Title: Mini Lit Review: Phospholipid Fatty Acid (PLFA) Soil Test

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Brief

- PFLA soil test as indication of soil microbiome

Executive Summary

- Phospholipid fatty acids (PLFA) are present within the cell membranes of microorganisms, therefore the measure of PLFA in soil samples is thought to provide a non-culturable way of estimating soil microbial communities and quantifying microbial biomass.

- PLFAs degrade rapidly following cell death, therefore PLFA is thought to be indicative of only the living microbiota within soils and therefore provides a “snap shot in time” of soil microbial composition.

- Although the technique describes promising results there are certain limitations with regards to its use, such as the selection of certain PLFAs for the identification of certain microbial groups where certain groups may share the same PLFA.

What a search of the literature found

Phospholipid fatty acid (PLFA) analysis is a non-culture technique used to estimate soil microbial communities and to quantify microbial biomass (Zhang et al., 2019). The idea behind PLFA analysis is based on all living cells containing fatty acids as components of phospholipids within their cellular membranes, therefore certain PLFAs can act as a biomarkers for certain groups of microbiota, where fatty acids are thought to differ between taxa (Zelles, 1999, Lewe et al., 2021). Moreover, phospholipids are not found in dead cells or in storage products and break down rapidly within soils, therefore PLFA analysis is thought to be indicative of the living microbiota within soil samples and can therefore provide indication of a soil sample as a “snap shot in time” (Zelles, 1999, Li et al., 2020, Zhang et al., 2019, Kao-Kniffin and Zhu, 2013).

<u>Extraction and Analysis Process</u>

The extraction process and analysis of soil samples can be found outlined in Figure 1 as summarised by Gorka et al. (2023). Briefly, there are four steps, the first being the extraction of lipids from soil samples using a two-phase extraction process. The second step involves the fractionation of lipids to yield neutral lipids (NL), glycolipids (GL) and phospholipids (PL). The third step involves the derivatisation of the lipids into fatty acid methyl esters (FAME) such as neutral lipid fatty acids, glycolipid fatty acids and phospholipid fatty acids. The final stage involves the analysis of the samples via gas chromatography mass spectroscopy or isotope ratio mass spectroscopy.

