higher in Sites 4 and 5 and decreased with decreasing clay content. The opposite trend was observed for the neutral metalloprotease gene (*npr*), for which the number of copies was significantly higher in the soil with highest sand content (Site 1), and the lowest at Site 5 (Table 2).

There were strong significant correlations ($p < .001$) between FAME markers and corresponding taxonomic gene copy numbers (Figure 2). The sum of the different markers for bacteria (Actinobacteria, Gram−, and Gram+) was strongly correlated with the bacterial 16S rRNA gene (16S; $r = .80$). Fungal markers, including both saprophytic and AMF, showed moderate correlation with the fungal 18S gene ($r = .58$). Among the functional genes evaluated, laccase strongly correlated with fungi and Actinobacteria markers with $r = .76$ and $r = .80$, respectively. In contrast, when analyzing Gram+ markers and the *npr* gene, which is predominantly found in Gram+ taxa, there was a weak negative ($r = -.29$) correlation.

3.3 | Prediction of genes as a function of FAME markers and abiotic factors

Models were used to predict genes as function of FAME markers and other covariates (Figure 3). The best model that predicted the 16S gene as a function of bacterial FAME markers included SiC, season, and irrigation as additional covariates (Supplemental Figure S1; Table 4). For example, the model predicted that 16S would increase 1.36 and 1.29 units per unit increase in bacteria FAME markers and SiC, respectively. Relative to drip irrigation, 16S would be 1.11 times higher under pivot irrigation and 0.95 times lower in dryland. The 16S model had moderate predictive accuracy and slightly over-predicted the average in years 2015–2017 (Figure 3a). For 18S as a function of fungal FAME markers, the model predicted increases of 1.01, 1.18, and 1.44 times per unit increase in fungal FAME markers, pH, and SOC, respectively, but decreases of 0.78 units relative to GWC.

The proteolytic genes *apr* and *npr* were predicted as functions of Gram− and Gram+ markers respectively (Figure 3c,d). The best model that predicted *apr* as a function of Gram− markers, included SiC, SOC, season, and irrigation as additional covariates. The model predicted that *apr* would increase 1.54 and 1.73 times per unit increase in Gram− and SiC, respectively, but decrease 0.91 times per unit increase in SOC (Table 4). The *apr* model had moderate predictive accuracy and slightly over-predicted the average in years 2015–2017 (Figure 3c). For *npr* gene as a function of Gram+, the model predicts that *npr* would increase 1.15 times per unit increase in Gram+ markers but decrease 0.86 and 0.55 per unit increase in SiC and GWC, respectively. However, the *npr* model had low predictive accuracy and under-predicted the average in all years (Figure 3d).

The laccase gene is associated with decomposers, and thus was predicted as a function of Actinobacteria and saprophytic fungi markers (Figure 3e,f). When predicting laccase as function of Actinobacteria, the best model included SiC, GWC, pH, and irrigation as additional covariates (Table 4). The

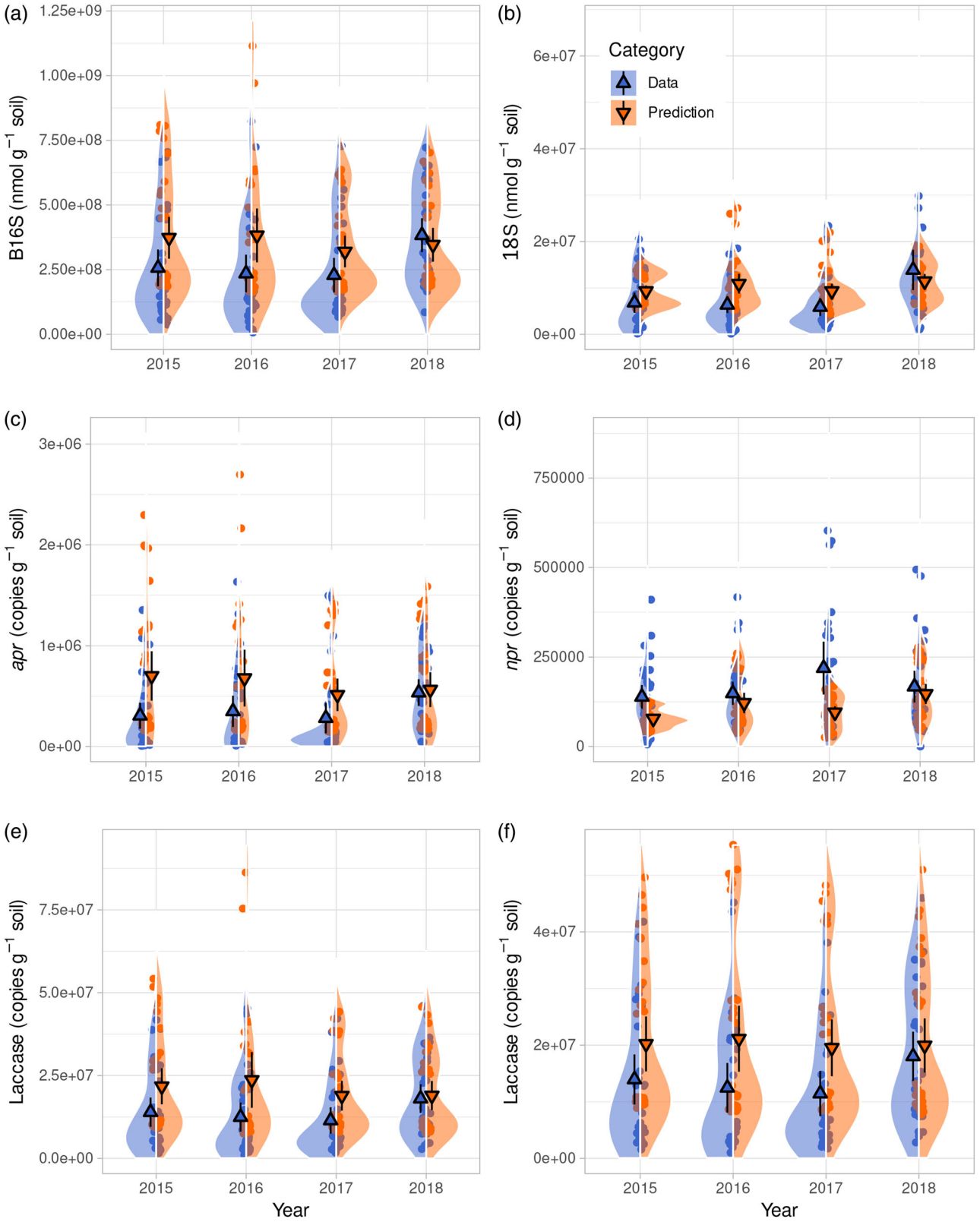

FIGURE 3 Models of predicted genes as a function of fatty acid methyl ester markers. Panels show the distributions of the out-of-sample predictions of the generalized linear model with gamma distributed dependent variable (orange) with the original data (blue). The triangles and black lines represent the mean estimate for the year and the standard errors, respectively. The shaded areas represent the distribution, while the jittered points represent the raw predictions or data. Panels a, b, c, d, e, and f describe the predictions of the best models for 16S using bacterial fatty acid methyl ester markers markers, 18S as a function of fungi markers, *apr* as a function of Gram-negative bacteria, *npr* as a function of Gram-positive bacteria, and laccase as a function of Actinobacteria and saprophytic fungi, respectively

