Interrelationships Among Methods of Estimating Microbial Biomass Across Multiple Soil Orders and Biomes

Buell, Z.W.,[1] Dabbs, J.,[2,A] Steinweg, J.M.,[3,B] Kluber, L.A.,[3,C] Phillips, J.R.,[2,] Yang, Z.K.,[3] Roth, S.W.[3,D], Miller, R.M.[4], Gutknecht, J.L.M.,[1] Schadt, C.W.,[3] and Mayes, M.A.[2*]

[1]Department of Soil Water and Climate, University of Minnesota, Saint Paul, MN
[2]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge TN
[3]Biosciences Division, Oak Ridge National Laboratory, Oak Ridge TN
[4]Environmental Sciences Division, Argonne National Laboratory, Lemont, IL
[A]Current Address: Tetra Tech, Inc., Superior, CO
[B]Current Address: Center for Excellence in Teaching and Learning for Students Success, Appalachian State University, Boone, NC
[C]Current Address: Novozymes Inc, Salem, VA
[D]Current Address: Pluton Biosciences, St. Louis, MO
* Corresponding author mayesma@ornl.gov

Buell, Z.W. buell028@umn.edu
Dabbs, J. Jenny.Dabbs@tetratech.com
Steinweg, J.M. steinwegjm@appstate.edu [ORCID: 0009-0007-7507-9648]
Kluber, L.A. laurel.kluber@gmail.com
Phillips, J.R. randolphjd1@ornl.gov] [ORCID: 0000-0001-9319-2336]
Yang, Z.K. zkooyang@gmail.com
Roth, S.W. spencer.w.roth@gmail.com [ORCHID: 0000-0001-9559-1154]
Miller, R.M. rmmiller745@gmail.com [ORCHID: 0000-0003-2114-7856]
Gutknecht, J.L.M. jgut@umn.edu [ORCHID: 0000-0001-7667-5272]
Schadt, C.W. schadtcw@ornl.gov [ORCHID: 0000-0001-8759-2448]
Mayes, M.A. mayesma@ornl.gov [ORCHID: 0000-0001-6368-9210]

Copyright notice: This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (http://energy.gov/downloads/doe-public-access-plan).

Manuscript Highlights

- Four common proxies for soil microbial biomass show significant correlations.
- Correlations are appropriate for mineral soils while organic soils require more study.

- Correlations are stronger when DNA-based proxies are corrected for clay content.
- Relationships facilitate different microbial data usage in soil carbon cycling models.

Abstract

Understanding the role of soil microbes is critical to ecosystem processes, and more thorough comparisons of measurement proxies for soil microbial biomass could broaden the inclusion of explicit microbial parameterization in soil carbon cycling and earth system models. We measured physical, chemical, and biological data from eight soil orders representing 11 major biomes and four climate regions. Four prominent methods to measure microbial abundance—chloroform fumigation extraction (CFE), total DNA yield, gene copy number by quantitative polymerase chain reaction (GCN), and phospholipid fatty acids (PLFA) —were compared to assess their relationships with each other and with soil characteristics. Correlations were observed when comparing methods, with CFE correlating strongly with total DNA yield, GCN, and PLFA; CFE with bacterial GCN and bacterial PLFA; and to a lesser extent, total PLFA and total DNA yield. Correlations improved with the removal of organic soils (Histosols, Gelisols). Comparisons involving extracted DNA were improved by correcting for clay content, due to DNA extraction inefficiencies in clay-rich soils. Correlations involving fungi (PLFA or GCN) were always less significant. These methods could serve as reliable, inter-relatable proxies for the estimation of total soil microbial biomass while recognizing that the proxies are less effective at parsing differences between bacteria and fungi. We provide specific equations to relate measures of soil microbial biomass by these four different methods to enable microbial models to utilize a greater diversity of observed data sources in parameterizations and simulations. Caveats for the equations and their values are also discussed.

Keywords: Soil microbial biomass; chloroform fumigation extraction; DNA; qPCR; PLFA; soil carbon cycle modeling

1 Introduction

Soils are an important carbon (C) sink, storing over two thirds of the global terrestrial carbon pool (De Deyn et al., 2008; Jobbagy and Jackson, 2000). More accurate measurements of the soil microbial communities that drive soil C turnover and greenhouse gas efflux, and improved representation of these communities in models, could improve our understanding of controls over the global soil C balance (Jastrow et al., 2007) and how climate change might alter C storage feedbacks (Dutta and Dutta, 2016). However, accurate estimates to quantify soil microbial biomass remain difficult to obtain and divergent approaches continue to be applied, despite the increasing incorporation of more microbial parameters in ecosystem models (Manzoni et al., 2014; Lawrence et al., 2009; Abramoff et al., 2022). A more accurate understanding of the relationships between various methods for estimating soil microbial biomass could improve the parameterization of microbially-explicit models across soil types and ecosystems, and thus provide a better understanding and more accurate projection of global climate change scenarios.

The high diversity in microbial communities, along with the difficulty in directly observing their numbers and growth, results in challenges in developing accurate methods to quantify soil microbial abundance (Fierer et al., 2007; Strickland and Rousk, 2010; Čapek et al., 2023). Additionally, the composition of soil microbial communities is important to consider, especially when trying to assess biogeochemical cycling and other ecosystem-scale dynamics. For example, the two major microbial groups in soils – bacteria and fungi – utilize contrasting mineralization pathways, where fungi dominate slower decomposition of more complex substrates and bacteria are generally more diverse in their metabolic strategies and life habits (Joergensen and Wichern, 2008; De Graaff et al., 2010). Thus, the relative abundances of bacteria and fungi can strongly affect carbon cycling rates and trajectories across soils (Waring, 2013; Manzoni et al., 2012). Recent microbial models have separated bacteria and fungi to better represent the diversity of soil carbon decomposition processes (He et al.; 2020, 2021).

Since microbes typically have similar amounts of C per cell or per unit biomass, the C in the microbial biomass is a useful proxy for total soil microbial biomass. Microbial biomass C is a parameter often used to quantify belowground microbes and their contributions to soil organic matter stabilization via secondary metabolites and

necromass residues (Khan et al., 2016). There are currently no methods for directly quantifying soil microbial biomass C, but indirect microbial biomass C measures have traditionally been obtained through chloroform fumigation-extraction (CFE) (Jenkinson and Powlson, 1976; Vance et al., 1987). CFE has been utilized frequently over the last 50 years, resulting in extensive evaluation of its assumptions, strengths, and weaknesses (Badalucco et al., 1992; Badalucco, et al., 1997; Jenkinson et al., 2004). Some key shortcomings of this procedure are its lack of taxonomic differentiation between bacterial and fungal biomass, as well as its varying accuracy and conversion factor efficacy across soil types, with underestimation of microbial biomass C often observed in soils of low porosity and overestimation of microbial biomass C in soils with high concentrations of organic matter (Fierer et al., 2009). Additionally, CFE is both labor- and time-intensive, utilizes toxic reagents, and requires large amounts of fresh, homogenized soil.

Phospholipid fatty acid (PLFA) analyses are another proxy for soil microbial biomass estimation. PLFAs are effective biomarkers for living organisms due to their short residence time in soil following cell death and hydrolysis (Zhang et al., 2019b), and are thus established as a prominent soil microbial biomass proxy (Bailey et al., 2002; Fierer et al., 2003; Leckie et al., 2004; Schmidt et al. 2015). PLFAs also offer a degree of taxonomic resolution useful in assessing microbial community composition, though this is limited to broad distinctions, for example bacterial vs. fungal lipids or gram negative vs. gram positive bacteria. Overlap in microbial and plant PLFAs in certain ecosystems can also reduce accuracy in estimating soil microbial biomass (Koskimies and Simola, 1980), while common PLFAs between bacteria and fungi reduce taxonomic resolution (Lekberg et al., 2022; Olsson et al., 2022). Additionally, archaea are not accounted for in PLFA analysis since their cell membranes do not contain ester lipids. Lastly, similar to CFE, PLFA analysis is a resource-intensive procedure requiring significant time and labor investment, specialized equipment and knowledge, and large volumes of toxic reagents and homogenized soil.

Other proxies for soil microbial biomass estimation include both extractable DNA yield (Marstorp et al., 2000, Leckie et al., 2004) and quantitative polymerase chain reaction (qPCR) of rRNA genes and other universal marker genes (Fierer et al., 2005). Both techniques can be used to rapidly evaluate soil microbial communities across

various ecosystems, and each have higher potential scaling throughput, reduced soil mass requirements, and reduced toxic reagent use compared to CFE and PLFA (Fierer et al., 2005, Dunbar et al., 2012). Contrastingly, while the reduced amounts of soil required for analyses of DNA based methods can be considered a throughput advantage, it may also mean that DNA sample sizes may be more susceptible to biases due to heterogeneities within any given sample. While qPCR can be used to determine the relative abundance of various groups in soil microbial communities (e.g. fungal to bacterial ratios) and overall abundance using the qPCR gene copy number (GCN), the relationships between gene abundance and soil microbial biomass have not been robustly established across studies and ecosystems (Strickland and Rousk, 2010; Fierer et al., 2021). Both DNA yield and qPCR methods can also be affected by variability in the DNA content per cell and GCN of rRNA genes per cell, and across phylogenetic groups and between groups, such as fungi relative to bacteria (Harris and Paul, 1994; Leckie et al., 2004; Lofgren et al., 2019). Due to differences in rRNA gene copy number between bacteria/archaea (usually <10 per genome) and fungi (often >100 per genome and highly variable), these methods may be especially problematic in comparison across soils that display large differences in degree of dominance between bacteria and fungi (Leckie et al., 2004). Both DNA yield and GCN can also be affected by choice of DNA extraction method (Harrison et al., 2021, Dunbar et al., 2012), and variability between labs has been observed (Dunbar et al., 2012). Additionally, both GCN and DNA yield methods may be vulnerable to overestimation due to so-called “relic DNA”, i.e., DNA in dead organisms or bound to clays (Carini et al., 2019). Despite the limitations of DNA yield and GCN, these methods are valuable proxies for soil microbial biomass and are widely used for microbial community ecology. Additionally, like PLFA, GCN methods use conserved markers to identify bacterial, archaeal and fungal composition within a single method (Kluber et al., 2020). Therefore, either alone or in combination with CFE, GCN could allow the partitioning of fungal, bacterial, and archaeal contributions to soil microbial biomass, thereby improving understanding of their roles in ecosystem processes and carbon cycle modeling.

Earth system and process models represent the decomposition of litter into soil organic C (SOC) pools, with decay rates modified by temperature, clay content, and soil

moisture. However, lack of agreement between observed and modeled SOC implies that the models remain insufficiently sophisticated to represent the complexity of the decomposition process (Todd-Brown et al., 2012). In particular, models often neglect the role of soil microbes with the use of implicit rather than explicit microbial biomass, activity, or function. A new generation of models has emerged that explicitly uses nonlinear equations to represent microbial processes, resulting in improved agreement between predicted and observed SOC (Schimel and Weintraub, 2003; Lawrence et al., 2009; Wang et al., 2013). While disagreement remains about how pools and fluxes should be conceptualized (Sulman et al., 2018), data on soil microbial biomass is needed to parameterize explicit microbial models. Models may use a single pool of soil microbial biomass (Wieder et al. 2014; Tang and Riley 2013; Wang et al., 2019), partition soil microbial biomass into rhizosphere and bulk soil (Sulman et al., 2014), or separate soil microbial biomass into fungal and bacterial biomass (He et al., 2020). Consequently, accurate measures of total soil microbial biomass, as well as separate measures of fungal biomass and bacterial biomass, are needed to effectively calibrate and test these kinds of models. Given this, a quantitative understanding of the relationships and correlations between these different observational methods described above are particularly needed, since observations available in the literature typically only use a single method. Additionally, modeling may require data assembled from a range of studies that use a diversity of soil microbial biomass proxies, and modeling outcomes should be improved with the ability to incorporate more than one type of soil microbial biomass data.