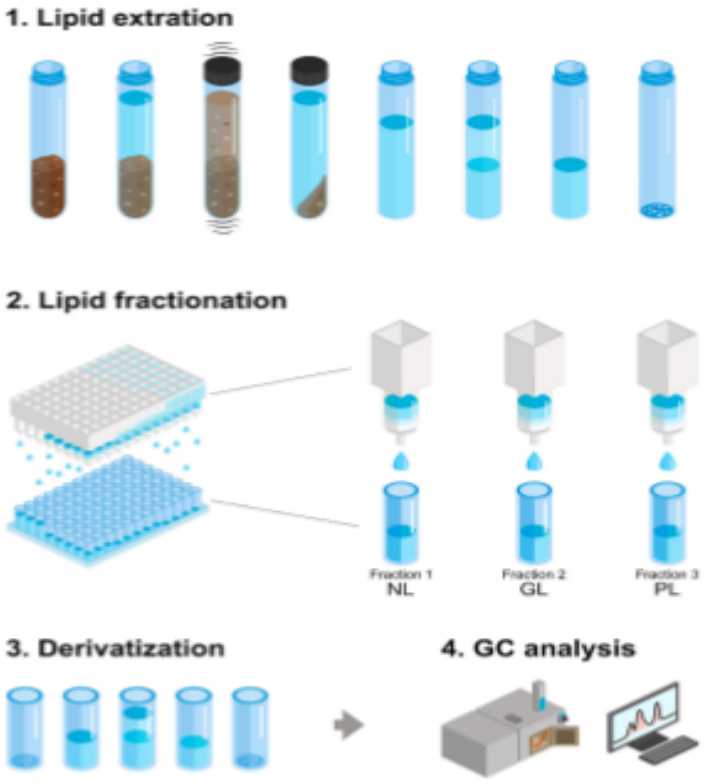


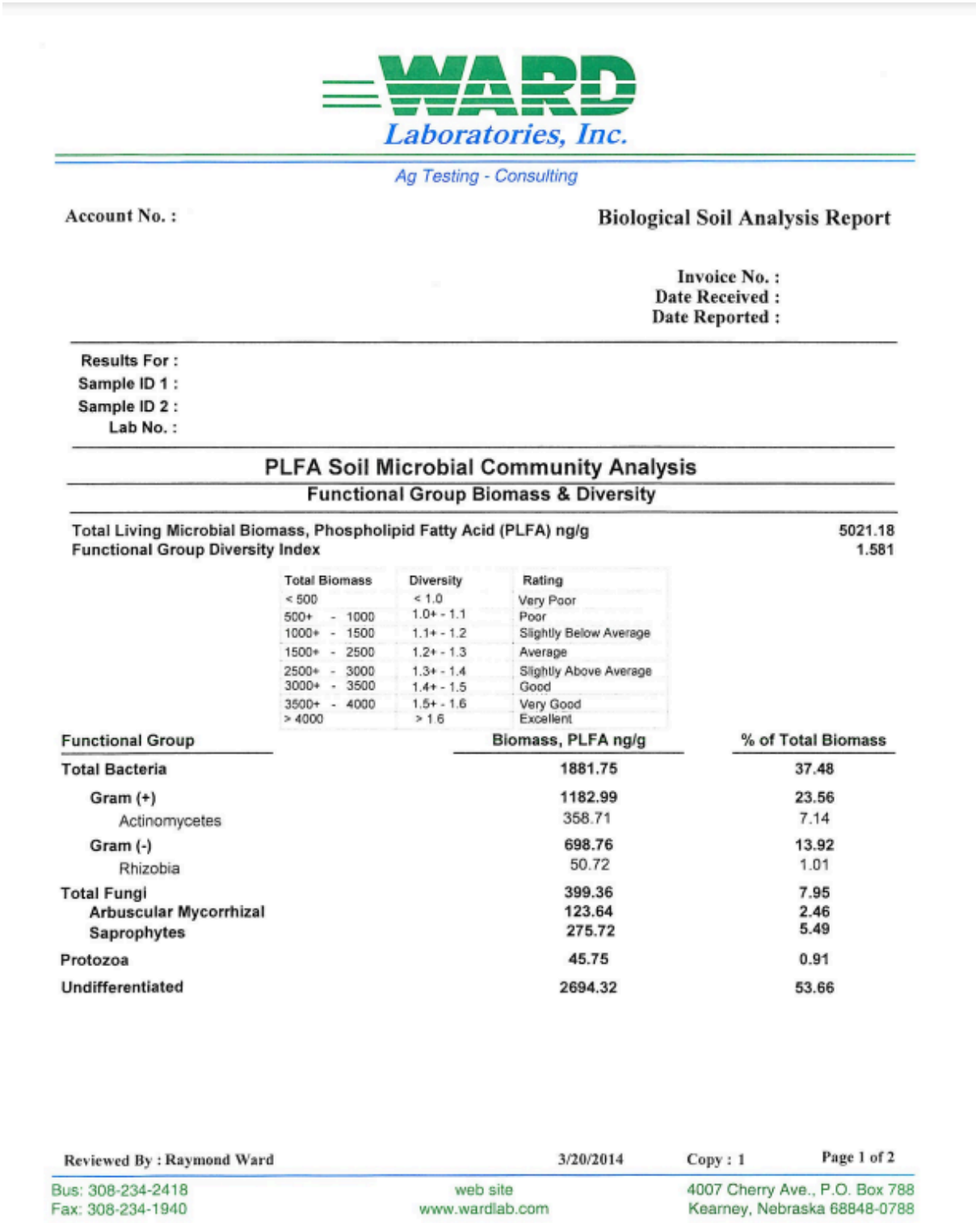
Figure 1: Graphical description of PLFA extraction and analysis from soil samples as described by ADDIN EN.CITE <EndNote><Cite><Author>Gorka</Author><Year>2023</Year><RecNum>745</RecNum><DisplayText>(Gorka et al., 2023)</DisplayText><record><rec-number>745</rec-number><foreign-keys><key app="EN" db-id="w0prsa9sfs9t0oesaewxpfzmxdsp2s0wavaf" timestamp="1720609162">745</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gorka, Stefan</author><author>Darcy, Sean</author><author>Horak, Julia</author><author>Imai, Bruna</author><author>Mohrlok,

What microorganisms are able to be detected?

From the results of a soil PLFA test, the quantification of PLFA within a sample can give estimation of the total microbial community composition and microbial biomass (Söderberg et al., 2004). This can be further broken down, where certain lipids are assigned to different genera of microorganisms based on findings within the literature, as such the lipids act as biomarkers for identification purposes. For example, certain bacteria and fungi have been demonstrated to have different fatty acid compositions in their phospholipids, therefore it is possible to distinguish between certain fungal and bacterial fatty acids and enable a ratio to be estimated (Bååth and Anderson, 2003). However, this ratio is not reflective of absolute biomass due to lack of conversion factors from PLFA to biomass, as such the ratio is commonly referred to as a biomass index (Bååth and Anderson, 2003).