TABLE 4 Best models to predict genes using EL-FAME markers, enzyme activities, and abiotic covariates

Response	Biological and environmental covariates	Season		Irrigation (relative to drip)		Covariates R^2	Predictive accuracy		
		growing	postharvest	pivot	dryland		PB (%)	RSE	R^2
Best model relative to EL-FAME markers and covariates									
16S	1.36 ± 0.16 FAME, 1.29 ± 0.15 SIC			1.11 ± 0.18	0.95 ± 0.16	0.93	−0.89	0.63	0.62
18S	1.01 ± 0.01 FAME, 1.44 ± 0.15 SOC, 1.18 ±0.07 pH, 0.78 ± 0.07 SiC					0.79	−2.20	0.91	0.17
apr	1.54 ± 0.41 FAME, 0.91 ± 0.30 SOC, 1.73 ± 0.28 SiC		0.82 ± 0.08	1.15 ± 0.04	0.84 ± 0.02	0.85	−3.67	1.43	0.55
npr	1.15 ± 0.24 FAME, 0.55 ± 0.14 GWC, 0.86 ± 0.20 SiC	0.72 ± 0.17		0.33 ± 0.11	0.56 ± 0.18	0.49	_	1.32	0.01
Laccase1	1.35 ± 0.11 FAME, 1.04 ± 0.04 pH, 1.10 ± 0.07 GWC, 1.32 ± 0.15 SiC			1.17 ± 0.17	0.93 ± 0.12	0.97	−1.16	1.06	0.60
Laccase2	1.06 ± 0.08 FAME, 1.34 ± 0.15 SOC, 1.04 ± 0.04 pH, 1.38 ± 0.17 SiC			1.01 ± 0.17	1.28 ± 0.24	0.96	−1.09	0.67	0.71
Best model relative to enzyme activity and covariates									
Bglu	1.02 ± 0.21 EA, 1.31 ± 0.31 SOC, 1.47 ± 0.29 SiC		0.74 ± 0.08	1.39 ± 0.44	0.88 ± 0.25	0.86	−1.18	0.48	0.70
phoD	1.89 ± 0.18 EA, 1.12 ± 0.54 pH		0.80 ± 0.09	0.74 ± 0.14	0.84 ± 0.15	0.88	−1.40	1.07	0.47
narG	1.04 ± 0.08 EA, 1.67 ± 0.37 SOC, 0.98 ± 0.07 pH, 1.39 ± 0.27 SiC			1.85 ± 0.46	1.15 ± 0.30	0.96	−1.75	0.51	0.59
nrfA	1.13 ± 0.11 EA, 1.03 ± 0.34 SOC, 1.05 ± 0.20 GWC, 2.42 ± 0.57 SiC		0.76 ± 0.15	0.18 ± 0.46	0.81 ± 0.25	0.86	−11.9	0.54	0.57
nxrA	1.12 ± 0.11 EA			1.63 ± 0.41	1.55 ± 0.57	0.82	−2.29	0.94	0.16

Note. EA, enzyme activity; FAME, fatty acid methyl ester; GWC, gravimetric water content; Laccase1, predicted as function of Actinobacteria FAME markers; Laccase2, predicted as function of saprophytic fungi markers; PB, percent bias; RSE, relative squared error; SiC, silt and clay; SOC, soil organic C.

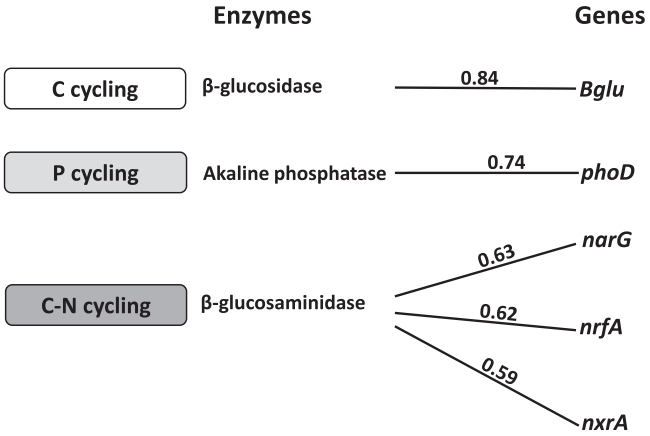

FIGURE 4 Correlations between enzyme activities using the bench-scale, *p*-nitrophenol method, and qPCR genes associated to carbon, phosphorous, and inorganic nitrogen cycling

model had moderate predictive accuracy and slightly over-predicted the average in years 2015–2017 (Figure 3e). For laccase as a function of saprophytic fungi, the best model included SiC, SOC, pH, and irrigation as other covariates, and predicted increases of 1.06 times per unit increase in saprophytic fungi markers, and 1.38, 1.04, and 1.34 times per unit increase in SiC, pH, and SOC, respectively. The model had high predictive accuracy, and slightly over-predicted the average in years 2015–2017 (Figure 3f).

3.4 | Comparisons between enzyme activities and genes of similar function

The potential EAs of β-glucosidase, β-glucosaminidase, and alkaline phosphatase were positively correlated with genes associated with C, N, and P cycling respectively. Of the three enzymes, β-glucosidase had the strongest correlation ($r = .84$) with the gene of similar function (*Bglu*), followed by alkaline phosphatase with *phoD* ($r = .74$) (Figure 4). Similarly, the genes *narG, nrfA, and nxrA* (associated with downstream N cycling) correlated ($r > .59$) with β-glucosaminidase. There were fluctuations associated with soil texture, sampling season (growing vs. postharvest), and year. Similarly to FAME data, EAs and the evaluated genes were significantly higher in the sites with higher clay content. For example, the potential enzyme activity of β-glucosidase and the number of copies of the *Bglu* gene showed similar temporal fluctuations (Supplemental Figure S3). Most sites showed decreases from 2015 (a year of record precipitation) to 2016, followed by increasing trends in 2017. However, at the site with the highest clay content (Site 5), there were decreases in β-glucosidase and the gene copies during the growing season of 2017, followed by increased measurements in 2018.

Although the potential enzyme activity of alkaline phosphatase and the *phoD* gene were positively correlated, there were contrasting trends based on soil texture (Supplemental Figure S4). The concentrations of alkaline phosphatase via EA were similar regardless of sampling season, but gene copies had lower concentrations during postharvest samplings (Supplemental Figure S4d). Additionally, there was higher variability in Site 5 and the trends were not as clear as those observed in soils with higher sand content. This variability was also observed for β-glucosaminidase and three genes involved in N cycling (Supplemental Figure S5). For example, there were fluctuations based on soil texture and year of sampling, but *narG* responded similar to β-glucosaminidase. Although *nxrA* and *nrfA* had the lowest and highest concentrations of N-related genes respectively, both showed similar patterns when compared to β-glucosaminidase.