The primary objective of this study was to broadly examine the relationships between four proxies for soil microbial biomass: CFE, PLFA, total DNA yield, and GCN across a diverse selection of soil types representing eight soil orders, four climate regions, and 11 distinct biomes. While others have compared methods for estimating soil microbial biomass, these studies were focused on a single soil type (Leckie et al., 2004; Buckeridge et al., 2013; Gong et al., 2021), organism type (Landeweert et al., 2003; Baldrain et al., 2013; Zhang et al., 2017), or occurred prior to the widespread use of qPCR (Bailey et al., 2002). Here we use a suite of soils from various biomes, soil orders, and horizons to establish quantitative relationships between these measures of microbial abundance, to better enable their cross-substitution as proxies for soil microbial biomass

and facilitate broader and more explicit inclusion of microbial parameters and processes into ecosystem and carbon cycling models. Towards this goal we provide explicit empirical equations that can be used to relate the proxies to each other.

2 Materials and methods

2.1 Soil collection and characterization

Fresh soils were collected from a variety of climate regions and soil orders (Table 1), including Aridisol (tropical), Oxisol (tropical), Ultisol (tropical and temperate), Inceptisol (temperate), Mollisol (temperate), Alfisol (temperate and Boreal), Histosol (southern Boreal) and Gelisol (sub-Arctic and Arctic). For most collection locations, a surface and a subsurface horizon were sampled, e.g., A and B horizons from mineral soils, although in some cases only an A horizon was collected. Most samples were collected by request to local investigators, who provided ca. 1 kg total of each horizon that was composited from 3 locations in close proximity and shipped chilled. Because the samples were composited in the field, there are no estimates of uncertainty for the values presented herein. For the Marcell Experimental Forest S-1 bog Histosol, samples were collected from peat near the surface and at depth, representing the acrotelm and catotelm layers, respectively. For permafrost soils, only the surface active layers were included. In total, 18 surface and 15 subsurface soil samples were used for this comparison. Soil samples were stored at -20 ^{o}C for fewer than five days before being sieved (2 mm) and subsampled (n = 3) for analyses of extractable organic carbon (EOC), soil microbial biomass, dry weight, and extraction of DNA. The Marcell Experimental Forest peat subsamples for qPCR and PLFA subsamples were stored at -80 ^{o}C prior to analysis. The full datasets described here are published (Buell et al. 2024a, b, c; Roth et al. 2024). DNA and CFE extraction methods are both independent of microbial growth stages.

Gravimetric moisture content (GMC) was calculated for each soil by oven-drying 5 g of field moist soil and then using the equation:

$$\theta_{dw} = \frac{m_f - m_d}{m_d}$$

where m_f is the mass of fresh field moist soil and m_d is the mass of the dried soil. This was used to correct all of the physical, chemical and microbial biomass measures below to a dry weight basis (g^{-1} dry soil). Total bulk soil C (TC) and nitrogen (TN) were

234 determined by a Leco Combustion Analyzer (Leco Corp., St. Joseph, MI) (Table 2). Total
235 organic C (TOC) concentrations were determined by the same method after treating
236 samples with 3*M* HCl for 1 hr (Nelson and Sommers, 1996). Particle size analysis for soil
237 texture was evaluated with the Bouyoucos hydrometer method (Gee and Or, 2002). Soil
238 pH was determined by shaking 1 part soil in 2 parts Milli-Q water and measuring the pH
239 of the supernatant (Thomas, 1996).
240 Subsamples (n = 3) of 7 g fresh soil were used to measure EOC for each soil type.
241 The soil was combined with 0.035 L of 0.5*M* K_2SO_4, and the samples were shaken on an
242 orbital reciprocating shaker for 1 hour. Afterward, the soil suspensions were gravity
243 filtered with Whatman No. 42 filter paper, and the extracts were immediately stored at -
244 20° C until analysis using a Shimadzu Total Organic Carbon Analyzer (Shimadzu Corp.,
245 Kyoto, Japan) to obtain values for EOC (Table 2). Analytical standards are used to derive
246 an approximate detection limit of 0.01 mg C l^{-1}.
247 **2.2 Microbial community characterization**
248 **2.2a Chloroform fumigation-extraction**
249 Subsamples (n = 3) of 7 g fresh soil were thawed from -20 °C and fumigated with
250 chloroform in a vacuum desiccator for a total of 48 h at 25° C to lyse the microbial cells.
251 The fumigated soil was then combined with 0.035 L of 0.5*M* K_2SO_4, and treated
252 identically to the EOC extractions in Section 2.1. On a subset of soils we measured CFE-
253 based microbial biomass on fresh, never frozen soil samples and obtained similar values
254 that were within the standard error of the samples frozen at -20 °C prior to fumigation.
255 Estimates for CFE biomass were then calculated using the equation
256 $$CFE = \frac{K_C}{E_C}$$
257 where K_C is the difference between extractable carbon before and after fumigation
258 corrected for dry weight, and E_C is the extraction efficiency coefficient (Fierer et al.,
259 2009). Although extraction efficiency will vary by individual soil, an E_C of 0.45 was
260 applied here, as this is a standard value typically applied for C from mineral soils (Fierer
261 et al., 2009; Vance at al., 1987).
262 **2.3b Phospholipid fatty acids**
263 Lipid analysis was performed according to an adaptation of the Bligh and Dyer
264 (1959) method, as outlined in Gray et al. (2011). Extractions were performed on freeze-

dried 2-g subsamples using a 1:2:0.8 ratio of chloroform, methanol, and phosphate buffer (pH 7.4). Water and chloroform were then added after three hours to produce a phase separation isolating total lipids in the chloroform layer. Phospholipids were separated using silicic acid column chromatography, then saponified and methylated using an alkaline solution to produce fatty acid methyl esters (FAMEs). Dried phospholipids were then reconstituted using a known concentration of FAME 19:0 as an internal standard to quantify PLFA concentrations. Samples from the Marcell Experimental Forest bog peat were processed using a modified Bligh and Dyer (1959) method for peat extractions (Felice et al. 2024). In contrast to the previously described method (Gray et al., 2011), a 1:1:0.9 ratio of chloroform, methanol, and citrate buffer (pH 4.0) was used to isolate total lipids and samples were not saponified prior to methylation. For the SPRUCE samples, Regen Ag used an Agilent 7890B GC equipped with split-splitless inlet and FID.

Nomenclature of fatty acids adheres to Frostegård et al. (1993). Specific PLFAs were used to quantify relative abundance of bacterial and fungal biomass. Calculations of total PLFAs comprise fatty acids with less than 20 carbons (Zelles, 1999). Bacterial biomass calculations included the sum of fatty acids 14:0, i15:0, a15:0, 15:0, i16:0, 16:0, 16:1w7, 10me16:0, i17:0, a17:0, cy17:0, 18:1w7, 18:0, 10me18:0, cy19:0a (Frostegård and Bååth, 1996; Zelles, 1999), while fungal biomass is represented by the sum of 18:2w6 (Zelles, 1999) and 18:1w9 (Zak et al., 1996). There are variations in the literature about which fatty acids are selected to quantify specific microbial groups.

Fungi, for example, have been represented by singular lipids (Landeweert et al. 2003; Fierer et al., 2003) or through various summations of multiple lipids (Bai et al., 2017; Clark et al., 2009; Gutknecht et al., 2012, Rakkar et al., 2023). All of these fatty acids are reliable indicators of total microbial biomass (Zelles, 1999) and bacterial and fungal biomass (Klamer and Bååth 2004; Rinklebe and Langer 2004) but an individual cell is composed of multiple types of fatty acids within an indicator group (i.e. Stahl and Klug 1996). Summing these multiple fatty acids is acceptable practice in order to have as complete a biomass representation as possible (i.e. Felice et al. 2024).

2.3c Microbial DNA yield and qPCR gene copy number

Subsamples (n = 3) of each soil type sample were stored at -80° C, and microbial DNA was extracted from 0.25 g of soil using the PowerSoil DNA Isolation Kit (MOBIO

Laboratories, Inc., CA, USA) or the E.Z.N.A. Soil DNA Kit (Omega Biotek, Norcross, GA, USA) for the SPRUCE Histosol samples. The extracted DNA was initially measured for purity using a NanoDrop Spectrophotometer (NanoDrop Technologies, Inc.), and the Quant-It PicoGreen dsDNA Assay Kit (Invitrogen) assay was used for quantitative DNA yield and corrected for dry weight.

To quantify GCN on a dry weight basis for bacteria, fungi, and archaea using qPCR, the same general approach outlined by Fierer et al. (2005) was used with some modifications (Kluber et al., 2020). Analyses for each soil were conducted in analytical triplicate and set up in optically clear 96-well plates. Each qPCR reaction consisted of 19 µl of MasterMix—5 µl H_2O, 2 µl of forward primer, 2 µl of reverse primer, and 10 µl of SYBR Green SuperMix (Bio-Rad Laboratories, Inc.)—combined with 1 µl of extracted microbial DNA or a 1:10 dilution. DNA extractions of pure culture standards of *Escherichia coli, Saccharomyces cerevisiae* and *Methanococcus maripaludis* of known DNA concentration were diluted to 1:10, 1:100, 1:1,000, and 1:10,000 and were used to generate a standard linear curve relating the log of the GCN to the measured threshold value (C_t) for eubacteria, fungi, and archaea, respectively (Table 3). These standard curves were then used to convert sample threshold values measured for each group on a CFX96 Real-Time System (Bio-Rad Laboratories, Inc., CA, USA) into GCN g^{-1} dry soil. Correlation coefficients and reaction efficiencies were calculated across the standard curves generated in each sample run. When correlations were < 0.95, or reaction efficiencies < 80% for bacteria or archaea reactions, or < 70% for fungi reactions, the sample run was repeated. Total GCN was then calculated as the sum of bacterial, fungal, and archaeal GCN. The approximate detection limit was determined using standard curves and resulting in values of 200 rRNA gene copies.

2.4 Statistical analysis

Linear single and multiple regression models were used to determine the relationships between the four soil microbial biomass estimation methods, and to explore relationships between each method and other soil characteristics. Analyses were performed using RStudio 2022.02.1.461 (RStudio Team, 2020) running R software version 4.0.3 (R Core Team, 2024) with the following packages: tidyverse (Wickham et al., 2019), broom (Robinson et al., 2020), readxl (Wickham & Bryan, 2019), and ggplot2 (Wickham et al.,

2016). Outliers were determined for each microbial measurement type using an Interquartile Range (IQR) method, determining values falling below Q1-1.5 IQR or above Q3+1.5 IQR. Other than a single mineral surface sample (Big Ridge, TN Inceptisol), all outliers identified were organic soils. Instead of treating the majority of organic soils as outliers for one or more comparisons, regression analyses were conducted for three data subsets: all soils (mineral and organic); mineral soils only; and mineral soils with a clay correction factor (Yankston and Steck, 2009) applied to GCN and DNA yields (Table 4). Coefficient error estimates were not calculated, since n = 1 for all sampling locations and depths because the samples were composited in the field.