From a search of companies offering PLFA analysis, it was evident that the use of the technique could help to quantify the microbial biomass of certain groups and aid the classification of microbiota within soil samples. Results of which could be portrayed as, total microbial community, gram positive

bacteria, gram negative bacteria, actinomycetes, mycorrhizal fungi and saprophytic fungi. Figure 2 displays a screen shot of a typical report from PLFA analysis from Ward lab (2024) as an example of the results you may expect from such analysis.



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Account No. :

Biological Soil Analysis Report

Invoice No. :
Date Received :
Date Reported :

Results For :
Sample ID 1 :
Sample ID 2 :
Lab No. :

PLFA Soil Microbial Community Analysis

Functional Group Biomass & Diversity

Total Living Microbial Biomass, Phospholipid Fatty Acid (PLFA) ng/g	5021.18
Functional Group Diversity Index	1.581

Total Biomass	Diversity	Rating
< 500	< 1.0	Very Poor
500+ - 1000	1.0+ - 1.1	Poor
1000+ - 1500	1.1+ - 1.2	Slightly Below Average
1500+ - 2500	1.2+ - 1.3	Average
2500+ - 3000	1.3+ - 1.4	Slightly Above Average
3000+ - 3500	1.4+ - 1.5	Good
3500+ - 4000	1.5+ - 1.6	Very Good
> 4000	> 1.6	Excellent

Functional Group	Biomass, PLFA ng/g	% of Total Biomass
Total Bacteria	1881.75	37.48
Gram (+)	1182.99	23.56
Actinomycetes	358.71	7.14
Gram (-)	698.76	13.92
Rhizobia	50.72	1.01
Total Fungi	399.36	7.95
Arbuscular Mycorrhizal	123.64	2.46
Saprophytes	275.72	5.49
Protozoa	45.75	0.91
Undifferentiated	2694.32	53.66

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Community Composition Ratios

Fungi:Bacteria 0.2122

Bacteria tend to dominate in systems with fewer organic inputs or residues possibly leading to a lower C:N ratio. In addition, bacteria can be more prominent in the early spring or late fall as soil temperatures are usually cooler and vegetation is less active or absent. Dry conditions, slightly alkaline to alkaline pH values, or increased land disturbance through prolonged and extensive tillage, grazing, or compaction may also favor bacteria. While bacteria are important and needed in the soil ecosystem, fungi are desired and more often considered indicators of good soil health. Increased use of cover crops and/or other organic inputs and less soil disturbance should help the soil support more fungi. Adjustments to pH may also be recommended in some more extreme circumstances.

Scale	Rating
< 0.05	Very Poor
0.05+ - 0.1	Poor
0.1+ - 0.15	Slightly Below Average
0.15+ - 0.2	Average
0.2+ - 0.25	Slightly Above Average
0.25+ - 0.3	Good
0.3+ - 0.35	Very Good
> 0.35	Excellent

Predator:Prey 0.0243

This ratio is also expressed as protozoa to bacteria. Protozoa feed on bacteria which helps release nutrients, especially nitrogen. A higher ratio indicates an active community where base level nutrients are sufficient to support higher trophic levels or predators. However, this ratio will always be a relatively low number because the prey will greatly outnumber the predators.

Scale	Rating
< 0.002	Very Poor
0.002+ - 0.005	Poor
0.005+ - 0.008	Slightly Below Average
0.008+ - 0.01	Average
0.01+ - 0.013	Slightly Above Average
0.013+ - 0.016	Good
0.016+ - 0.02	Very Good
> 0.02	Excellent

Gram (+):Gram (-) 1.6930

Gram (+) bacteria typically dominate early in the growing season and/or following a fallow period. They also survive better under certain environmental conditions or stressors such as drought or extreme temperatures due to their ability to form spores. Therefore, it is common to see higher values when the community is coming out of dormancy or is stressed. These values will typically begin to approach those of a more balanced bacterial community as the soil conditions become more favorable throughout the growing season. A gram (-) dominated soil may be due to anaerobic conditions or other stressors such as pesticide application or heavy metal contamination.