3.5 | Prediction of genes as a function of enzyme activities and abiotic factors

Genes involved in C, N, and P cycling were predicted as a function of EAs and other covariates (Figure 5). The best model to predict the *Bglu* gene as a function of β-glucosidase included irrigation, season, SOC, and SiC as additional covariates (Supplemental Figure S2; Table 4). Partial correlations showed that the controlling covariates explained more of the variance than the enzyme. For example, the model predicted increases in *Bglu* of 1.02 times per unit increase in β-glucosidase, and of 1.47 and 1.31 per unit increase in SiC and SOC respectively. During postharvest, *Bglu* would be 0.74 times lower than during the growing season. Relative to drip irrigation, *Bglu* would be 1.39 times higher under pivot irrigation and 0.88 times lower in dryland. When predicting the patterns for years 2015–2018, the model slightly over-predicted the average for years 2015–2017 (Figure 5a). However, the model had high predictive accuracy and the SE of the predictions overlap those of the data for all time periods.

Three genes involved in N cycling were predicted as function of β-glucosaminidase and abiotic factors (Figure 5b–e). For *narG* as a function of β-glucosaminidase, the model predicted increases of 1.04 times per unit increase in β-glucosaminidase. It also predicted increases of 1.39, 1.67, and 0.98 times per unit increase in SiC, SOC, and pH, respectively. The model slightly over-predicted the average for years 2015–2017, and slightly under-predicted in 2018 (Figure 5b). Nevertheless, the SE of the predictions overlaps those of the data for all time periods resulting in moderate predictive accuracy. For *nrf*A as a function of β-glucosaminidase the model predicted increases of 1.13 and 2.42 times per unit increase of the enzyme and SiC, respectively. During postharvest, *nrfA* would be 0.76 times lower than during the growing season. The model had moderate predictive accuracy and slightly

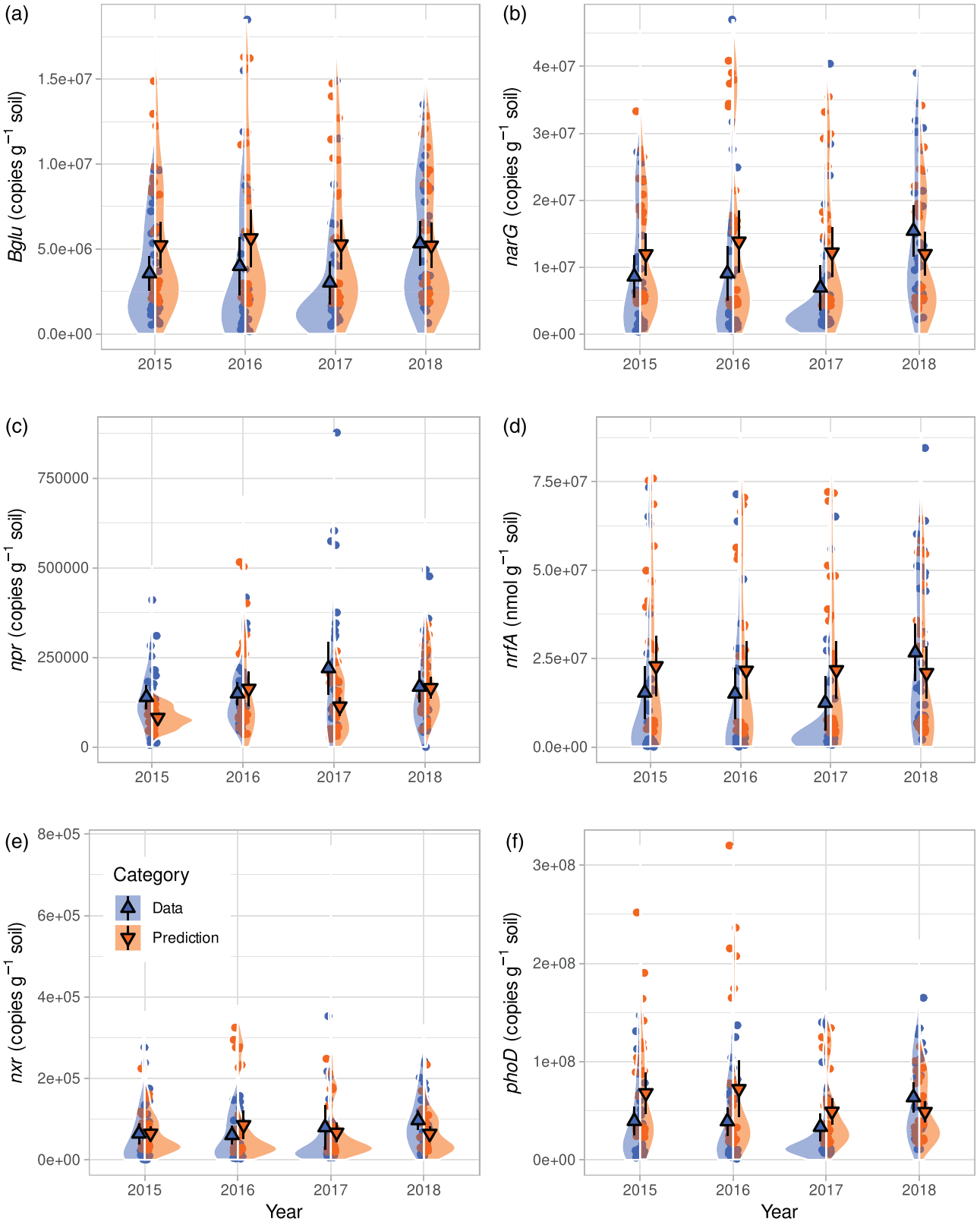

FIGURE 5 Models of predicted genes as a function of enzyme activities. Panels show the distributions of the out-of-sample predictions of the generalized linear model with gamma distributed dependent variable (orange) with the original data (blue). The triangles and black lines represent the mean estimate for the year and the SE, respectively. The shaded areas represent the distribution, while the jittered points represent the raw predictions or data. Panel a describes the predictions of the best model for *Bglu* gene as a function of β-glucosidase. Panels b, c, d, and e show the best models for predicting *narG, npr, nrfA*, and *nxrA* and respectively, as a function of β-glucosaminidase. Panel e shows *phoD* as a function of alkaline phosphatase

over-predicted the average in years 2015–2017 and slightly under-predicted in 2018 (Figure 5d). For the *nxrA* gene as a function of β-glucosaminidase, the best model included SOC and irrigation as additional covariates. The model predicted increases of 1.12 times in *nxrA* with a unit increase in the enzyme. Relative to drip irrigation, *nxrA* would be 1.63 times higher under pivot irrigation and 1.55 times higher in dryland. However, the model had low predictive accuracy and slightly over-predicted the average in years 2015–2017 and slightly under-predicted in 2018 (Figure 5e).

When predicting the *phoD* gene as a function of alkaline phosphatase, the best model included pH, GWC, season, and irrigation as other covariates. *phoD* was predicted to increase 1.89 times with a unit increase in alkaline phosphatase and 1.12 times with a unit increase in pH. During postharvest, *phoD* would be 0.80 times lower than in the growing season. Relative to drip irrigation, *phoD* would be 0.74 times lower under pivot irrigation and 0.84 times lower in dryland. The model had moderate predictive accuracy and slightly over-predicted the average in years 2015–2017 and slightly under-predicted in 2018 (Figure 5f).