3. Results

3.1. Relationships between soil physical, chemical, and their microbiological properties

Physical and chemical properties were examined for each soil sample to provide context and possible explanatory information for differences in soil microbial biomass proxy measurements. Soil physicochemical properties varied significantly across different soil types (Table 2). TOC and TC were strongly positively correlated with each other, so correlations with TOC are not shown. The least variable property across soils was pH, which displayed no correlations with other measured soil properties. As expected, most measurements of microbial biomass decreased with depth across nearly all soil types (Buell et al., 2024a). CFE, total DNA yield, GCN, and PLFA each displayed a correlation with bulk soil TC and TN (Fig. S1). With the exception of PLFA, the relationships increased in significance with the removal of organic soils (data not shown); PLFA retained similar correlations. Tthe samples were composited during field sampling, and therefore the values and the relationships shown here lack estimates of uncertainty.

3.2 Relationships between microbial analyses

3.2a. CFE, GCN, and DNA yield

A positive relationship was found between CFE and total GCN (Fig. 1a) and between CFE and total DNA yield (Fig. 1d). These relationships explained more variance when organic soils (Gelisols & Histosols) were excluded (Fig. 1b, e). Table 4 provides the correlation statistics and the equations for the positive correlations observed; complete information about all correlations analyzed is provided in Table S1.

CFE was correlated with bacterial GCN (Fig. S2a). CFE showed a weakly correlated positive relationship with fungal GCN (Fig. S2d). There was no relationship between CFE and archaeal GCN ($R^2 = 0.03$). Fungal and archaeal GCN displayed little to no correlation with CFE, though fungal correlations improved with both the removal of organic soils and after correcting for clay content (Fig. S2). With the exception of the correlation between CFE and archaeal GCN, all of these relationships explained more variance when organic soils were removed (Table 4).

CFE and total GCN regression residual values for mineral soils correlated positively with the clay content of soils, suggesting DNA extraction bias of clays may account for some observed variation in the model. When the experimental values for GCN are weighted to account for DNA extraction bias from the clay component of each soil, where extraction efficiency from clay is estimated at 7.3% (Yankston and Steck, 2009), relationships strengthen between CFE and total GCN. Relationships also strengthened slightly for CFE and total DNA yield after clay correction.

3.2b. CFE and PLFA

A relationship was found for CFE with both total PLFAs (Fig. 2b), but only after the removal of organic soils. Bacterial and fungal PLFAs were also correlated with CFE with all soil types included, and both relationships strengthened slightly with the removal of organic soils, but neither were substantially different compared to total PLFAs.

3.3c. PLFA, GCN, and DNA yield

A correlation was observed between total PLFAs and total GCN (Fig. 3a), and between total PLFAs and total DNA yield (Fig. 3d). These relationships strengthened after removing organic soils (Fig. 3b, e), and after correcting for the clay component of each mineral soil (Fig. 3c, f; Table 4). There were also relationships between bacterial PLFAs and bacterial GCN when comparing all soil types (Fig. S3a). The relationship was not improved by removing the organic soils (Fig. S3b) but was improved by correcting for clay content (Fig. S3c).

4 Discussion

4.1. *Compatibility of methods as microbial biomass proxies*

Our analysis demonstrated correlations between all four methods for overall soil microbial biomass carbon estimation, although the strengths of these correlations and slopes of the relationships between them vary. Our findings suggest that total and bacterial biomass calculated with PLFA indicators can serve as reliable alternatives for CFE in mineral soils. CFE is the method most frequently used in microbial soil carbon cycling models, so our findings serve to expand the potential data sources that can be used in models and we provide quantitative equations to translate amongst the different methods (Table 4). Similarly strong relationships have been observed between CFE- and PLFA-based biomass C in mineral soils (Bailey et al., 2002) and forest humus (Leckie et al., 2004), and our correlations remained significant with the inclusion of the Fairbanks, AK Gelisol samples. However, given the remaining organic soils were treated as outliers for CFE or PLFAs, these soils may not be appropriate for the proposed modeling equations. No studies have compared CFE and PLFA for Histosols nor Gelisols, and our soil sample size is too limited (n = 5) to reliably assess the relationships between these soil microbial biomass methods within highly organic contexts alone. This constrains our proposed equations for earth system models to mineral soils in more temperate and tropical regions and warrants more thorough examination of these methodological relationships in organic soils, given the outsized role of these ecosystems in soil C storage and their projected increases in C emissions with climate change through accelerated microbially-mediated decomposition (Nichols and Peteet, 2019; Frolking et al., 2011).

Fungal and archaeal GCN displayed little to no correlation with CFE methods, though fungal correlations improved with both the removal of organic soils and after correcting for clay content. The high variability in fungal GCN compared to bacterial GCN, along with the lack of correlation between fungal GCN and CFE may reflect increased variability in DNA per unit biomass of fungi relative to bacteria (Leckie et al., 2004). GCN may not fully illustrate the true soil abundance of various microbial groups for several additional reasons. First, GCN measurements are subject to additional DNA extraction biases, where bacterial and fungal groups have differing extraction efficiencies (Strickland and Rousk 2010; Fierer et al., 2005). For example, Feinstein at al. (2009) found that extraction of fungal DNA, particularly in highly organic soils, may be

inhibited due to greater protection of fungal cells that grow within small organic particles. Second, many microbes may contain multiple and variable GCN of the amplified target gene per genome, resulting in overestimation of abundance (Strickland and Rousk 2010, Lofgren et al. 2019). Lastly, the DNA content of fungi per unit biomass is both lower and more variable than that of bacteria, making DNA content a potentially less reliable measure of fungal biomass (Harris and Paul, 1994; Leckie et al., 2004).

The weakest correlations between the proxies for soil microbial biomass were observed for GCN and PLFA, and between total DNA yield and PLFA, though these relationships strengthened with the removal of organic soils and application of a clay correction factor (Fig. 3). Previous studies have demonstrated a lack of correlation between DNA yield and total PLFA biomass (Leckie et al., 2004). In part, this may be because of variability in the DNA content per cell and in GCN of rRNA genes per cell (Harris and Paul, 1994; Leckie et al., 2004; Lofgren et al., 2019). DNA extraction methods can also affect results for GCN and total DNA yield (Harrison et al., 2021; Dunbar et al., 2012). Finally, "relic DNA", i.e., DNA adsorbed from microbial necromass, can artificially increase both GCN and total DNA yield results (Carini et al. 2019). PLFA, in contrast, may be affected by overlap in PLFA markers between plants and microbes, and underestimation due to a lack of representation of archaea, which do not contain the ester-linked fatty acids PLFA procedures are designed to extract (Gattinger et al., 2003; Willers et al., 2015). Finally, there are varying ratios of fungal-specific PLFAs to total PLFAs as compared to bacterial-specific PLFAs to total PLFAs. The utility of using GCN and total DNA yields in combination with PLFA methods to improve soil biomass estimates had been demonstrated (Widmer et al., 2001), but the authors focused these studies within limited soils, so did not conduct direct correlative analyses between these methods across diverse soil types, as done here.

4.2. Soil factors affecting soil microbial biomass measurements

Our results show that clay content was a significant soil factor to consider when comparing soil microbial biomass proxy methods. The relationship between extracted DNA yield; total, bacterial, and fungal GCN; and both CFE and PLFA improved where the nucleic acid-derived data were corrected for influence of clay content on extraction efficiency (Yankston and Steck, 2009). Evidence that nucleic acid extraction was

inhibited in high clay soils was observed elsewhere in this study. For example, the subsurface Ultisols and Alfisols displayed some of the highest clay percentages while also consistently displaying low DNA yields. Ultisols are typically rich in metal oxides with a clay-rich subsurface horizon, while subsurface Alfisols generally accumulate silicate clay and are often enriched in Al- and Fe-bearing minerals. Such soils pose particular challenges for consistent nucleic acid extraction because nucleic acids adsorb to reactive soil minerals, which results in lower extraction efficiencies in high clay content soils in particular (Frostegård et al., 1999; Feinstein at al., 2009). Similarly, extraction of nucleic acids from soils with iron-rich clay components is inhibited as iron and other multivalent cations sorbed to clay surfaces bind readily with nucleic acids (Hurt et al., 2014). Conversely, the possible overestimation of microbial biomass with CFE in clay-rich soils can occur due to the adsorption of excess chloroform and release of dissolved organic C during the incubation (Alessi et al., 2011). Here, the positive correlation of CFE-qPCR residuals with clay content points to the predominance of decreased DNA extraction efficiency due to adsorption of DNA onto clays. In the future, modifying procedures to account for soil characteristics (e.g. clay content) could improve our understanding of potential extraction biases that affect these relationships. For example, recent studies have shown increased DNA yield (Hurt et al., 2014; Guerra et al., 2020) and microbial diversity (Hurt et al., 2014) in clay and/or iron-rich soils following treatment with a phosphate buffer to encourage nucleic acid desorption.

This study demonstrated that soils with high organic matter may present a problem in correlating microbial methods across soil types. Generally, nucleic acid methods have been underexplored and are inconsistent predictors of soil microbial biomass, especially in highly organic soils. For example, Marstorp et al. (2000) found a strong correlation ($r = 0.96$) between CFE and total DNA yield in a loamy mineral soil, whereas Leckie et al. (2004) found no correlation between total DNA yield and CFE in forest humus characterized by high organic matter, low pH, and variable fungal to bacterial ratios. Additionally, it can be difficult to extract nucleic acids, PLFAs, or other molecules from soils containing high levels of organic matter or humic substances entrapping these biomolecules (Frostegård et al., 1999; Yankston and Steck, 2009).

Total soil C and N were both significantly correlated with all four microbial biomass estimates (Fig. S1). It is unlikely that these relationships represent true causality; rather, they indicate co-varying relationships in ecosystems that respond broadly to edaphic conditions (Bradford et al. 2014). Both C and N additions can alter microbial carbon use efficiency, microbial community composition, and thereby affect elemental stoichiometry (Feng et al., 2022; Hu et al., 2022). Conversely, microbial growth and nitrogen use efficiency can influence soil N mineralization and retention (Zhang et al., 2019a). The relationship between CFE-derived microbial biomass C and N and other microbial methods such as GCN have not been thoroughly explored, though significant relationships have recently been observed between microbial DNA yield and CFE in arid and semi-arid environments (Gong et al., 2021) and in five distinct soil types across Japan (Yokoyama et al., 2017). However, such correlations may be too simplified, as there are too many factors that affect stoichiometry in soils and in microbes (Bradford et al. 2016). Given the relatively high concentration of N in microbes, especially in bacteria and archaea, microbial biomass N measured through CFE should be included in future comprehensive soil microbial biomass comparison studies to improve the measurement of soil microbes and the parameterization of their contributions to ecosystem models, particularly given most models now include both C and N cycles.

