

PLFA: AN INDICATOR FOR SOIL HEALTH?



TECH MEMO 0019-#13

SOLVITA SOIL HEALTH NOTES

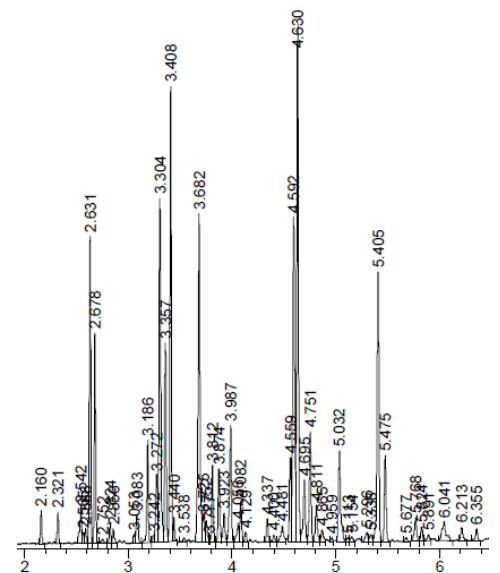
Phospholipid fatty acid, popularly called “PLFA”, is an analytical concept that emerged in the late 1980’s for estimating composition of soil microbial communities. Contrary to popular belief that it determines types of microbes in soils, PLFA reveals content of differing extractable phosphorylated-lipids known to be cell-wall constituents of microbes¹ conveniently extractable from soil with organic solvents. To interpret the test, specific lipids are assigned as “biomarkers” or signatures for certain genera of organisms, based on casual research literature corroborating the relationships.

Unfortunately, PLFA compounds are not specific to organisms and are shared across microbial communities. For example, PLFA 16:1ω5 is common in arbuscular mycorrhiza, but also found in bacteria¹. PLFAs 18:2ω6,9 and 18:1ω9, both common in fungi are also common in other organisms including plants. Some PLFA’s are good indicators but not if soil bacterial biomass is depleted. The notion that PLFA patterns change rapidly enough to use them for management trials is not well supported, and the view that ratios of PLFA’s such as trans/cis, indicate community “stress” has not been substantiated consistently². Moreover, the very popular use of PLFA to calculate ecological indexes such as Shannon diversity, Species Richness or Evenness is regarded as flawed.¹

Studies comparing PLFA to other methods of microbial community structure such as DNA and CLSU have shown good differentiation by all techniques, but all together, they fail in relative relatedness³. This means that used alone, PLFA could result in conclusions about microbial communities that are contradicted by other biology tests. It is important to note that plant roots share similar phospholipids as bacteria and fungi⁴. Soil health tests are popular to assess effects from cover crops, a practice that significantly increases fine root hair presence in soil. Moreover, high speed soil grinding common in soil labs alters soil biological properties⁵. In addition to refining roots into the sample for detection, grinding damages protected structures, resulting in a further increase of apparent PFLA⁶.

An internal study of two PLFA labs using 4 well-characterized soils from the Solvita proficiency program revealed large discrepancies in findings. PLFA biomass recovered differed by a factor of 2 between the labs. The fungi:bacteria ratios, diversity index and stress rankings were substantially different and opposite in two of the samples. One PLFA lab assigned the most optimal result to the most depleted sample of a 30-year continuous corn trial soil which had very low CO₂ respiration, low carbon and poor structure.⁷ This indicates interpretation issues.

Differences could be explained by different lab handling practices or the choice by labs of extraction solvents



PLFA analysis detects at least 115 different compounds which are inconsistently selected by labs as “markers” specific to a group or genera of microbes.

¹ Frostegård, Å., et al., 2010. Use and misuse of PLFA measurements in soils, *Soil Biology & Biochemistry* (2010).

² Fischer, J., et al., 2010. The trans/cis ratio of unsaturated fatty acids is not applicable as biomarker for environmental stress in case of long term contaminated habitats. *Applied Microbiology and Biotechnology* 87, 365-371.

³ F. Widmer et al. 2001. Assessing soil biological characteristics: a comparison of bulk soil community DNA-, PLFA-, and Biolog analyses. *Soil Biology & Biochemistry* 33:1029-1036

⁴ Harwood, JL, and NJ Russel. 1984. *Lipids in Plants and Microbes*. George Allen Press

⁵ Brinton et al. 2009. Grinding affects. SSSA Mtgs, Denver CO.

⁶ Allison V.J and M. Miller. 2005. Soil grinding increases the relative abundance of eukaryotic PLFA’s. *Soil sci. Soc. Am. J.*, vol. 69:423

⁷ Brinton, W., Vallotton, J., 2018. PLFA analysis of soil proficiency samples by two commercial labs. Woods End Lab manuscript

known to influence PLFA profiles⁸ but there is no way to know this. There have been no round-robin proficiency trials between PLFA labs ever to our knowledge and therefore there is no way to assign variability expectations. There is no general agreement on precise methodology nor on which biomarkers are selected as signatures. Even if all labs agreed to the same biomarkers for group identification, this could install false confidence since there is no certainty that those signatures are correct or relevant to the assessment or for that group.

These facts do not mean PLFA tests cannot yield meaningful data, particularly on long term management comparisons with samples run in the same lab. Studies comparing organic vs conventional management have shown consistent differences in PLFA-profiles particularly when specific sub-groups of markers are selected, and also when compared to CLSU^{9 10}. In several cases, however, structural changes (indicated by PLFA) are reported without evidence for corresponding functional changes (such as by microbiological utilization), which in the worst case would suggest methodological issues and in the best case indicates functional redundancy of microbes¹¹.

Nevertheless, PLFA is a chemistry stand-in for microbiology since organisms are not actually being identified and their activities are unknown. Without cross comparisons with other methods including more difficult traditional microbiology, community substrate utilization tests and overall respiration, little may be learned about soil health even when the analytical differences are significant between samples. The sense gained from much of the published work on PLFA continues to be that differences are detected but cannot be interpreted definitively. This may be why, despite decades of PLFA work, no practical application of PLFA for soils has emerged¹².

⁸ Papadopoulou, ES. et al. 2011. Extraction Significantly Influence Profile of PLFAs Extracted from Soils. *Microb Ecol* (2011) 62:704–714

⁹ Buyer J.S, L.E. Drinkwater 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. *Journal of Microbiological Methods* 30 (1997) 3–11

¹⁰ Esperschutz et al. 2007. Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations. *FEMS Microbiol Ecol* 61 (2007) 26–37

¹¹ Elfstrand et al. 2007. Soil enzyme activities, microbial community composition and function after 47 years of continuous green manuring. *Applied Soil Ecology* 35 (2007) 610–621

¹² Buyer, J 2016. personal communication