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Use of Sherlock™ X for Automated Characterization of Phospholipid Fatty Acids in Soil Samples by GC-MS

Application Note – Soil Microbial Community Analysis | PLFA

Abstract

The *Sherlock X* software package developed by MIDI, Inc. automatically characterizes phospholipid fatty acid (PLFA) compounds originating from the soil microbial community (soil microbiota). This specialized software replaces the manual process of compound naming currently practiced by many soil scientists. Automation saves time and generates a more complete analysis of PLFA compounds, including characterization by type of fatty acid as well as individual compound.

Introduction

Analysis of phospholipid fatty acids (PLFAs) in soil samples is an important step in determining the health and structure of the soil microbiota. The chemical extraction procedure is described in [Buyer, 2012] and yields an extract with a large variety of PLFAs, including straight-chain saturated, mono- and poly- unsaturated, 10-methyl, and cyclopropane fatty acids. Unlike microbes grown in the laboratory, soil may contain a number of conflating compounds from the background, such as hydrocarbons from vehicle exhaust or from pollution sources. The final chromatogram is typically extremely complex, often with more than twenty-five identifiable peaks (Figure 1).

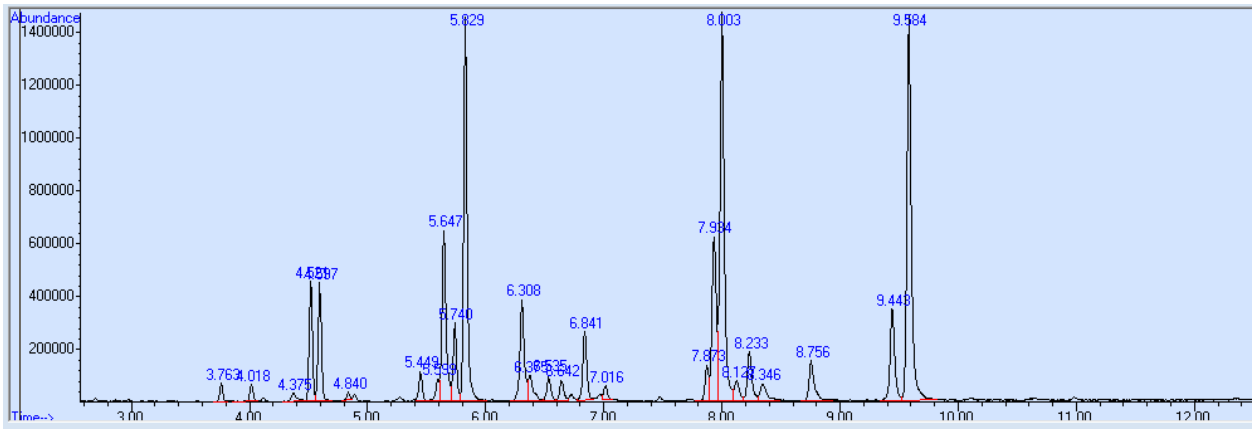


Figure 1. GCMS Total Ion Chromatogram of soil PLFA

The manual approach to analyzing a soil chromatogram is for a highly-skilled analyst to identify each major peak, which requires considering both the retention time of the peak and also the spectral match against an industry spectral library. Retention time alone is insufficient due to instrument changes and the potential for interference from background compounds, while spectral matching rarely gives a unique, well-separated identification, with the correct choice often not being the first one.

Rank	Name	Ref No.	MW	Qual
1	9-Octadecenoic acid, methyl ester, (E)-	115457	296	99
2	8-Octadecenoic acid, methyl ester	115429	296	99
3	11-Octadecenoic acid, methyl ester	115447	296	99
4	9-Octadecenoic acid, methyl ester	115431	296	99

Figure 2. Spectral match showing multiple compounds with high quality factors.

The experienced analyst develops expected retention times for the compounds of interest from samples of known composition; these retention times are then applied along with the spectral library matches and information about specific ions to manually determine peak names. Changes to the system, such as installing a new column, may require re-assigning these retention times.

Adding more complexity, not all compounds are fully resolved, yielding overlapping peaks that can be difficult to characterize. The peak in Figure 3 shows three unresolved peaks: 18:2w6c and two 18:1 isomers.