One primary objective of this study was to compare different estimates of soil microbial biomass across a variety of soil biomes, orders, and horizons to develop equations for correlations between these different methods and enable improved representation of microbial communities in ecosystem models. Specifically, we developed equations relating the different measures of soil microbial biomass (Table 4) so that the latest generation of soil carbon cycling and earth system models will have more options for parameterizing microbial biomass and testing outcomes against a wider variety of microbial observations (e.g., He et al., 2020; Wang et al., 2019). Consequently, future modeling efforts would be able to convert among different measures of microbial biomass carbon, and thereby use a broader spectrum of available microbial data, potentially improving both the development and application of microbial soil carbon cycling models. However, we acknowledge that the widescale applicability of our proposed coefficients is constrained by limited sample sizes reducing adequate

representation of uncertainty, especially at the site level (n = 1). We recommend future studies increase site-level sample numbers to both improve representation of sampling locations (many with inherently heterogeneous soils) and uncertainty representation overall. Additionally, the relationships shown here are quite coarse—samples of opportunity were assembled together from around the world, with identification only at the biome and soil order levels. The quantitative relationships are best considered as useful (but not definitive) for model parameterization, particularly for large-scale models. Different relationships would likely emerge within a soil order or a biome, for example, as well as for finer scales (Le Noë et al. 2023). Finally, additional data is still needed to better understand if soil microbial biomass proxies can be correlated for organic soils, and the exact mechanisms affecting DNA extractions from clay-rich soils. There are limitations with DNA-based extraction methods and with PLFA for quantifying fungal biomass, due to inefficiencies in both extraction and quantification, that could potentially be improved by further study.

Data availability

The data described in this manuscript are available in Buell et al. (2024a,b,c) and Roth et al. (2024).

Acknowledgements

The authors gratefully acknowledge the contribution of soils provided by researchers from around the globe, including Yuri Zinn of Universidade Federal de Lavras in Brazil and Sindhu Jagadamma of the University of Tennessee (Lavras soils); Melissa C. Schrock of The Reckoning International and Sindhu Jagadamma (Kakamega soils); Steve Sebestyen and Randy Kolka at the U.S. Department of Agriculture (USDA) Forest Service Northern Research Station (Marcell Experimental Forest (MEF) soils); Paul Hanson of Oak Ridge National Laboratory (ORNL) who manages the U.S. Department of Energy (DOE) Spruce and Peatland Response to Changing Environments (SPRUCE) experiment located in the MEF; Jeffrey S. Dukes of the Carnegie Institution for Science and Carol Goranson of the University of Massachusetts in Boston (BACE soils); Matthew D. Wallenstein of Colorado State University (PHACE soils); Steve Allison and

Sarah Evans of the University of California at Irving (Loma Ridge soils); Julie Jastrow of the U.S. Department of Energy Argonne National Laboratory (Fermi soils); Jennifer Z. Williams of The Pennsylvania State University and Ashlee Dere of the University of Nebraska at Omaha (Critical Zone soils and Big Ridge soils); Anna M. Wagner of the U.S. Army Engineer Research and Development Center's Cold Regions Research and Engineering Laboratory (Barrow and Fairbanks soils); and Roger McCoy and Sarah Nicley of the Tennessee Department of Environment and Conservation and Brian Lester of UT-Battelle (Big Ridge soils). We also thank Carol Loopstra at the University of Minnesota Department of Soil, Water, and Climate for laboratory assistance. We also acknowledge two anonymous reviewers whose suggestions improved this manuscript. This work was partially financially supported by the U.S. DOE Office of Biological and Environmental Research through the Terrestrial Ecosystem Science Scientific Focus Area at ORNL. ORNL is managed by UT-Battelle, LLC, under contract DE-AC05-00OR22725 with the U.S. DOE. The USDA Forest Service supported the long-term research program at the MEF (FS#21-CR-11242307-051). This work was also supported by SeaGrant grant number CON000000070744-00085442.

References

Abramoff, R. Z., Guenet, B., Zhang, H., Georgiou, K., Xu, X., Viscarra Rossel, R. A., Yuan, W., & Ciais, P. (2022). Improved global-scale predictions of soil carbon stocks with Millennial Version 2. *Soil Biology and Biochemistry*, 164, 108466. https://doi.org/10.1016/j.soilbio.2021.108466

Alessi, D. S., Walsh, D. M., & Fein, J. B. (2011). Uncertainties in determining microbial biomass C using the chloroform fumigation–extraction method. *Chemical Geology*, 280(1), 58–64. https://doi.org/10.1016/j.chemgeo.2010.10.014

Badalucco, L., Gelsomino, A., Dell'Orco, S., Grego, S., & Nannipieri, P. (1992). Biochemical characterization of soil organic compounds extracted by 0.5 m K_2SO_4 before and after chloroform fumigation. *Soil Biology and Biochemistry*, 24(6), 569–578. https://doi.org/10.1016/0038-0717(92)90082-9

Badalucco, L., De Cesare, F., Grego, S., Landi, L., & Nannipieri, P. (1997). Do physical properties of soil affect chloroform efficiency in lysing microbial biomass? *Soil Biology and Biochemistry*, 29(7), 1135–1142. https://doi.org/10.1016/S0038-0717(96)00307-0

Bai, Z., Ma, Q., Wu, X., Zhang, Y., & Yu, W. (2017). Temperature sensitivity of a

PLFA-distinguishable microbial community differs between varying and constant temperature regimes. *Geoderma*, 308, 54–59. https://doi.org/10.1016/j.geoderma.2017.08.026

Bailey, V. L., Peacock, A. D., Smith, J. L., & Bolton, Jr., H. (2002). Relationships between soil microbial biomass determined by chloroform fumigation–extraction, substrate-induced respiration, and phospholipid fatty acid analysis. *Soil Biology and Biochemistry*, 34(9), 1385–1389. https://doi.org/10.1016/S0038-0717(02)00070-6

Baldrian, P., Větrovský, T., Cajthaml, T., Dobiášová, P., Petránková, M., Šnajdr, J., & Eichlerová, I. (2013). Estimation of fungal biomass in forest litter and soil. *Fungal Ecology*, 6(1), 1–11. https://doi.org/10.1016/j.funeco.2012.10.002

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911–917. https://doi.org/10.1139/o59-099

Borneman, J. & Hartin, R. J. (2000). PCR primers that amplify fungal rRNA genes from environmental samples. *Applied and Environmental Microbiology*, 66(10), 4356–4360. https://doi.org/10.1128/aem.66.10.4356-4360.2000

Bradford, M., Warren II, R., Baldrian, P., Crowter, T. W., Maynard, D. S., Oldfield, E. E., Wieder, W. R., Wood, S. A., & King, J. R. (2014). Climate fails to predict wood decomposition at regional scales. *Nature Climate Change*, 4, 625–630. https://doi.org/10.1038/nclimate2251

Bradford, M. A., Berg, B., Maynard, D. S., Wieder, W. R. & Wood, S. A. (2016). Understanding the dominant controls on litter decomposition. *Journal of Ecology*, 104, 229–238. https://doi.org/10.1111/1365-2745.12507

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 and Biochemistry*, 65, 338–347. https://doi.org/10.1016/j.soilbio.2013.06.012

Buell, Z. W., Dabbs, J., Steinweg, J. M., Phillips, J. R., Kluber, L. A., Yang, Z. K., Miller, R. M., Gutknecht, J. L. M., Schadt, C. W. & Mayes, M. A. (2024a). Interrelationships among methods of estimating microbial biomass across multiple soil orders and biomes: Supporting data. Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/ornlsfa.031/2274949.

Buell, Z., Phillips, J., Ottinger, S., Lowe, K., Schadt, C.W., & Mayes, M.A. (2024b). Chloroform fumigation extraction for microbial biomass and dissolved organic carbon from SPRUCE, 2021-2022. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.109/1998876.

Buell, Z., Felice, M., Phillips, J., Ottinger, S., Lowe, K., & Gutknecht J.L.M. (2024c). SPRUCE Phospholipid Fatty Acid (PLFA) abundances, August 2021-June 2022. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.112/1998897.

Čapek, P., Choma, M., Kaštovská, E., Tahovská, K., Glanville, H. C., & Šantrůčková, H. (2023). Revisiting soil microbial biomass: Considering changes in composition

with growth rate. *Soil Biology and Biochemistry*, 184, 109103. https://doi.org/10.1016/j.soilbio.2023.109103

Carini, P., Marsden, P. J., Leff, J. W., Morgan, E. E., Strickland, M. S. and Fierer, N. (2016). Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. *Nature Microbiology*, 2(3), 16242. https://doi.org/10.1038/nmicrobiol.2016.242

Clark, J. S., Campbell, J. H., Grizzle, H. W., & Acosta-Martinez, V. (2009). Soil microbial community response to drought and precipitation variability in the Chihuahuan Desert. (2024). *Microbial Ecology* 57(2), 248–260. https://doi.org/10.1007/s00248-008-9475-7

De Deyn, G. B., Cornelissen, J. H. C., & Bardgett, R. D. (2008). Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters*, 11(5), 516–531. https://doi.org/10.1111/j.1461-0248.2008.01164.x

De Graaff M-A., Castro H., Classen A.T., Garten C.T. Schadt C.W. (2010). Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. *New Phytologist*, 188(4), 1055–1064. https://doi.org/10.1111/j.1469-8137.2010.03427.x

Dunbar, J., Eichorst, S.A., Gallegos-Graves, L.V., Silva, S., Xie, G., Hengartner, N.W., Evans, R.D., Hungate, B.A., Jackson, R.B., Megonigal, J.P. and Schadt, C.W. (2012). Common bacterial responses in six ecosystems exposed to 10 years of elevated atmospheric carbon dioxide. *Environmental Microbiology*, 14(5), pp.1145–1158. https://doi.org/10.1111/j.1462-2920.2011.02695.x

Dutta, H., & Dutta, A. (2016). The microbial aspect of climate change. *Energy, Ecology and Environment*, 1, 209–232. https://doi.org/10.1007/s40974-016-0034-7

Feinstein, L. M., Sul, W. J., & Blackwood, C. B. (2009). Assessment of bias associated with incomplete extraction of microbial DNA from soil. *Applied and Environmental Microbiology*, 75(16), 5428–5433. https://doi.org/10.1128/AEM.00120-09

Felice, M., Blake, C. M., Sebestyen, S., and Gutknecht, J. L. (2024). Microbial abundances and carbon use under ambient temperature or experimental warming in a southern boreal peatland. *Biogeochemistry*, 167(5), 631–650. https://doi.org/10.1007/s10533-024-01129-z

Feng, X., Qin, S., Zhang, D., Chen, P., Hu, J., Wang, G., ... & Chen, L. (2022). Nitrogen input enhances microbial carbon use efficiency by altering plant–microbe–mineral interactions. *Global Change Biology*, 28(16), 4845–4860.

Hu, J., Huang, C., Zhou, S., & Kuzyakov, Y. (2022). Nitrogen addition to soil affects microbial carbon use efficiency: Meta-analysis of similarities and differences in 13C and 18O approaches. *Global Change Biology*, 28, 4977–4988.

Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biology and Biochemistry*, 35(1), 167–176. https://doi.org/10.1016/S0038-0717(02)00251-1

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. https://doi.org/10.1128/AEM.71.7.4117-4120.2005

667 Fierer, N., Bradford, M. A., & Jackson, R. B. (2007). Toward an ecological classification
668 of soil bacteria: New directions in microbial ecology. *Ecology*, 88(6), 1354–1364.
669 https://doi.org/10.1890/05-1839
670 Fierer, N., Strickland, M. S., Liptzin, D., Bradford, M. A., & Cleveland, C. C. (2009).
671 Global patterns in belowground communities. *Ecology Letters*, 12(11), 1238–
672 1249. https://doi.org/10.1111/j.1461-0248.2009.01360.x
673 Fierer, N., Wood, S. A., & Bueno de Mesquita, C. P. (2021). How microbes can, and
674 cannot, be used to assess soil health. *Soil Biology and Biochemistry*, 153, 108111.
675 https://doi.org/10.1016/j.soilbio.2020.108111
676 Frolking, S., Talbot, J., Jones, M.C., Treat, C.C., Kauffman, J.B., Tuittila, E.S., &
677 Roulet, N. (2011). Peatlands in the Earth's 21st century climate system.
678 Environmental Reviews, 19, 371–396. http://www.jstor.org/stable/envirevi.19.371
679 Frostegård, Å., Tunlid, A., & Bååth, E. (1993). Phospholipid fatty acid composition,
680 biomass, and activity of microbial communities from two soil types
681 experimentally exposed to different heavy metals. *Applied and Environmental*
682 *Microbiology*, 59(11), 3605–3617. https://doi.org/10.1128/aem.59.11.3605–
683 3617.1993
684 Frostegård, Å., & Bååth, E. (1996). The use of phospholipid fatty acid analysis to
685 estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, 22,
686 59–65. https://doi.org/10.1007/BF00384433
687 Frostegård, Å., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin,
688 P., Nesme, X., & Simonet, P. (1999). Quantification of bias related to the
689 extraction of DNA directly from soils. *Applied and Environmental Microbiology*,
690 65(12), 5409–5420. https://doi.org/10.1128/AEM.65.12.5409–5420.1999.
691 Gattinger, A., Günthner, A., Schloter, M. and Munch, J.C. (2003) Characterisation of
692 Archaea in soils by polar lipid analysis. *Acta Biotechnology* 23, 21–28.
693 Gee, G. W., & Or, D. (2002). Particle-size analysis. *In:* Dane, J. H. & Topp, G. C.,
694 Eds., Methods of Soil Analysis. Part 4, Physical methods. SSSA Book Series 5,
695 255–293, Madison, WI, USA. https://doi.org/10.2136/sssabookser5.4
696 Gong, H., Du, Q., Xie, S., Hu, W., Akram, M. A., Hou, Q., Dong, L., Sun, Y., Manan, A.,
697 Deng, Y., Ran, J., & Deng, J. (2021). Soil microbial DNA concentration is a
698 powerful indicator for estimating soil microbial biomass C and N across arid and
699 semi-arid regions in northern China. *Applied Soil Ecology*, 160, 103869.
700 https://doi.org/10.1016/j.apsoil.2020.103869
701 Gray, S. B., Classen, A. T., Kardol, P., Yermakov, Z., & Mille, R. M. (2011). Multiple
702 climate change factors interact to alter soil microbial community structure in an
703 old-field ecosystem. *Soil Science Society of America Journal*, 75(6), 2217–2226.
704 https://doi.org/10.2136/sssaj2011.0135
705 Guerra, V., Beule, L., Lehtsaar, E., Liao, H.-L., & Karlovsky, P. (2020). Improved
706 protocol for DNA extraction from subsoils using phosphate lysis buffer.
707 *Microorganisms*, 8(4), 532. https://doi.org/10.3390/microorganisms8040532
708 Gutknecht, J. L., Field, C. B., & Balser, T. C. (2012). Microbial communities and their
709 responses to simulated global change fluctua*te greatly over multiple years. Global*
710 *Change Biology*, 18(7), 2256-2269.
711 Harris, D. & Paul, E. A. (1994). Measurement of bacterial growth rates in soil. *Applied*
712 *Soil Ecology*, 1(4), 277–290. https://doi.org/10.1016/0929-1393(94)90005-1

Harrison, J. G., Calder, W. J., Shuman, B. & Buerkle, A. C. (2021). The quest for absolute abundance: The use of internal standards for DNA-based community ecology. *Molecular Ecology Resources*, 21(1), 30–43. https://doi.org/10.1111/1755-0998.13247

He, L., Lipson, D. A., Mazza Rodrigues, J. L., Mayes, M., Björk, R. G., Glaser, B., Thornton, P., & Xu, X. (2021). Dynamics of fungal and bacterial biomass carbon in natural ecosystems: Site-level applications of the CLM-Microbe Model. *Journal of Advances in Modeling Earth Systems*, 13(2), e2020MS002283. https://doi.org/10.1029/2020MS002283

He, L., Mazza Rodrigues, J. L., Soudzilovskaia, N. A., Barceló, M., Olsson, P. A., Song, C., Tedersoo, L., Yuan, F., Yuan, F., Lipson, D. A., & Xu, X. (2020). Global biogeography of fungal and bacterial biomass carbon in topsoil. *Soil Biology and Biochemistry*, 151, 108024. https://doi.org/10.1016/j.soilbio.2020.108024

Hurt, Jr., R. A., Robeson, II, M. S., Shakya, M., Moberly, J. G., Vishnivetskaya, T. A., Gu, B., & Elias, D. A. (2014). Improved yield of high molecular weight DNA coincides with increased microbial diversity access from iron oxide cemented sub-surface clay environments. *PloS One*, 9(7), e102826. https://doi.org/10.1371/journal.pone.0102826

Jastrow, J. D., Amonette, J. E., & Bailey, V. L. (2007). Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. *Climatic Change*, 80(1-2), 5–23. https://doi.org/10.1007/s10584-006-9178-3

Jenkinson, D. S., & Powlson, D. S. (1976). The effects of biocidal treatments on metabolism in soil—I. Fumigation with chloroform. *Soil Biology & Biochemistry*, 8(3), 167–177. https://doi.org/10.1016/0038-0717(76)90001-8

Jenkinson, D. S., Brookes, P. C., & Powlson, D. S. (2004). Measuring soil microbial biomass. *Soil Biology and Biochemistry*, 36(1), 5–7. https://doi.org/10.1016/j.soilbio.2003.10.002

Jobbágy, E. G., & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, 10(2), 423–436. https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2

Joergensen, R. G., & Wichern, F. (2008). Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biology and Biochemistry*, 40(12), 2977–2991. https://doi.org/10.1016/j.soilbio.2008.08.017

Khan, K. S., Mack, R., Castillo, X., Kaiser, M., & Joergensen, R. G. (2016). Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. *Geoderma*, 271, 115–123. https://doi.org/10.1016/j.geoderma.2016.02.019

Klamer, M., & Bååth, E. (2004). Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18: 2ω6, 9. *Soil Biology and Biochemistry*, 36(1), 57-65.

Kluber, L. A., Johnston, E. R., Allen, S. A., Hendershot, J. N., Hanson, P. J. and Schadt, C. W. (2020). Constraints on microbial communities, decomposition and methane production in deep peat deposits. *PLoS One*, 15(2), e0223744. https://doi.org/10.1371/journal.pone.0223744

Koskimies, K., & Simola, L. K. (1980). The fatty acid composition of some *Sphagnum* species. *Canadian Journal of Botany*, 58(2), 259–263. https://doi.org/10.1139/b80-026

Landeweert, R., Veenman, C., Kuyper, T. W., Fritze, H., Wernars, K., & Smit, E. (2003). Quantification of ectomycorrhizal mycelium in soil by real-time PCR compared to conventional quantification techniques. *FEMS Microbiology Ecology*, 45(3), 283–292. https://doi.org/10.1016/S0168-64960300163-6

Lane, D. (1991). 16S/23S rRNA Sequencing. *In:* Stakenbrandt, E., & Goodfellow, M., Eds. Nucleic Acid Techniques in Bacterial Systematics. John Wiley and Sons, Ltd., New York. pp. 115–175.

Lawrence, C. R., Neff, J. C., & Schimel, J. P. (2009). Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. *Soil Biology and Biochemistry*, 41(9), 1923–1934. https://doi.org/10.1016/j.soilbio.2009.06.016

Le Noë, J., Manzoni, S., Abramoff, R. *et al.* (2023). Soil organic carbon models need independent time-series validation for reliable prediction. *Communications in Earth & Environment*, 4, 158. https://doi.org/10.1038/s43247-023-00830-5

Leckie, S. E., Prescott, C. E., Grayston, S. J., Neufeld, J. D., & Mohn, W. W. (2004). Comparison of chloroform fumigation-extraction, phospholipid fatty acid, and DNA methods to determine microbial biomass in forest humus. *Soil Biology and Biochemistry*, 36(3), 529–532. https://doi.org/10.1016/j.soilbio.2003.10.014

Lekberg, Y., Bååth, E., Frostegård, Å., Hammer, E., Hedlund, K., Jansa, J., Kaiser, C., Ramsey, P. W., Řezanka, T., Rousk, J., Wallander, H., Welc, M., & Olsson, P. A. (2022). Fatty acid 16:1ω5 as a proxy for arbuscular mycorrhizal fungal biomass: current challenges and ways forward. *Biology and Fertility of Soils*, 58, 835–842. https://doi.org/10.1007/s00374-022-01670-9

Lofgren, L. A., Uehling, J. K., Branco, S., Bruns, T. D., Martin, F. & Kennedy, P. G. (2019). Genome-based estimates of fungal rDNA copy number variation across phylogenetic scales and ecological lifestyles. *Molecular Ecology*, 28(4), 721–730. https://doi.org/10.1111/mec.14995

Manzoni, S., Schaeffer, S. M., Katul, G., Porporato, A., & Schimel, J. P. (2014). A theoretical analysis of microbial eco-physiological and diffusion limitations to carbon cycling in drying soils. *Soil Biology and Biochemistry*, 73, 69–83. https://doi.org/10.1016/j.soilbio.2014.02.008

Manzoni, S., Piñeiro, G., Jackson, R. B., Jobbágy, E. G., Kim, J. H., & Porporato, A. (2012). Analytical models of soil and litter decomposition: Solutions for mass loss and time-dependent decay rates. *Soil Biology and Biochemistry*, 50, 66–76. https://doi.org/10.1016/j.soilbio.2012.02.029

Marstorp, H., Guan, X., & Gong, P. (2000). Relationship between dsDNA, chloroform labile C and ergosterol in soils of different organic matter contents and pH. *Soil Biology and Biochemistry*, 32(6), 879–882. https://doi.org/10.1016/S0038-0717(99)00210-2

Muyzer, G., De Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and*

Environmental Microbiology, 59(3), 695–700. https://doi.org/10.1128/aem.59.3.695-700.1993

Nelson, D. W., & Sommers, L. E. (1996). Total carbon, organic carbon, and organic matter. *In:* Sparks, D.L. Page, A.L. Helmke, P.A. Loeppert, R.H. Soltanpour, P. N. Tabatabai, M. A. Johnston, C. T. Sumner M. E., Eds., Methods of Soil Analysis. Part 3, Chemical Methods. SSSA Book Series 5, 961–1010, Madison, WI, USA. https://doi.org/10.2136/sssabookser5.3

Nichols, J. E., & Peteet, D. M. (2019). Rapid expansion of northern peatlands and doubled estimate of carbon storage. *Nature Geoscience*, 12(11), 917–921. https://doi.org/10.1038/s41561-019-0454-z

Olsson, P. A., & Lekberg, Y. (2022). A critical review of the use of lipid signature molecules for the quantification of arbuscular mycorrhiza fungi. *Soil Biology and Biochemistry*, 166, 108574. https://doi.org/10.1016/j.soilbio.2022.108574

R Core Team (2024). R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.