4 | DISCUSSION

There is a significant gap in knowledge on whether results from different biological indicator methods provide comparable information on soil health. Different research groups employ different methods according to equipment availability, specific expertise, and budgetary constraints. Conclusions derived from such methods vary depending on what has been evaluated (e.g., diversity, abundance, function) and the target molecule (e.g., lipids, DNA, enzymes) used for quantification. These varied approaches can make it challenging to compare results, establish trends and reach consensus on, for example, how climate and management alter soil health. To address this knowledge gap, we measured current and emerging biological indicators of soil biological health in five agricultural semi-arid soils with different textural classes, which were collected during years of climatic variability. Our primary goal was to obtain a better understanding of how different groups of biological indicators compared, both directly and with respect to temporal (growing season vs. postharvest) and management (irrigation vs. dryland) driven trends of soil health.

We developed a model to predict functional and taxonomic genes as a function of Tier 2 indicators and abiotic factors, which was tested by making out-of-sample predictions for the years 2015–2018. These years were ideal to test the model's predictive power because they represented extreme drying and wetting cycles. For example, the year 2015 brought record precipitation for the region (693 mm vs. 465 mm), while 2016 was characterized by extreme high temperatures. A regression model using local soil temperature (ST) data for these sites showed that ST (0–10 cm) in 2016 was almost 7 ˚C warmer than in 2015, and over 2 ˚C warmer than the mean ST from 2005–2018 (Pérez-Guzmán et al., 2020). The year 2017 had average precipitation during the growing season, whereas 2018 had higher temperatures and low precipitation during the growing season. Since the model was tested against these challenging predictive conditions ensuring a thorough assessment of its predictive power, we considered models with $R^2 > .40$ and $R^2 > .70$ to have moderate and strong predictive accuracy, respectively. Overall, the five evaluated sites varied greatly in their soil physicochemical properties, with major differences in soil texture and SOC which, in turn, impacted EAs, microbial community structure, and functional diversity via EL-FAME and qPCR genes. In most cases, the model identified Tier 2 indicators, soil texture, and SOC as moderate to strong predictors of the genes of interest.

4.1 | Bacterial and fungal FAME markers were strongly related to taxonomic and C cycling genes

An ongoing challenge in soil microbiology is determining the links between microbial communities and the biogeochemical cycling processes they provide or support. Recent studies have demonstrated that microbial diversity and abundance can be used to predict ecosystem functioning and sustainability (e.g., Salles, Le Roux, & Poly, 2012; Wagg, Bender, et al., 2014; Wagg, Schlaeppi, et al., 2019). For example, Delgado-Baquerizo et al. (2016) reported a positive relationship between microbial diversity and multifunctionality suggesting that loss of diversity would adversely impact soil fertility and other services. In our study, there were strong positive correlations between certain FAME markers and genes of similar functions or taxonomic groups measured via qPCR. The sum of fungal (saprophytic and AMF) and the sum of all bacterial markers were linked to the fungal 18S and bacterial 16S rRNA genes, respectively. Although no similar research comparing these two methods has been done, there are studies that have used other fatty acid profiling assays along with DNA analysis. For example, the abundance of a plant pathogen (mold) measured via whole-cell fatty acid analysis was highly correlated with the amplified DNA from the organism's spores found in the plant (Sundelin, Møller, Lübeck, Bødker, & Jensen, 2010). Similarly, a soil microcosm study on the effect of pesticides on microbial communities found significant decreases in the abundance of Gram+ and Gram− groups via PLFA and qPCR (Karpouzas et al., 2014). In a study by Buckeridge, Banerjee, Siciliano, and Grogan (2013), seasonal shifts in the microbial community via PLFA were strongly associated with nutrient availability; however, qPCR results showed less variations.

The researchers attributed the discrepancy to the stability of DNA in soils compared to phospholipids, which are quickly degraded upon cell death. Our models showed that, in order to accurately predict 16S and 18S from FAME markers in these soils, abiotic factors such as soil texture and SOC need to be included.

Laccases, which are capable of oxidizing diverse aromatic compounds such as lignin, were strongly correlated with Actinobacteria and fungal markers. Both fungi and bacteria (especially Actinobacteria) produce laccases (Baldrian, 2006; Sinsabaugh, 2010), which enable them to break down lignolyic compounds in soil. These enzymes play an important role in C cycling as they are involved in humification, C mineralization, and dissolved organic C export (Kellner, Luis, Zimdars, Kiesel, & Buscot, 2008; Sinsabaugh, 2010). Recently, the use of degenerate primers has allowed the identification of different laccases from fungal groups and from bacterial communities, especially Actinobacteria (Kellner, Luis, & Buscot, 2007; 2008; Sinsabaugh, 2010; Fernandes, da Silveira, Passos, & Zucchi, 2014). The direct correlative results found between the different markers in this study, along with the moderate to strong predictive power of our models, suggests that the abundance of these decomposer groups may be used as a proxy for laccase abundance.

4.2 | Gram− bacteria via FAME markers were strongly correlated with proteolytic gene abundance

Proteases are a large group of hydrolytic enzymes that catalyze proteins to release peptides and amino acids, thus playing an important role in N mineralization (Ladd & Jackson, 1982; Landi, Renella, Giagnoni, & Nannipieri, 2011). Although there are several proteolytic genes identified in soil, *apr* and *npr* metallopeptidase genes are thought to be the most important in determining overall soil protease activity (Sakurai, Suzuki, Onodera, Shinano, & Osaki, 2007). Alkaline proteases are typically found in Gram− bacteria, while neutral proteases are found in Gram+ bacteria (Bach, Hartmann, Schloter, & Munch, 2001; Sakurai et al., 2007). For example, the extracellular peptidases encoded by *apr* genes have been identified in members of the Gram− genera *Pseudomonas*, *Serratia*, and *Erwinia*, while *npr* genes are almost exclusively found in Gram+ *Bacilli* (Bach et al., 2001; Baraniya et al., 2016). In this study, we found a strong positive relationship between *apr* gene abundance and FAME markers for Gram− bacteria, and the model had moderate predictive power. However, contrary to expectation, we found a weakly negative relationship between *npr* and Gram+ FAME markers, and the model showed small predictive accuracy. In contrast to all the other biological parameters measured in this study, there were significantly higher copy numbers of *npr* proteolytic genes in sites with higher sand content.

At least one other study has found that *Bacillus*-related *npr* genes are more abundant in agricultural soils with low organic matter under desiccation stress (Phillips et al., 2015). *Bacillus* spp. are often classified as K-strategists due to their stability in resource-limited conditions; *Firmicutes* (a bacilli) have been shown to be unaffected by C limitation (Fierer, Bradford, & Jackson, 2007) such as would occur in our sandy soils. In these nutrient-limited soils, *npr*-containing *Bacilli* may have a competitive advantage over r-strategist type *apr*-containing taxa, and be better able to compete for the available resources and niche space. High numbers of K-strategists within a microbial community may result in high resistance to disturbances associated with climate change (de Vries & Shade, 2013). Recently, our group found significant increases in Gram+ under natural drought and extreme high temperatures and that microbial communities (via FAME) in the sandy soils were more resistant and recovered faster than those from soils with higher clay content (Pérez-Guzmán et al., 2020). Similarly, under controlled laboratory conditions, Bérard et al. (2011) found that Gram+ markers increased after prolonged drought demonstrating the acclimation of these organisms to cope with stress. The lack of a positive relationship between *npr* genes and Gram+ markers in our current study may simply be due to the fact that while the majority of *npr*-containing *Bacilli* are Gram+, not all Gram+ bacteria are *Bacilli*. Alternately, we may be detecting *npr* genes in dormant *Bacilli* (e.g., within endospores; Higgins & Dworkin, 2012). These discrepancies along with the small predicted accuracy of our model suggest that in our soils one assay cannot replace the other.

