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Phospholipid Fatty Acid (PLFA) Analysis: A Robust Indicator for Soil Health?

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Opinion

Phospholipid fatty acid, commonly referred to as PLFA, is an analytical concept that emerged in the late 1980's for estimating composition of soil microbial communities [1]. While it is popular to describe the test as determining types of microbes in soils, in fact PLFA reveals the content of differing extractable phosphorylated-lipids recognized to be cell-wall constituents of microbes [2] extractable from soil with organic solvents. The advantage of PLFA is frequently characterized by contrasting it to the recognized difficulties that traditional microbiological cultural methods encounter in successful isolation and culturing of species present. PLFA is an indirect and multi-step process. After successful delineation of the range of lipids observed the analyst normally proceeds further to decide on assignments of groups of specific lipids as biomarkers or "signatures" for classes or genera of organisms. This selection process is not necessarily straight-forward and is based on accumulated evidence corroborating structural aspects of the relationships. The assignments generally include terminally branched saturated fatty acids to gram-positive bacteria, monounsaturated fatty acids to gram-negative bacteria, mid-branched saturated fatty acids for actinomycetes, and polyunsaturated fatty acids to fungi.

Unfortunately, PLFA compounds are not at all specific to organisms and are shared across microbial communities. For example, PLFA 16:1ω5 is common in arbuscular mycorrhiza, but also found in bacteria [1]. PLFAs 18:2u6,9 and 18:1u9, both common in fungi are also common in other organisms including plants. Some PLFA's are suitable 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 consistently [3]. Moreover, the very popular use of PLFA to calculate ecological indexes such as Shannon diversity, Species Richness or Evenness is regarded as certainly flawed [2].

Studies comparing PLFA to other methods of microbial community structure such as DNA and community level substrate utilization (CLSU) have shown good differentiation by all techniques, but all together, they fail in relative relatedness [4]. 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 tissue including either active roots, cover-crop residues or turned-under green manures share similar phospholipids as bacteria and fungi [5]. Soil health tests are most popular to assess effects from improved management such as cover-cropping and organic farming, practices that significantly increase fresh plant matter deposition and fine root hair presence in soil. Moreover, high speed soil grinding common in soil labs alters soil biological properties [6]. In addition to refining roots into the sample for ordinary detection, including suggesting the nutrients are soil-present, grinding damages protected soil structures, resulting in increases of apparent PFLA [7]. A recent study in our laboratory of 4 well-characterized soils processed and shipped to recognized PLFA labs revealed just how large discrepancies in findings can be. The average total PLFA biomass recovered from the soils differed by a factor of 2 between the two labs. The ratio of fungi to bacteria (TF/B), the diversity index and stress rankings were substantially different, and in two of the samples, opposite to each other. One PLFA lab assigned the most optimum result to the most depleted sample taken from a 30-year continuous corn trial soil, a Ultisol from North Carolina which also had very low CO2 respiration, low carbon and poor structure [8].

Differences such as these between labs offering a similar routine analyses indicate a complicated dilemma of methodological and interpretation issues. The differences could be explained by a particular lab handling practice or the choice of extraction solvents, known to influence PLFA profiles [9]; however, there is no way to know this precisely. There have not been any

reported round-robin proficiency trials between PLFA labs to our knowledge and therefore there is no way to assign variability expectations. There is no standardization on 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 or that particular constellation of soil-environment factors. These facts do not mean PLFA tests cannot yield meaningful data, particularly on long term management comparisons with similarly derived samples run in the same laboratory. Studies comparing organic versus conventional management have shown consistent differences in PLFA-profiles particularly when specific sub-groups of markers are selected, and also when compared to CLSU [10,11]. In several cases, however, structural changes, such as indicated by PLFA, have been reported without evidence for corresponding functional changes, such as determined by microbiological substrate utilization, which could suggest problematic methodological issues, or functional redundancy of microbes [12], or both.

PLFA is a chemistry stand-in for microbiology and organisms are not actually being identified nor their activities becoming known. 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, and certainly there are no practical applications comparable to yield-response curves associated with crop nutrients as a basis for interpreting extracted nutrients for fertilizer-rate applications. Of questionable basis is the apparent practical use of the total fungal: bacterial (TF/B) ratios from PLFA analysis to decide on the success of management practices. This appears to be the case with a curious emerging preference for fungal domination even in arable farming, undoubtedly resulting from the reported sensitivity of filamentous fungi to physical disturbances (tillage) [13].

The fact that PLFA provides challenges for general usage should not be taken to mean that other integrative forms of soil health analysis necessarily have better chances. Integrative analyses use multiple indicators of a chemical, biological and possibly physical nature, combined. For example, two popular such test modules called CASH for Cornell comprehensive assessment of soil health and HSHT for Haney soil health test have both been recently found either not to indicate differences in regional soils with large practiced management differences [14], or to be insufficiently sensitive to identify obvious within-crop soil management practices leading to large yield differences [15]. Still, it is compelling that PLFA for soils as an analysis method appears to be here to stay [16]. It may not be a contradiction that this is the case on the basic research level and at the same time, despite decades of PLFA work, no practical application of PLFA for soils has yet presented..

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