Rakkar, M., Jungers, J. M., Sheaffer, C., Bergquist, G., Grossman, J., Li, F., & Gutknecht, J. L. (2023). Soil health improvements from using a novel perennial grain during the transition to organic production. *Agriculture, Ecosystems & Environment*, 341, 108164.

Rinklebe, J., & Langer, U. (2010). Relationship between soil microbial biomass determined by SIR and PLFA analysis in floodplain soils. *Journal of Soils and Sediments*, 10, 4-8.

Robinson, D., Hayes, A., and Couch, S. (2021) Broom: Convert Statistical Objects into Tidy Tibbles. https://cran.r-project.org/package=broom

Roth, S., Buell, Z., Kristy, B., Philips, J., Ottinger, S., Lowe, K., Mayes, M. A., & Schadt C. W. (2024). SPRUCE quantitative PCR of microbial gene copy numbers, 2021-2022. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/spruce.110/1998878.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.

Schimel, J. P., & Weintraub, M. N. (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry*, 35(4), 549–563. https://doi.org/10.1016/S0038-0717(03)00015-4

Schmidt, J., Schulz, E., Michalzik, B., Buscot, F., & Gutknecht, J. L. M. (2015). Carbon input and crop-related changes in microbial biomarker levels strongly affect the turnover and composition of soil organic carbon. *Soil Biology and Biochemistry*, 85, 39–50. https://doi.org/10.1016/j.soilbio.2015.02.024

Stahl, P. D., & Klug, M. J. (1996). Characterization and differentiation of filamentous fungi based on fatty acid composition. *Applied and Environmental Microbiology*, 62(11), 4136-4146.

Strickland, M. S., & Rousk, J. (2010). Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. *Soil Biology and Biochemistry*, 42(9), 1385–1395. https://doi.org/10.1016/j.soilbio.2010.05.007

Sulman, B. N., Oishi, A. C., Phillips, R. P., Shevliakova, E., & Pacala, S. (2014). Microbe-driven turnover offsets mineral-mediated storage of soil carbon under

elevated CO_2. *Nature Climate Change*, 4, 1099–1102. https://doi.org/10.1038/nclimate2436

Sulman, B. N., Moore, J. A. M., Abramoff, R., Averill, C., Kivlin, S., Georgiou, K., Sridhar, B., Hartman, M., Wang, G., Wieder, W. R., Bradford, M. A., Lou, Y., Mayes, M. A., Morrison, E., Riley, W. J., Salazar, A., Schimel, J. P., Tang, J., & Classen, A. T. (2018) Multiple models and experiments underscore large uncertainty in soil carbon dynamics. *Biogeochemistry*, 141, 109–123 https://link.springer.com/article/10.1007/s10533-018-0509-z

Tang, J. Y., & Riley, W. J. (2013) A total quasi-steady-state formulation of substrate uptake kinetics in complex networks and an example application to microbial litter decomposition. *Biogeosciences*, 10(12), 8329–8351. https://doi.org/10.5194/bg-10-8329-2013

Thomas, G. W. (1996). Soil pH and soil acidity. *In:* Sparks, D.L. Page, A.L. Helmke, P.A. Loeppert, R.H. Soltanpour, P. N. Tabatabai, M. A. Johnston, C. T. Sumner M. E., Eds., Methods of Soil Analysis. Part 3, Chemical Methods. SSSA Book Series 5, 475–490, Madison, WI, USA. https://doi.org/10.2136/sssabookser5.3

Todd-Brown, K. E. O., Hopkins, F. M., Kivlin, S. N. Talbot, J. M. & Allison, S. D. (2012) A framework for representing microbial decomposition in coupled climate models. *Biogeochemistry*, 109, 19–33. https://doi.org/10.1007/s10533-011-9635-6

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, 19(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Wang, G., Post, W. M., & Mayes, M. A. (2013). Parameterizing an enzyme-mediated soil organic carbon decomposition model. *Ecological Applications*, 23(1): 255–272. https://doi.org/10.1890/12-0681.1

Wang, G., Huang, W., Mayes, M. A., Liu, X., Zhang, D., Zhang, Q., Han, T., and Zhou, G. (2019) Soil moisture drives microbial controls on carbon decomposition in two subtropical forests. *Soil Biology and Biochemistry*, 130, 185–194. https://doi.org/10.1016/j.soilbio.2018.12.017

Waring, B. G. (2013). Exploring relationships between enzyme activities and leaf litter decomposition in a wet tropical forest. *Soil Biology and Biochemistry*, 64, 89–95. https://doi.org/10.1016/j.soilbio.2013.04.010

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

Wickham, H. and Bryan, J. (2019) Readxl: Read Excel Files R Package Version 1.3.1. https://CRAN.R-project.org/package=readxl

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., and Yutani, H. (2019) Welcome to the Tidyverse, *Journal of Open Source Software*, 4(43), 1686. https://doi.org/10.21105/joss.01686, 2019.

Widmer, F., Fließbach, A., Laczkó, E., Schulze-Aurich, J., & Zeyer, J. (2001). Assessing soil biological characteristics: A comparison of bulk soil community DNA-, PLFA-, and Biolog™-analyses. *Soil Biology and Biochemistry*, 33(7), 1029–1036. https://doi.org/10.1016/S0038-0717(01)00006-2

Wieder, W. R., Boehnert, J., & Bonan, G. B. (2014). Evaluating soil biogeochemistry parameterizations in Earth system models with observations. *Global Biogeochemical Cycles*, 28(3), 211–222. https://doi.org/10.1002/2013GB004665

Willers, C., Jansen van Rensburg, P. j., & Claassens, S. (2015). Microbial signature lipid biomarker analysis – an approach that is still preferred, even amid various method modifications. Journal of Applied Microbiology, 118(6), 1251–1263. https://doi.org/10.1111/jam.12798

Yankson, K. K., & Steck, T. R. (2009). Strategy for extracting DNA from clay soil and detecting a specific target sequence via selective enrichment and real-time (quantitative) PCR amplification. *Applied and Environmental Microbiology*, 75(18), 6017–6021. https://doi.org/10.1128/AEM.00211-09

Yokoyama, S., Yuri, K., Nomi, T., Komine, M., Nakamura, S., Hattori, H., & Rai, H. (2017). The high correlation between DNA and chloroform-labile N in various types of soil. *Applied Soil Ecology*, 117–118, 1–9. https://doi.org/10.1016/j.apsoil.2017.04.002

Yu, Y., Lee, C., Kim, J., Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnology and Bioengineering*, 89(6), 670–679. https://doi.org/10.1002/bit.20347

Zak, D. R., Ringelberg, D. B., Pregitzer, K. S., Randlett, D. L., White, D. C., & Curtis, P. S. (1996). Soil microbial communities beneath populus grandidentata grown under elevated atmospheric CO_2. *Ecological Applications*, 6(1), 257–262. https://doi.org/10.2307/2269568

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: A review. *Biology and Fertility of Soils*, 29(2), 111–129. https://doi.org/10.1007/s003740050533

Zhang, S., Zheng, Q., Noll, L., Hu, Y., & Wanek, W. (2019a). Environmental effects on soil microbial nitrogen use efficiency are controlled by allocation of organic nitrogen to microbial growth and regulate gross N mineralization. *Soil Biology and Biochemistry*, 135, 304-315.

Zhang, Y., Zheng, N., Wang, J., Yao, H., Qiu, Q., & Chapman, S. J. (2019b). High turnover rate of free phospholipids in soil confirms the classic hypothesis of PLFA methodology. *Soil Biology and Biochemistry*, 135, 323–330. https://doi.org/10.1016/j.soilbio.2019.05.023

Zhang, Z., Qu, Y., Li, S., Feng, K., Wang, S., Cai, W., Liang, Y., Li, H., Xu, M., Yin, H., & Deng, Y. (2017). Soil bacterial quantification approaches coupling with relative abundances reflecting the changes of taxa. *Scientific Reports*, 7, 4837. https://doi.org/10.1038/s41598-017-05260-w.

Tables

Table 1. Sampling site characteristics

Location	Abbreviation	Soil Type	Climate	MAT (°C)	MAP (cm)	Latitude-Longitude
Lavras, Brazil	BU (A/B)	Ultisol	Tropical	19.4	153.0	21°12'S 44°60'W
Lavras, Brazil	BO (A/B)	Oxisol	Tropical	19.4	153.0	21°12'S 44°60'W
Kakamega, Kenya	KA	Aridisol	Tropical	20.5	192.1	0°14'N 34°52'E
BACE[1], MA, USA	BA (A/B)	Inceptisol	Temperate	10.8	111.3	42°15'N 71°08'W
PHACE[2], WY, USA	WY (A/B)	Mollisol	Temperate	7.9	41.4	41°11′N, 104°54′W
MOFLUX[3], MO, USA	MO (A/B)	Mollisol	Temperate	13.3	117.6	38°45′N 92°11′W
Milan, TN, USA	ML (A/B)	Alfisol	Temperate	14.9	136.9	35°55'N 88°44'W
Marcell Forest, MN, USA	MF (A/B)	Alfisol	Boreal	3.5	74.4	47°36'N 93°41'W
SPRUCE[4] site, MN, USA	M (S/SS)	Histosol	Boreal	3.3	76.8	47°30'N 93°29'W
Loma Ridge, CA, USA	CA	Alfisol	Temperate	18.5	36.3	33°44'N 117°43'W
Melton Branch, TN, USA	MB (A/B)	Inceptisol	Temperate	14.9	129.3	35°54'N 84°18'W
Walker Branch, TN, USA	WB (A/B)	Ultisol	Temperate	14.9	129.3	35°57'N 84°16'W
Fermi, IL, USA	FM (A/B)	Mollisol	Temperate	10.1	95.8	41°50'N 88°13'W
Critical Zone, PA, USA	CZI (A/B)	Inceptisol	Temperate	10.1	100.6	40°40' N 77°54'W
Critical Zone, PA, USA	CZU (A/B)	Ultisol	Temperate	10.1	100.6	40°40' N 77°54'W
Fairbanks, AK, USA	FAK	Gelisol	Boreal	-1.8	31.2	64°50'N 147°39'W
Barrow, AK, USA	BAK	Gelisol	Arctic	-11.2	11.4	71°17'N 156°47'W
Big Ridge TN, USA	BR (A/B)	Inceptisol	Temperate	13.3	137.4	36°15'N 83°56W

[1]Boston Area Climate Experiment
[2]Prairie Heating and Carbon Dioxide Enrichment Experiment
[3]Missouri Ozark Forest AmeriFlux Site
[4]Spruce and Peatland Responses Under Changing Environments experiment, located in the Marcell Experimental Forest S-1 bog
[5]Fermi National Accelerator Laboratory

938
939 **Table 2. Selected physicochemical characteristics of composited soil samples at each site, by depth horizon**
940