4.3 | Enzyme activities and functional genes involved in C, N, and P cycling were positively correlated

Enzyme activities play a major role in the capacity of a soil to degrade SOM. Although no single enzyme is able to capture the entire metabolic activity of the soil, certain activities provide important information about degradation pathways involved in nutrient cycling, and have been positively correlated with several indicators of soil health and labile fractions of SOM (Acosta-Martínez, Zobeck, Gill, & Kennedy, 2002; Stott et al., 2009; Veum et al., 2014). The activities of β-glucosidase, β-glucosaminidase, and alkaline phosphatase evaluated in this study provide information on C, C and N, and P cycling, respectively (Dick 2011; Tabatabai, 1994), and hydrolyze various chemical bonds to release bioavailable sources of energy to plants and other organisms. We found comparable relationships between these EAs and the abundance of related microbial functional genes, with

strong positive correlations and similar responses to the different sampling times. Among the enzymes, β-glucosidase had the strongest correlation with the gene encoding that enzyme, and similar trends were observed regardless of sampling time (growing season vs. postharvest), year or soil textural class. β-glucosidase's response to organic C fluctuations associated with management (e.g., Bandick & Dick, 1999; Cotton & Acosta-Martínez, 2018; Veum et al., 2015) and climate variability (Acosta-Martínez et al., 2014; Kardol, Cregger, Campany, & Classen, 2010) has been demonstrated. Moreover, β-glucosidase activity has been included as an indicator of C cycling in the Soil Management Assessment Framework due to its sensitivity to changes in SOC (Stott et al., 2009). Since both methods provided similar results, at least for these five sites, the presence of the *Bglu* gene has the potential to be used as a proxy of the enzyme activity.

Phosphatases are involved in the cycling of P, which, after N, is the second most limiting nutrient for plants. Both alkaline- phosphatase and acid-phosphatase hydrolyze phosphomonoesters, which could represent up to one third of the soil organic P (Acosta-Martínez & Tabatabai, 2011). The potential activity of alkaline phosphatase and the *phoD* gene were positively correlated across all soils; however, temporal (i.e., season and year) trends from these methods were more evident in the sites with higher sand content. Additionally, the model predicted lower abundance of *phoD* during postharvest when compared to the growing season. There are numerous reasons that may explain this finding, including different levels of phosphatase contribution by other organisms in the soils and relative differences in P pools, organic matter, and clay content. While alkaline phosphatase may have different origins (e.g., plant, microbes) and be stabilized in the soil matrix, the *phoD* gene measured in this study is primarily found in bacteria. A global meta-analysis of phosphatase activity found that alkaline phosphatase activity responds strongly to shifts in readily bioavailable P (Margalef et al., 2017), which may be more inconsistent in nutrient-poor sandy soils. Soils with higher clay content also have a higher potential for stabilizing extracellular enzymes (Ragot, Kertesz, & Bünemann, 2015), leading to a greater possible disparity between potential enzyme activity and gene abundance than might be found in sandy soils. It was beyond the scope of this study to evaluate the relative contributions of intracellular vs. extracellular enzyme activity and how those relate to *phoD* gene abundance, but our results suggest that these two approaches require further analysis (e.g., analyzing other phosphatase genes) to determine whether one can be used as a proxy for the other.

The activity of β-glucosaminidase provides information about C and N cycling as it hydrolyzes chitin, which is a major source of mineralized N, and releases amino sugars for plants and microorganisms to use (Ekenler & Tabatabai, 2004). Although there are qPCR primers that target soil chitinases (Williamson, Brian, & Wellington, 2000), we were unsuccessful at implementing these assays and so we instead evaluated β-glucosaminidase with respect to three genes involved in downstream inorganic N cycling. The three N genes followed similar trends to those of β-glucosaminidase, with similar responses to changes associated with texture and year of sampling. A recent report on N functional genes showed similar changes in abundance for nitrate reducers (e.g., *narG*) and chitin degraders (e.g., *chiA*), especially in the topsoil (Turner, Mikutta, Guggenberger, & Schaarschmidt, Schippers, 2019). Their study also found strong correlation between those two genes and total N. Although our study showed positive correlations between these indicators and TN, abiotic factors such as soil properties, climate, and management controlled the presence of the genes, thus evaluation of additional N genes and β-glucosaminidase is needed.

5 | CONCLUSIONS

Our study showed that Tier 2 biological indicators such as fatty acid profiling and potential enzyme activity, as well as next generation indicators (i.e., genes) evaluated, provided comparable results for five agricultural sites that varied in soil types and sampling times exhibiting climate variability. Although the trends did not represent a perfect (1:1) relationship, their strong correlations and the moderate to strong predictive accuracy of Tier 2 indicators resulted in similar trends and conclusions. However, it is important to note that environmental factors and management practices influenced the biological indicators and, in some cases, explained most of their variability. Furthermore, the methods were sensitive for detecting changes in these soils characterized by low SOM content. Despite this, it is imperative to acknowledge that the methods we employed measure diverse aspects of the microbial component (e.g., taxonomy, functional diversity) by means of different cellular and molecular components, and as such present tradeoffs in terms of labor, equipment needed, and costs. However, a major accomplishment of our results is that it highlighted the benefits of using qPCR as a next generation of biological indicators for a more comprehensive understanding of how the soil microbial community is responding to changes in management and climate.

AUTHOR CONTRIBUTIONS

Lumarie Pérez-Guzmán, Investigation, Data curation, Investigation, Data curation, Methodology, Formal analysis, Visualization, Writing original draft, Writing-review & editing. Lori A. Phillips, Formal analysis, Methodology, Supervision, Writing-review, & editing. Miguel A. Acevedo: Data curation, Formal analysis, Visualization, Writing-review & editing. Veronica Acosta-Martínez: Conceptualization, Resources, Visualization, Formal analysis, Methodology, Supervision, Writing-review & editing.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

DISCLAIMER

Trade names and company names are included for the benefit of the reader and do not infer any endorsement or preferential treatment of the product by USDA-ARS. USDA-ARS is an equal opportunity provider and employer.

ORCID

Verónica Acosta-Martínez https://orcid.org/0000-0001-7203-7142

REFERENCES

Acosta-Martínez, V., & Cotton, J. E. (2017). Lasting effects of soil health improvements with management changes in cotton-based cropping systems in a sandy soil. *Biology and Fertility of Soils*, *53*, 1–14. http://doi.org/10.1007/s00374-017-1192-2

Acosta-Martínez, V., Mikha, M. M., & Vigil, M. F. (2007). Microbial communities and enzyme activities in soils under alternative crop rotations compared to wheat-fallow for the Central Great Plains. *Applied Soil Ecology*, *37*, 41–52. http://doi.org/10.1016/j.apsoil.2007.03.009

Acosta-Martínez, V., Moore-Kucera, J., Cotton, J., Gardner, T., & Wester, D. (2014). Soil enzyme activities during the 2011 Texas record drought/heat wave and implications to biogeochemical cycling and organic matter dynamics. *Applied Soil Ecology*, *75*, 43–51. http://doi.org/10.1016/j.apsoil.2013.10.008

Acosta-Martínez, V., Pérez-Guzmán, L., Veum, K. S., Núnes, M. R., & Dick, R. P. (2021). Metabolic activity: Enzymes. In D. L. Karlen, D. E. Stott, & M. M. Mikha (Eds.), *Soil health: Laboratory methods for soil health assessment* (Vol. *2*). Madison, WI: SSSA. (in press).