Scale	Rating
< 0.5	Gram (-) Dominated
0.5+ - 1.0	Slightly Gram (-) Dominated
1.0+ - 2.0	Balanced Bacterial Community
2.0+ - 3.0	Slightly Gram(+) Dominated
3.0+ - 4.0	Gram(+) Dominated
> 4.0	Very Gram(+) Dominated

Stress and Community Activity Ratios

Sat:Unsat 2.6072

Bacteria alter their membranes under various environmental conditions in order to maintain optimal fluidity for nutrient and waste transport into and out of the cell. Saturated fatty acids may reflect a better adapted community to current environmental conditions. Communities under stressed conditions will increase their proportion of unsaturated fatty acids. This will likely occur most often as a result of low soil moisture or drastic changes in temperature. In general, a higher number indicates a healthier and more stable community.

Mono:Poly 10.2902

The ratio of monounsaturated to polyunsaturated fatty acids is used along with the sat:unsat ratio to further indicate the degree of community stress. A higher ratio indicates less stress, while a lower ratio would depict higher levels of prolonged stress due to conditions such as temperature, moisture, pH, or nutrient availability (starvation).

Pre 16:1ω7c:cy17:0 7.0141

Pre 18:1ω7c:cy19:0 6.9336

Cyclo (cy) fatty acids are more prominent during stationary phases of growth or under high stress conditions that influence membrane fluidity and growth rates such as temperature, pH, moisture, and nutrient availability. In general, a higher number or all Pre16/Pre18 is better and indicates an actively growing community experiencing fewer stressors. These values are typically higher early in the growing season (planting) when the community is becoming active and experiencing fast growth. The values may begin to drop towards the end of the growing season (harvest) following a decrease in plant growth activity or as the community approaches a stationary growth phase as the temperature/moisture changes between the seasons.

All ratios should be looked at separately, but should also be taken into context and compared with one another to better understand the big picture. These are general guidelines and statements regarding soil microbial communities. In addition, the scales and ranges presented here are specific for the type of extraction and analytical methods used for PLFA analysis at Ward Laboratories, Inc. They will not necessarily reflect ranges derived from other methods of analysis or the literature. The scales can and should be adjusted slightly depending on the time of year and conditions at sampling along with the climate and soil type of specific regions where comparisons are being made. Conditions such as time of year, past and present crop, moisture, pH, and fertility should be noted or measured close to sampling for PLFA analysis for a more in depth interpretation of results.

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Limitations

There are limitations associated with PLFA analysis. A paper by Frostegård et al. (2011) discusses the use and misuse of PLFA analysis, which is here on summarised in this section, as such the reference above accounts for all information in this section. The analysis of PLFA data and comparing different PLFAs can be used to answer the question "have there been changes in the community due to a specific treatment?" (Frostegård et al., 2011). However, identifying and estimating the microbial biomass of different groups through PLFA analysis can be challenging. It was suggested in the paper that the association of PLFA biomarkers to certain groups of microbiota is often derived from pure culture studies and there are reported to be inconsistencies between studies within the literature. For example, it is often the case that different groups of microorganisms can share the same PLFAs, for example, the PLFAs cy17:00 and cy19:00 that are usually used to identify gram negative bacteria can also be seen present within certain gram-positive bacteria. Moreover, the PLFA 16:1 ω5 identified in arbuscular mycorrhizal fungi has also been identified in certain bacteria. Furthermore, it was suggested that certain PLFA used to identify microbiota groups can also be found present in plants and therefore issues may incur should samples contain plant material e.g. roots. Furthermore, certain ratios of PLFA have been used to indicate stress orstarvation, under the hypothesis that certain bacteria alter the PLFAs in their membranes in response to stress from environmental conditions. Examples of this include trans/ cis or cyclo/ mono-unsaturated precursors. Although this may be true, there is suggestion that these changes may also be a result of shifts in community structure and there is no such way of distinguishing between the two circumstances as of yet as such presenting a limitation in this area.

Conclusion

In conclusion, PLFA analysis is an alternative non-culture method for measuring community structure and quantifying microbial biomass in soil samples. The method involves the identification of groups of microbes and quantification of them as a result of certain PLFA which are found within the membranes of microbiota. PLFA rapidly degrade in soil samples and following soil death, therefore PLFA analysis is thought to be provide a "snap shot" of living microbiota in soil samples. Although the technique sounds promising there are certain limitations that should be recognized the first being that certain PLFAs used as biomarkers may be present across different groups/ taxa of microbiota thereby presenting a limitation when trying to identify just one group. Likewise, the results of the analysis can not determine absolute microbial abundance but instead provide an estimation and indication of community structure.

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