Location	Horizon	Grav. Moisture Content	pH	Total N (µg N g^{-1} dry soil)	Total C (µg C g^{-1} dry soil)	C:N	TOC¶ (µg C g^{-1} dry soil)	EOC§ (µg C g^{-1} dry soil)	Clay content (%)
Lavras, Brazil (U)	A	0.30	5.4	1,798	25,409	14.1	18,261	43.3	21.2
	B	0.29	5.0	218	6,210	28.5	4,269	39.2	28.7
Lavras, Brazil (A)	A	0.20	4.4	3,590	54,486	15.2	46,454	31.2	30.1
	B	0.25	4.7	887	24,221	27.3	20,445	40.3	27.6
Kakamega, Kenya	A	0.03	5.6	410	7,734	18.9	6,088	16.9	13.5
BACE[1], Waltham, MA, USA	A	0.39	5.5	5,967	59,290	9.9	47,493	22.3	7.3
	B	0.38	6.0	3,574	41,510	11.7	31,229	21.7	2.2
PHACE site[2], WY, USA	A	0.04	6.8	2,259	22,726	10.1	14,500	14.5	7.2
	B	0.08	6.8	1,306	13,121	10.0	7,966	10.3	11.1
MOFLUX site[3], MO, USA	A	0.24	6.8	1,996	26,326	13.2	21,784	12.3	14.8
	B	0.20	5.0	671	8,626	12.9	6,785	16.4	22.4
Experimental farm[4], Milan, TN, USA	A	0.05	6.4	1,185	10,817	9.1	10,148	8.4	17.4
	B	0.14	5.8	373	5,035	13.5	3,849	6.7	27.5
Marcel Forest, MN, USA	A	0.27	5.6	1,617	28,307	17.5	24,099	25.6	7.3
	B	0.11	5.3	BDL*	3,371	BDL*	2,979	11.4	13.4
Loma Ridge, CA, USA	A	0.03	6.4	1,018	12,596	12.4	9,197	9.1	8.5

Melton Branch, TN, USA	A	0.30	5.5	2,406	29,326	12.2	25,611	12.6	14.5
	B	0.23	5.3	477	7,941	16.6	6,329	8.9	13.6
Walker Branch, TN, USA	A	0.18	4.7	1,043	28,289	27.1	21,393	31.0	8.5
	B	0.09	4.7	295	9,391	31.8	7,273	17.5	13.3
Fermilab[5], Batavia, IL, USA	A	0.32	7.0	4,061	7,441	9.5	35,041	9.3	26.0
	B	0.07	7.5	502	19,209	38.3	8,004	4.4	32.2
Critical Zone, PA, USA (I)	A	0.19	4.7	1,255	16,419	13.1	14,101	38.3	10.5
	B	0.16	4.6	788	13,780	17.5	10,360	28	10.6
Critical Zone, PA, USA (U)	A	0.18	4.0	875	16,557	18.9	11,665	27.4	13.1
	B	0.15	4.4	549	6,225	11.3	4,514	20.5	10.6
Fairbanks, AK, USA	Surface	0.27	4.8	2,791	59,736	20.1	43,534	47.9	8.5
	Sub-surface	0.41	4.9	2,791	59,736	20.1	31,723	47.9	9.8
Barrow, AK, USA	Surface	0.70	4.6	9,167	150,940	16.5	125,640	43.7	11.0
Big Ridge, TN, USA	A	0.35	4.1	3,373	66,599	19.7	57,121	32.2	16.0
	B	0.22	4.1	822	11,367	13.8	8,575	18.4	23.6
SPRUCE[6] site, MN, USA	Surface	8.49	3.7	1,220	49,1133	41.6	49,921	2298.7	n/a
	Sub-surface	6.84	3.9	1,964	53,650	29.0	51,849	1,987.6	n/a

941

[1]Boston Area Climate Experiment
[2]Prairie Heating and Carbon Dioxide Enrichment Experiment
[3]Missouri Ozark Forest AmeriFlux Site
[4]University of Tennessee Experimental Agricultural Farm

[5]Fermi National Accelerator Laboratory
[6]Spruce and Peatland Response Under Climatic and Environmental Change experiment, located in the Marcell Experimental Forest S-1 bog
[7]Data source: https://doi.org/10.25581/spruce.102/1878603
¶ Total organic carbon
§ Extractable organic carbon
* Below detection limit

942
943

944

Table 3. Standard DNA source information, primers, and specifications used for the qPCR protocols

	Eubacteria	**Fungi**	**Archaea**
Standard	*Echerichia coli*	*Saccharomyces cerevisiae*	*Methanococcus maripaludis* S2
Genome Size	4640000	12500000	1661137
rRNA Copy Number	7	150	3
Forward Primer	Eub338[1,2]	nuSSU1196F[3]	915F[4]
	ACT CCT ACG GGA GGC AGC AG	GGAAACTCACCAGGTCCAGA	AGGAATTGGCGGGGGAGCA
Reverse Primer	Eub518	nuSSU1536R	1059R
	ATT ACC GCG GCT GCT GG	ATTGCAATGCYCTATCCCCA	GCCATGCACCWCCTCT
Denaturation	95 °C, 30 s	95 °C, 30 s	95 °C, 30 s
Annealing	53 °C, 30 s	56 °C, 30 s	61 °C, 30 s
Elongation	72 °C, 1 min	72 °C, 1 min	72 °C, 30 s

945

1. Lane, 1991.
2. Muyzer et al., 1993.
3. Borneman and Hartin, 2000.
4. Yu et al., 2005.

Table 4. Summary of correlative relationships between soil microbial biomass measurements

CFE = chloroform fumigation extraction biomass; DNA yield = total quantity of DNA extracted, GCN = gene copy number using quantitative polymerase chain reaction; PLFA = biomass determined through phospholipid fatty acid extractions. Correlations were performed for all samples, for mineral soils only (i.e., Histosols and Gelisols are removed), or for mineral soils corrected for clay content.

	All samples	**Mineral Soils Only**	**Mineral Soil with Clay Correction**	**Equation: Mineral Soils & Clay Correction**
CFE & DNA yield	R^2 = 0.58 p-value = 7.8e-07 n = 30	R^2 = 0.74 p-value = 6.6e-09 n = 27	R^2 = 0.81 p-value = 1.2e-10 n = 27	CFE = 0.02(DNA yield) + 45.44
CFE & GCN	R^2 = 0.55 p-value = 1.1e-06 n = 32	R^2 = 0.66 p-value = 2.6e-07 n = 27	R^2 = 0.85 p-value = 5.7e-12 n = 27	CFE = 1.99e-07(GCN) + 32.02
CFE & bacterial GCN	R^2 = 0.55 p-value = 2.4e-06 n = 30	R^2 = 0.68 p-value = 1.2e-07 n = 27	R^2 = 0.86 p-value = 4.7e-12 n = 27	CFE = 4.84e-07(GCN_{bact}) + 97.35
CFE & fungal GCN	R^2 = 0.14 p-value = 0.04 n = 32	R^2 = 0.19 p-value = 0.02 n = 27	R^2 = 0.26 p-value = 0.005 n = 27	CFE = 1.85e-07(GCN_{fung}) + 52.74
CFE & PLFA	R^2 = 0.06 p-value = 0.16 n = 32	R^2 = 0.75 p-value = 1.9e-09 n = 27		CFE = 8.3(PLFA) + 75.9
CFE & bacterial PLFA	R^2 = 0.76 p-value = 3.2e-10 n = 30	R^2 = 0.76 p-value = 1.7e-09 n = 27		CFE = 16.1($PLFA_{bact}$) + 88.7
PLFA &	R^2 = 0.39	R^2 = 0.42	R^2 = 0.55	

DNA yield	p-value = 3.2e-04 n = 29	p-value = 2.9e-04 n = 27	p-value = 8.7e-07 n = 27	PLFA = 1.6e-03(DNA yield) + 8.95
PLFA & GCN	R^2 = 0.13 p-value = 0.05 n = 32	R^2 = 0.40 p-value= 4.5e-04 n = 27	R^2 = 0.63 p-value= 9.1e-07 n = 27	PLFA = 1.8e-8(GCN) + 6.6
Bacterial PLFA & bacterial GCN	R^2 = 0.42 p-value = 8.1e-05 n = 31	R^2 = 0.38 p-value = 6.1e-04 n = 27	R^2 = 0.63 p-value = 6.7e-07 n = 27	$PLFA_{bact}$ = 9.0e-09(GCN_{bact}) + 3.64

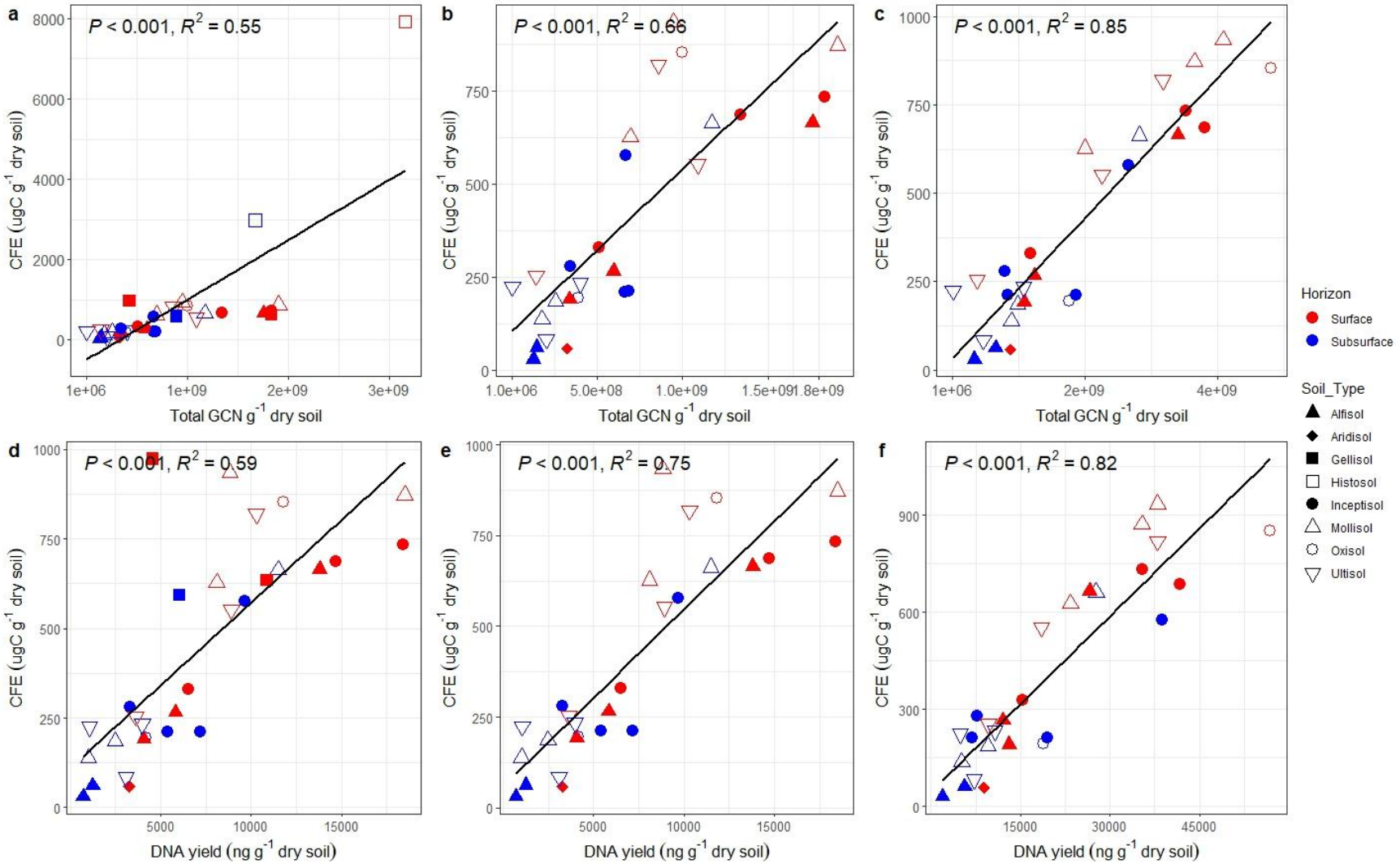
946

Figure Captions

Figure 1. Relationship between chloroform fumigation extraction (CFE) and total gene copy number (GCN) (a-c) and total DNA yield (d-f), where (a, d) show the observed relationship with organic soils included, (b, e) show mineral soils only, and (c, f) show the relationships after total GCN and DNA yield have been transformed based on clay content and an assumed clay extraction efficiency of 7.3% (Yankston and Steck, 2010). Histosols are only displayed in panel (a), as DNA yield data was not collected for these samples.