Acosta-Martínez, V., & Tabatabai, M. A. (2011). Phosphorous cycling enzymes. In R. P. Dick (Ed.), *Methods of soil enzymology* (pp. 161–184). Madison, WI: SSSA. https://doi.org/10.2136/sssabookser9.c8

Acosta-Martínez, V., Zobeck, T. M., Gill, T. E., & Kennedy, A. C. (2002). Enzyme activities and microbial structure in semiarid agricultural soils. *Biology and Fertility of Soils*, *38*, 216–227. http://doi.org/10.1007/s00374-003-0626-1

Bach, H-J., Hartmann, A., Schloter, M., & Munch, J. C. (2001). PCR primers and functional probes for amplification and detection of bacterial genes for extracellular peptidases in single strains and in soil. *Journal of Microbiological Methods*, *44*, 173–182. http://doi.org/10.1016/S0167-7012(00)00239-6

Baldrian, P. (2006). Fungal laccases-occurrence and properties. *FEMS Microbiology Reviews*, *30*, 215–242. http://doi.org/10.1111/j.1574-4976.2005.00010.x

Balota, E. L., Kanashiro, M., Colozzi Filho, A., Andrade, D. S., & Dick, R. P. (2004). Soil enzyme activities under long-term tillage and crop rotation systems in subtropical agro-ecosystems. *Brazilian Journal of Microbiology*, *35*, 300–306. http://doi.org/10.1590/S1517-83822004000300006

Bandick, A. K., & Dick, R. P. (1999). Field management effects on soil enzyme activities. *Soil Biology & Biochemistry*, *31*, 1471–1479.

Baraniya, D., Puglisi, E., Ceccherini, M. T., Pietramellara, G., Giagnoni, L., Arenella, M., Nannipieri, P., & Renella, G. (2016). Protease encoding microbial communities and protease activity of the rhizosphere and bulk soils of two maize lines with different N uptake efficiency. *Soil Biology & Biochemistry*, *96*, 176–179.

Bardgett, R. D., & van der Putten, W. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*, 505–511. http://doi.org/10.1038/nature13855

Bérard, A., Bouchet, T., Sévenier, G., Pablo, A. L., & Gros, R. (2011). Resilience of soil microbial communities impacted by severe drought and high temperature in the context of Mediterranean heat waves. *European Journal of Soil Biology*, *47*, 333–342. http://doi.org/10.1016/j.ejsobi.2011.08.004

Bhowmik, A., Fortuna, A. M., Cihacek, L. J., Bary, A. I., & Cogger, C. G. (2016). Use of biological indicators of soil health to estimate reactive nitrogen dynamics in long-term organic vegetable and pasture systems. *Soil Biology & Biochemistry*, *103*, 308–319.

Brandt, K., Amézquita, A., Backhaus, T., Boxall, A., Coors, A., Heberer, T., Lawrence, J. R., Lazorchak, J., Schönfeld, J., Snape, J., Zhu, Y-G., & Topp, E. (2015). Ecotoxicological assessment of antibiotics: A call for improved considerations of microorganisms. *Environment International*, *85*, 189–205. http://doi.org/10.1016/j.envint.2015.09.013

Buckeridge, K. M., Banerjee, S., Siciliano, S. D., & Grogan, P. (2013). The seasonal pattern of soil microbial community structure in mesic low arctic tundra. *Soil Biology & Biochemistry*, *65*, 338–347.

Canty, A., & Ripley, B. D. (2020). boot: Bootstrap R (S-Plus) functions. *R package version 1.3-25*. https://CRAN.R-project.org/package=boot

Cantrell, S. A., Molina, M., Lodge, D. J., Rivera-Figueroa, F. J., Ortiz-Hernández, M. L., Marchetti, A. A., Cyterski, M. J., & Pérez-Jiménez, J. R. (2014). Effects of a simulated hurricane disturbance on forest floor microbial communities. *Forest Ecology and Management*, *332*, 22–31. http://doi.org/10.1016/j.foreco.2014.07.010

Cañizares, R., Benítez, E., & Ogunseitan, O. A. (2011). Molecular analysis of β-glucosidase diversity and function in soil. *European Journal of Soil Biology*, *47*, 1–8. http://doi.org/10.1016/j.ejsobi.2010.11.002

Cotton, J., & Acosta-Martínez, V. (2018). Intensive tillage converting grassland to cropland immediately reduces soil microbial community size and organic carbon. *Agricultural & Environmental Letters*, *3*, 1–4.

Cotton, J., Acosta-Martínez, V., Moore-Kucera, J., & Burow, G. (2013). Early changes due to sorghum biofuel cropping systems in soil microbial communities and metabolic functioning. *Biology and Fertility of Soils*, *49*, 403–413. http://doi.org/10.1007/s00374-012-0732-z

Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., Berdugo, M., Campbell, C. D., & Singh, B. K. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nature Communications*, *7*(1). 10541, 1–8. https://doi.org/10.1038/ncomms10541

de Vries, F. T., & Shade, A. (2013). Controls on soil microbial community stability under climate change. *Frontiers in Microbiology*, *4*(265), 1–16.

Dick, R. P. (Ed.). (2011). *Methods of soil enzymology* (Vol. *9*). Madison, WI: SSSA. https://doi.org/10.2136/sssabookser9

Dick, W. A. (1984). Influence of long-term tillage and crop rotation combinations on soil enzyme activities. *Soil Science Society of America Journal*, *48*, 569–574. http://doi.org/10.2136/sssaj1984.03615995004800030020x

Dose, H. L., Fortuna, A-M., Cihacek, L. J., Norland, J., DeSutter, T. M., Clay, D. E., & Bell, J. (2015). Biological indicators provide short term soil health assessment during sodic soil reclamation. *Ecological Indicators*, *58*, 244–253. http://doi.org/10.1016/j.ecolind.2015.05.059

Eivazi, F., & Tabatabai, M. A. (1977). Phosphatases in soils. *Soil Biology & Biochemistry*, *9*, 167–172.

Ekenler, M., & Tabatabai, M. A. (2004). β-glucosaminidase activity as an index of nitrogen mineralization in soils. *Communications in Soil Science and Plant Analysis*, *35*, 1081–1094. http://doi.org/10.1081/CSS-120030588

Fernandes, T. A. R., da Silveira, W. B., Passos, F. M. L., & Zucchi, T. D. (2014). Laccases from Actinobacteria–What we have and what to expect. *Advances in Microbiology*, *4*, 285–296. http://doi.org/10.4236/aim.2014.46035

Fierer, N., Bradford, M. A., & Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology*, *88*(6), 1354–164. http://doi.org/10.1890/05-1839

Fierer, N., Jackson, J. A., Vilgalys, R., & Jackson, R. B. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology*, *71*(7), 4117–4120. http://doi.org/10.1128/AEM.71.7.4117-4120.2005

Frankenberger, W. T., & Dick, W. A. (1983). Relationships between enzyme activities and microbial growth and activity indices in soil. *Soil Science Society of America Journal*, *47*, 945–951. http://doi.org/10.2136/sssaj1983.03615995004700050021x

Frostegård, Å., & Bååth, E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, *22*, 59–65. http://doi.org/10.1007/BF00384433

García-Ruíz, R., Ochoa, V., Hinojosa, M. B., & Carreira, J. A. (2008). Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. *Soil Biology & Biochemistry*, *40*, 2137–2145.