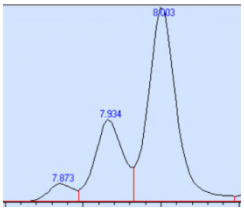


Figure 3 Peaks without baseline resolution

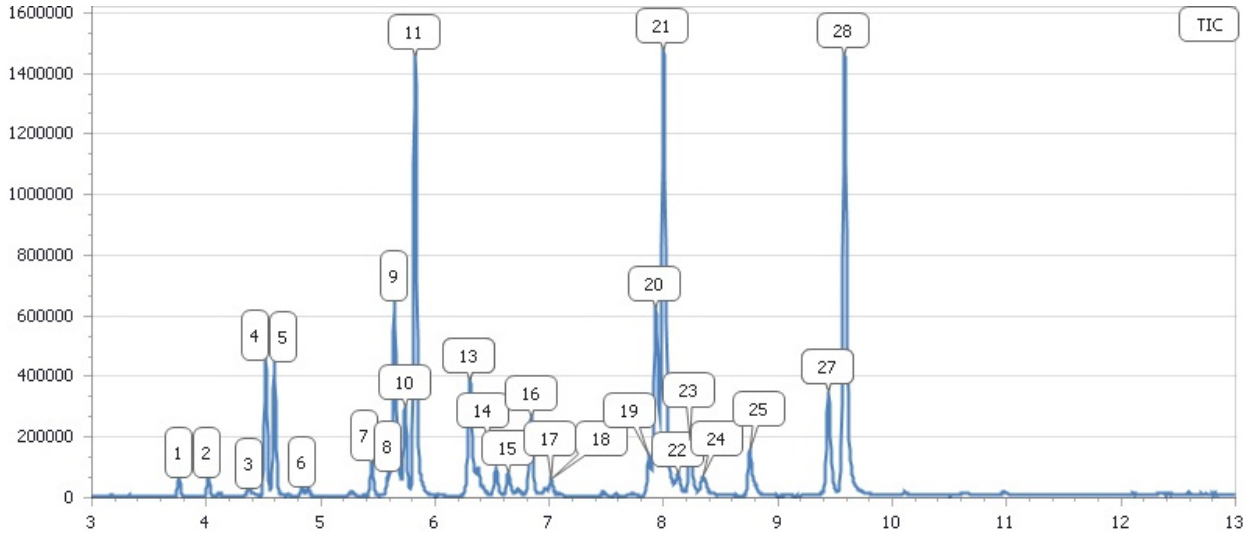
A more efficient and objective solution to this complex problem is automation of peak naming. *Sherlock X* is intelligent software that evaluates both retention time and spectral match information to identify compounds; it can determine when compounds coelute and separate the components, and it reports not just the individual named compounds but also the total abundance of compound types.

Given the individually named compounds and the relative abundances of compound types, a technician can quickly and confidently proceed with evaluating and interpreting the results.

A Sherlock X Example

The chromatogram shown in Figure 1 was run on a 7890 GC / 5977A MSD with an Agilent HP5-MS column (30m x 0.25 mm, 0.25 µm). The temperature program was as follows: starting temperature 190°C; ramp to 288°C at 5°C/minute; a final ramp of 60°C/minute to 310°C holding for 2 minutes to remove less volatile compounds.

Following the GCMS run, Sherlock X automatically characterizes the peaks, naming 28 fatty acids, and annotating the chromatogram (Figure 4).



1. 14:0 iso	11. 16:0	21. 18:1 w7c
2. 14:0	12. 17:1 iso w10c	22. 18:1 w5c
3. 15:1 iso w6c	13. 16:0 10-methyl	23. 18:0
4. 15:0 iso	14. 17:0 iso	24. 18:1 w7c 10-methyl
5. 15:0 anteiso	15. 17:0 anteiso	25. 18:0 10-methyl
6. 15:0	16. 17:0 cyclo w7c	26. 19:0 cyclo w9c
7. 16:0 iso	17. 17:0	27. 19:0 cyclo w7c
8. 16:1 w9c	18. 17:1 w7c 10-methyl	28. 19:0
9. 16:1 w7c	19. 18:2 w6c	
10. 16:1 w5c	20. 18:1 w9c	

Figure 4. Chromatogram from Figure 1 automatically characterized by Sherlock X

Precise Peak Naming by ECL

The Sherlock X system names peaks precisely by using not just the spectral information but also the retention time information converted into Equivalent Carbon Length (ECL). At the start of each batch a calibration sample is run with known straight-chain fatty acids, from C10 through C24. The software assigns these fatty acids ECL values 10.000 through 24.000 respectively. Peaks that fall between these known ECLs are assigned ECLs by interpolation.