Figure 2. Relationship between soil microbial biomass proxies as measured by chloroform fumigation extraction (CFE) and total phospholipid fatty acids (PLFA). Panel (a) displays all soil samples, whereas panel (b) displays mineral soils only.

Figure 3. Relationship between total phospholipid fatty acids (PFLA) and total gene copy numbers (GCN) (a-c) and total DNA yield (d-f) where (a, d) shows the observed relationship with organic soils included, (b, e) show mineral soils only, and (c, f) show the relationships after total GCN and DNA yield have been transformed based on clay content and an assumed clay extraction efficiency of 7.3% (Yankston and Steck, 2010). Histosols are only displayed in panel (a), as DNA yield data was not collected for these samples.


a
P < 0.001, R2 = 0.55
CFE (ugC g-1 dry soil)
Total GCN g-1 dry soil
b
P < 0.001, R2 = 0.66
c
P < 0.001, R2 = 0.85
d
P < 0.001, R2 = 0.59
DNA yield (ng g-1 dry soil)
e
P < 0.001, R2 = 0.75
f
P < 0.001, R2 = 0.82
Horizon
Surface
Subsurface
Soil_Type
Alfisol
Aridisol
Gellisol
Histosol
Inceptisol
Mollisol
Oxisol
Ultisol

967
968

Figure 1. Relationship between chloroform fumigation extraction (CFE) and total gene copy number (GCN) (a-c) and total DNA yield (d-f), where (a, d) show the observed relationship with organic soils included, (b, e) show mineral soils only, and (c, f) show the relationships after total GCN and DNA yield have been transformed based on clay content and an assumed clay extraction efficiency of 7.3% (Yankston and Steck, 2010). Histosols are only displayed in panel (a), as DNA yield data was not collected for these samples.

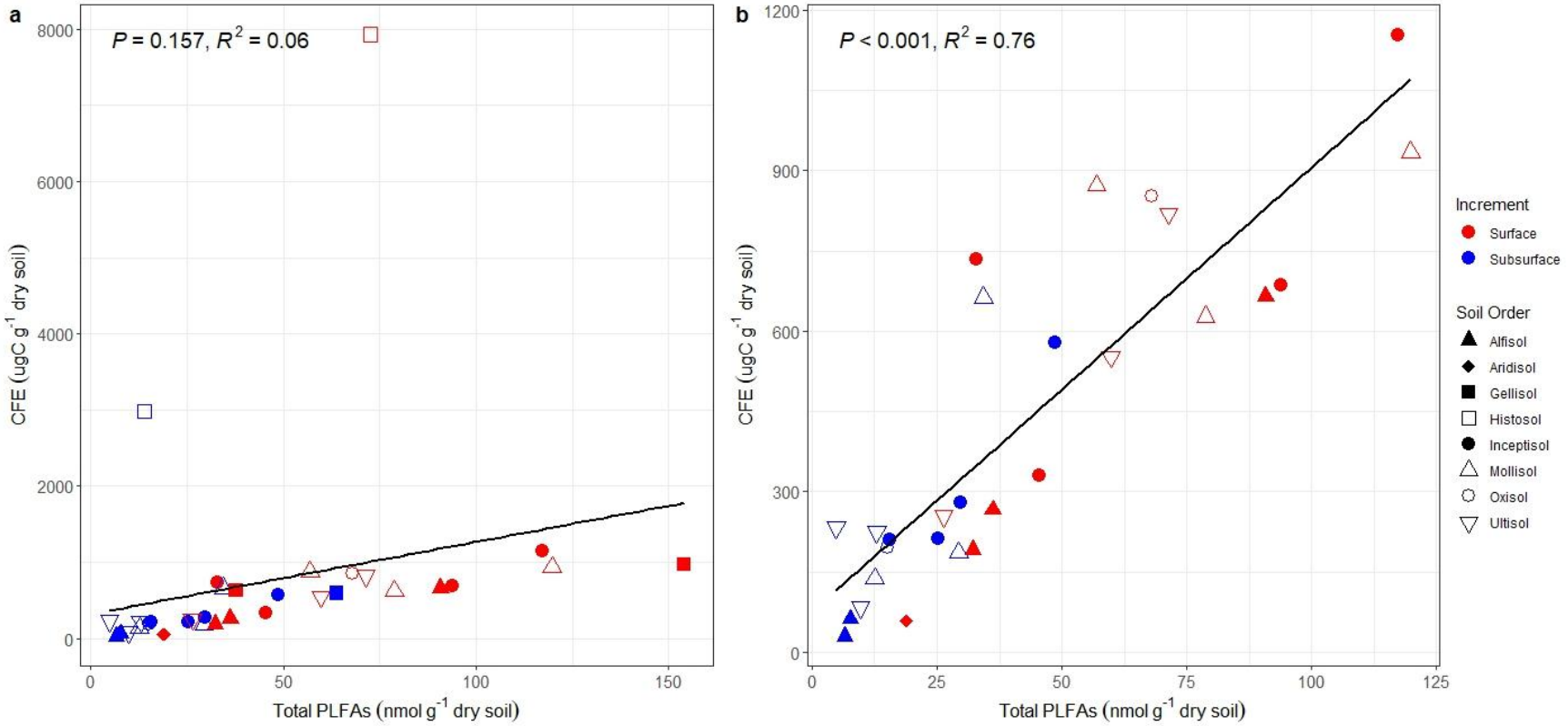
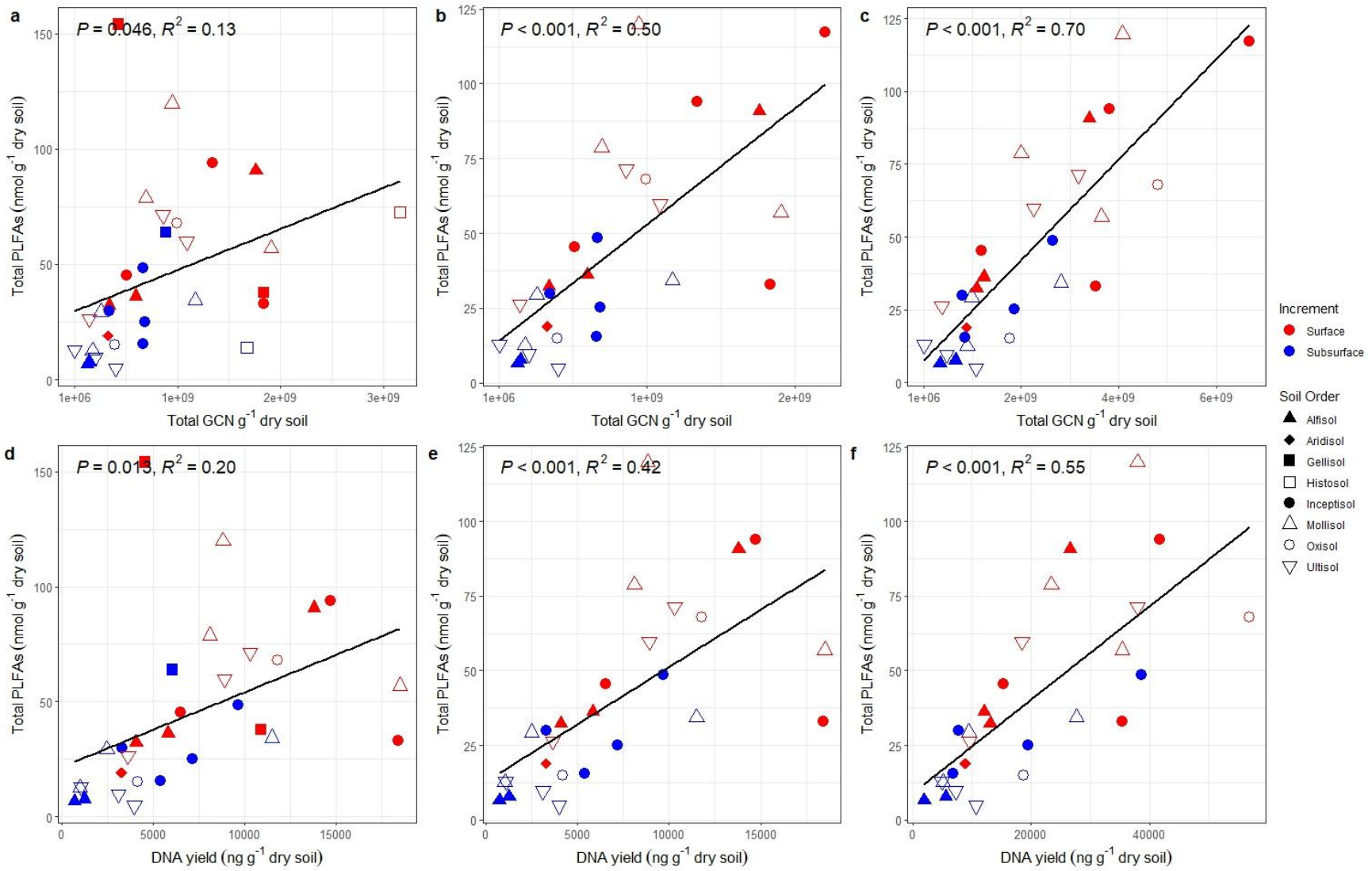


Figure 2. Relationship between soil microbial biomass proxies as measured by chloroform fumigation extraction (CFE) and total phospholipid fatty acids (PLFA). Panel (a) displays all soil samples, whereas panel (b) displays mineral soils only.


a
P = 0.046, R2 = 0.13
b
P < 0.001, R2 = 0.50
c
P < 0.001, R2 = 0.70
d
P = 0.013, R2 = 0.20
e
P < 0.001, R2 = 0.42
f
P < 0.001, R2 = 0.55
Total PLFAs (nmol g-1 dry soil)
Total GCN g-1 dry soil
DNA yield (ng g-1 dry soil)
Increment
Surface
Subsurface
Soil Order
Alfisol
Aridisol
Gellisol
Histosol
Inceptisol
Mollisol
Oxisol
Ultisol

981

Figure 3. Relationship between total phospholipid fatty acids (PFLA) and total gene copy numbers (GCN) (a-c) and total DNA yield (d-f) where (a, d) shows the observed relationship with organic soils included, (b, e) show mineral soils only, and (c, f) show the relationships after total GCN and DNA yield have been transformed based on clay content and an assumed clay extraction efficiency of 7.3% (Yankston and Steck, 2010). Histosols are only displayed in panel (a), as DNA yield data was not collected for these samples.