Gregory, L. G., Karakas-Sen, A., Richardson, D. J., & Spiro, S. (2000). Detection of genes for membrane-bound nitrate reductase in nitrate-respiring bacteria and in community DNA. *FEMS Microbiology Letters*, *183*, 275–279. http://doi.org/10.1111/j.1574-6968.2000.tb08971.x

Higgins, D., & Dworkin, J. (2012). Recent progress in Bacillus subtilis sporulation. *FEMS Microbiology Reviews*, *36*, 121–148. http://doi.org/10.1111/j.1574-6976.2011.00310.x

Kardol, P., Cregger, M. A., Campany, C. E., & Classen, A. T. (2010). Soil ecosystem functioning under climate change: Plant species and community effects. *Ecology*, *91*(3), 767–781. http://doi.org/10.1890/09-0135.1

Karpouzas, D. G., Kandeler, E., Bru, D., Friedel, I., Auer, Y., Kramer, S., Vasileiadis, S., Petric, I., Udikovic-Kolic, N., Djuric, S., & Martin-Laurent, F. (2014). A tiered assessment approach based on standardized methods to estimate the impact of nicosulfuron on the abundance and function of the soil microbial community. *Soil Biology & Biochemistry*, *75*, 282–291.

Kellner, H., Luis, P., & Buscot, F. (2007). Diversity of laccase-like multicopper oxidase (LMCO) genes in Morchellaceae: Identification of genes potentially involved in extracellular activities related to plant litter decay. *FEMS Microbiology Ecology*, *61*, 153–163. http://doi.org/10.1111/j.1574-6941.2007.00322.x

Kellner, H., Luis, P., Zimdars, B., Kiesel, B., & Buscot, F. (2008). Diversity of laccase-like multicopper oxidase genes in forest and grassland Cambisol soil samples. *Soil Biology & Biochemistry*, *40*, 638–648.

Ladd, J. N., & Jackson, R. B. (1982). Biochemistry of ammonification. In F. J. Stevenson (Ed.), *Nitrogen in agricultural soils* (pp. 173–228). Agronomy Monograph 22, Madison, WI: ASA, CSSA, SSA. https://doi.org/10.2134/agronmonogr22.c5

Landi, L., Renella, G., Giagnoni, L., & Nannipieri, P. (2011). Activities of proteolytic enzymes. In R. P. Dick (Ed.), *Methods of soil enzymology* (pp. 247–260). Madison, WI: SSSA

Lehman, R. M., Acosta-Martínez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., Halvorson, J. J., Jin, V. L., Johnson, J. M. F., Kremer, R. J., Lundgren, J. G., Manter, D. K., Maul, J. E., Smith, J. L., & Stott, D. E. (2015). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, *70*(1), 12A–18A. http://doi.org/10.2489/jswc.70.1.12A

Li, C., Cano, A., Acosta-Martínez, V., Veum, K. S., & Moore-Kucera, J. (2020). A comparison between fatty acid methyl ester profiling methods (PLFA and EL-FAME) as soil health indicators. *Soil Science Society of America Journal*, *84*(4), 1153–1169. https://doi.org/10.1002/saj2.20118</bib>

Li, C., Fultz, L. M., Moore-Kucera, J., Acosta-Martínez, V., Kakarla, M., & Weindorf, D. C. (2018). Soil microbial community restoration in Conservation Reserve Program semi-arid grasslands. *Soil Biology & Biochemistry*, *118*, 166–177.

Lüdecke, D. (2019). *sjPlot: Data visualization for statistics in social science*. https://strengejacke.github.io/sjPlot/

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowni-Horas, R., Janssens, I. A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D., & Peñuelas, J. (2017). Global patterns of phosphatase activity in natural soils. *Scientific Reports*, *7*, 1337. http://doi.org/10.1038/s41598-017-01418-8

Mbuthia, L. W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., & Eash, N. (2015). Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biology & Biochemistry*, *89*, 24–34.

Miller, W. P., & Miller, D. M. (1987). A micro-pipette method for soil mechanical analysis. *Communications in Soil Science and Plant Analysis*, *18*, 1–15. http://doi.org/10.1080/00103628709367799

Miura, T., Makoto, K., Niwa, S., Kaneko, N., & Sakamoto, K. (2017). Comparison of fatty acid methyl ester methods for characterization of microbial communities in forest and arable soil: Phospholipid fractions (PLFA) versus total ester linked fatty acids (EL-FAME). *Pedobiologia*, *63*, 14–18.

Moore-Kucera, J., & Dick, R. P. (2008). PLFA profiling of microbial community structure and seasonal shifts in a Douglas-fir chronosequence. *Microbial Ecology*, *55*, 500–511. http://doi.org/10.1007/s00248-007-9295-1

Mundepi, A., Cabrera, M., & Norton, J., & Habteselassie, M. (2019). Ammonia oxidizers as biological health indicators of elevated Zn and Cu in poultry litter amended soil. *Water, Air, and Soil Pollution*, *230*. https://doi.org/10.1007/s11270-019-4283-x

NOAA. National Oceanic and Atmospheric Administration. (2020). *Global Climate Report-Annual 2019*. https://www.ncdc.noaa.gov/sotc/global/201913

Olsson, P. A., Bååth, E., Jakobsen, I., & Söderström, B. (1995). The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. *Mycological Research*, *99*, 623–629. http://doi.org/10.1016/S0953-7562(09)80723-5

Ouyang, Y., Reeve, J. R., & Norton, J. M. (2018). Soil enzyme activities and abundance of microbial functional genes involved in nitrogen transformations in an organic farming system. *Biology and Fertility of Soils*, *54*, 437–450. https://doi.org/10.1007/s00374-018-1272-y

Parham, J. A., & Deng, S. P. (2000). Detection, quantification and characterization of β-glucosidase activity in soil. *Soil Biology & Biochemistry*, *32*, 1183–1190.

Pérez-Guzmán, L., Acosta-Martínez, V., Phillips, L. A., & Mauget, S. A. (2020). Resilience of the microbial communities and their enzymatic activities in semi-arid agricultural soils during natural climatic variability events. *Applied Soil Ecology*, *149*. https://doi.org/10.1016/j.apsoil.2019.103487

Phillips, L. A., Schefe, C. R., Fridman, M., O'Halloran, N., Armstrong, R. D., & Mele, P. M. (2015). Organic nitrogen cycling microbial communities are abundant in a dry Australian agricultural soil. *Soil Biology & Biochemistry*, *86*, 201–2011.