In this manner, *Sherlock X* is able to name compounds for instruments at different sites with different columns, handling changing conditions over time.

As well as showing the annotated image, the detailed Sherlock X report includes a profile of the named peaks determined by applying the ECLs to a peak naming table developed specifically for PLFAs. A section of the profile is shown in Table 1.

Table 1: Sherlock X Profile (partial)

RT	Response	ECL	Index	Percent	Name
3.7613	1078041	13.5995	25	0.66	14:0 iso
4.0154	1004381	13.9887	32	0.62	14:0
4.3742	354664	14.4274	36	0.22	15:1 iso w6c
4.5200	7243995	14.6048	40	4.45	15:0 iso
4.5948	7383074	14.6958	42	4.53	15:0 anteiso
4.8377	351019	14.9912	50	0.22	15:0
5.4470	2064154	15.6084	59	1.27	16:0 iso
5.5965	1354957	15.7595	61	0.83	16:1 w9c
5.6451	13569059	15.8086	63	8.33	16:1 w7c
5.7385	5654039	15.9030	65	3.47	16:1 w5c
5.8282	27560158	15.9936	68	16.91	16:0
6.2842	751965	16.3942	73	0.46	17:1 iso w10c
6.3066	7883053	16.4139	74	4.84	16:0 10-methyl

In the calibration prior to the sample shown in Table 1, the C14 peak eluted at 4.8449 minutes and the C15 peak eluted at 4.0228 minutes. Thus, a peak that elutes at 4.5200 minutes is assigned an interpolated ECL of 14.6048. Based on sample runs of *Sherlock X*, the *15:0 iso* peak is known to elute with ECL 14.6100 ± 0.0200, so this peak is assigned the name *15:0 iso*.

Given the named peaks and the GCMS responses for each peak, a percent is calculated for each compound.

Category Analysis

Sherlock X also categorizes the identified compounds into user-defined groups. Table 2 shows categorized results for the sample shown in Figure 1.

Table 2: PLFA Analysis by Type

Index	Response	Percent	Name
1	75334557	35.52	Straight Chain
2	24174121	11.40	Branched Chain
3	77798578	36.68	Monounsaturates
4	2924734	1.38	Polyunsaturates
7	15838222	7.47	10-methyls
8	14807265	6.98	Cyclopropanes
11	1230704	0.58	Mixed FAs

Categorization is useful for determining, for example, the relative amount of branched chain fatty acids and monounsaturated fatty acids.

Different types of microorganisms produce different fatty acids; Gram-positive bacteria, for example, produce branch chain fatty acids, while Actinomycetes produce 10-methyl fatty acids. One can categorize the results by the type of organism generating the fatty acids found. Table 3 shows this type of categorization.

Table 3: Categorization by Microbial Type

Index	Response	Percent	Name
1	75334557	35.52	General FAME
2	41265995	19.46	Gram-positives
3	70441123	33.21	Gram-negatives
4	2924734	1.38	Fungi
5	6303550	2.97	AM Fungi
7	15838222	7.47	Actinomycetes

In this manner one can discern changes in microbial community over time or with various treatments.

Conclusion

Using *Sherlock X* to characterize PLFA compounds in a soil sample provides many benefits:

- Saves the technician time and tedium of manual analysis (~30 minutes/sample)
- Names more compounds automatically than would be done manually
- Categorizes results automatically, reducing errors
- Generates visually appealing, easily understood reports with annotated chromatograms, which is optimal for publication

References

1. **Buyer, J.S. & Sasser, M. (2012).** High throughput phospholipid fatty acid analysis of soils. *Applied Soil Ecology* **61**, 127-130.

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