Poly, F., Wertz, S., Brothier, E., & Degrange, V. (2008). First exploration of Nitrobacter diversity in soils by a PCR cloning-sequencing approach targeting functional gene nxrA. *FEMS Microbiology Ecology*, *63*, 132–140. http://doi.org/10.1111/j.1574-6941.2007.00404.x

Ragot, S. A., Kertesz, M. A., & Bünemann, E. K. (2015). phoD alkaline phosphatase gene diversity in soil. *Applied and Environmental Microbiology*, *81*(20), 7281–7289. http://doi.org/10.1128/AEM.01823-15

RStudio Team (2018). *RSTudio: Integrated development for R*. Boston, MA: RSTudio, Inc. http://www.rstudio.com/

Sakurai, M., Suzuki, K., Onodera, M., Shinano, T., & Osaki, M. (2007). Analysis of bacterial communities in soil by PCR-DGGE targeting protease genes. *Soil Biology & Biochemistry*, *39*(11), 2777–2784.

Salles, J. F., Le Roux, X., & Poly, F. (2012). Relating phylogenetic and functional diversity among denitrifiers and quantifying their capacity to predict community functioning. *Frontiers in Microbiology*, *3*, 209. https://doi.org/10.3389/fmicb.2012.00209

Schutter, M. E., & Dick, R. P. (2000). Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. *Soil Science Society of America Journal*, *64*, 1659–1668. http://doi.org/10.2136/sssaj2000.6451659x

Schutter, M. E., & Dick, R. P. (2002). Microbial community profiles and activities among aggregates of winter fallow and cover-cropped. *Soil Science Society of America Journal*, *66*, 142–153. http://doi.org/10.2136/sssaj2002.1420

Sinsabaugh, R. L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology & Biochemistry*, *42*, 391–404.

Stewart, R. D., Jian, J., Gyawali, A. J., Thomason, W. E., Badgley, B. D., Reiter, M. S., & Strickland, M. S. (2018). What we talk about when we talk about soil health. *Agricultural & Environmental Letters*, *3*, 180033. https://doi.org/10.2134/ael2018.06.0033

Stone, D., Ritz, K., Griffiths, B. G., Orgiazzi, A., & Creamer, R. E. (2015). Selection of biological indicators appropriate for European soil monitoring. *Applied Soil Ecology*, *97*, 12–22. http://doi.org/10.1016/j.apsoil.2015.08.005

Stott, D. E. (2019). *Recommended soil health indicators and associated laboratory procedures*. Soil health Technical Note No. 450-03. USDA and NRCS. https://directives.sc.egov.usda.gov/OpenNonWebContent.aspx?content=44475.wba

Stott, D. E., Andrews, S. S., Liebig, A. M., Wienhold, B. J., & Karlen, D. K. (2009). Evaluation of β-glucosidase activity as a soil quality indicator for the Soil Management Assessment Framework. *Soil Science Society of America Journal*, *74*(1), 107–119. http://doi.org/10.2136/sssaj2009.0029

Sundelin, T., Møller, K., Lübeck, M., Bødker, L., & Jensen, B. (2010). In planta quantification of Plasmodiophora brassicae using signature fatty acids and real-time PCR. *Plant Disease*, *94*(4), 432–438. http://doi.org/10.1094/PDIS-94-4-0432

Tabatabai, M. A. (1994). Soil enzymes. In R. W. Weaver, J. S. Angle, & P. S. Bottomley (Eds.), *Methods of soil analysis: Part 2. Microbiological and biochemical properties* (Vol. *5*, pp. 775–833). Madison WI: SSSA.

Tabatabai, M. A., & Dick, W. A. (2002). Enzymes in soil: Research and developments in measuring activities. In R. G. Burns & R. P. Dick (Eds.), *Enzymes in the environment: Activity, ecology and applications* (pp. 567–596). New York: Marcel Dekker.

Thiele-Bruhn, S., Schloter, M., Wilk, B-M., Beaudette, L. A., Martin-Laurent, F., Cheviron, N., Mougin, C., & Römbke, J. (2020). Identification of new soil microbial functional standards for soil quality assessment. *Soil*, *6*, 17–34. http://doi.org/10.5194/soil-6-17-2020

Trasar-Cepeda, C., Leirós, M. C., Seoane, S., & Gil-Sotres, F. (2000). Limitations of soil enzymes as indicators of soil pollution. *Soil Biology & Biochemistry*, *32*, 1867–1875.

Turner, S., Mikutta, R., Guggenberger, G., & Schaarschmidt, F., & Schippers, A. (2019). Distinct pattern of nitrogen functional gene abundance in top- and subsoils along a 120,000-year ecosystem development gradient. *Soil Biology & Biochemistry*, *132*, 111–119.

Vanio, E. J., & Hantula, J. (2000). Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycological Research*, *104*(8), 927–936. http://doi.org/10.1017/S0953756200002471

Veum, K. S., Goyne, K. W., Kremer, R. J., Miles, R. J., & Sudduth, K. A. (2014). Biological indicators of soil quality and soil organic matter characteristics in an agricultural management continuum. *Biogeochemistry*, *117*, 81–99. http://doi.org/10.1007/s10533-013-9868-7

Veum, K. S., Kremer, R. J., Sudduth, K. A., Kitchen, N. R., Lerch, R. N., Baffaut, C., Stott, D. E., Karlen, D. L., & Sadler, E. J. (2015). Conservation effects on soil quality indicators in the Missouri Salt River Basin. *Journal of Soil and Water Conservation*, *70*, 232–246. http://doi.org/10.2489/jswc.70.4.232

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystems multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(14), 5266–5270. http://doi.org/10.1073/pnas.1320054111

Wagg, C., Schlaeppi, K., Banerjee, S., Kuramae, E. E., & van der Heijden, M. G. A. (2019). Fungal-bacterial diversity and microbiome complexity predict ecosystem functioning. *Nature communications*, *10*, 4841, 1–10. https://doi.org/10.1038/s41467-019-12798-y

Watanabe, K., Kodama, Y., & Harayama, S. (2001). Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. *Journal of Microbiological Methods*, *44*, 253–262. http://doi.org/10.1016/S0167-7012(01)00220-2

Welsh, A., Chee-Sanford, J. C., Connor, L. M., Löffler, F. E., & Sanford, R. S. (2014). Refined NrfA phylogeny improves PCR-based nrfA gene detection. *Applied and Environmental Microbiology*, *80*(7), 2110–2119. http://doi.org/10.1128/AEM.03443-13

Wessén, E., & Hallin, S. (2011). Abundance of archaeal and bacterial ammonia oxidizers: Possible bioindicator for soil monitoring. *Ecological Indicators*, *11*(6), 1696–1698. http://doi.org/10.1016/j.ecolind.2011.04.018

Wickham H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag. https://ggplot2.tidyverse.org

Willers, C., Jansen van Rensburg, P. J., & Claassens, S. (2015). Phospholipid fatty acid profiling of microbial communities: A review of

interpretations and recent applications. *Journal of Applied Microbiology*, *119*, 1207–1218. http://doi.org/10.1111/jam.12902

Williamson, N., Brian, P., & Wellington, E. (2000). Molecular detection of bacterial and streptomycete chitinases in the environment. *Antonie Van Leeuwenhoek*, *78*, 315–321 (2000). https://doi.org/10.1023/A:1010225909148

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: A review. *Biology and Fertility of Soils*, *29*, 111–129. http://doi.org/10.1007/s003740050533

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Pérez-Guzmán L, Phillips LA, Acevedo MA, Acosta-Martínez V. Comparing biological methods for soil health assessments: EL-FAME, enzyme activities, and qPCR. *Soil Sci Soc Am J*. 2021;*85:*636–653. https://doi.org/10.1002/saj2.20211