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Soil Lipidomics: A LC-MS/MS based workflow with advanced data processing for biomarker discovery in soil communities

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The vital role played by soil microbial communities, including bacteria, fungi, and other microorganisms, in the provision of several essential terrestrial ecosystem services cannot be overstated. To gain a more comprehensive understanding of their multifaceted ecosystem services, it is essential to identify and examine the functional role of these microbial communities and of the pathways by which they facilitate soil organic matter and plant necromass decomposition and nutrient cycling. The determination of microbial community composition in soils, and extending this to the complex decomposer soil food web, has remained a significant challenge. To address this challenge, various techniques such as 16S and 18S rRNA gene sequencing and phospholipid fatty acid (PLFA)-based biomarker analysis have been applied. While PLFA analysis has been used to characterize these communities for over three decades, recent advancements in liquid chromatography (LC) and high-resolution mass spectrometry (HRMS) now enable comprehensive analysis of the soil lipidome based on intact polar lipids, providing greater though unknown opportunities to discover biomarkers of soil microbes and other food web members. In light of this, we developed an untargeted lipidomics workflow using reverse phase liquid chromatography and electrospray ionization tandem mass spectrometry (RPLC ESI MSMS) for the analysis of lipidomes in soil and pure cultures of archaeal, bacterial, and fungal organisms, to be extended to soil fauna and plants. This workflow includes techniques for the rapid and accurate identification and quantification of lipid molecules in complex samples, utilizing internal standards, quality control strategies, Orbitrap based instrument setups, and an advanced data processing pipeline. Key features of the pipeline include compound annotation for unknowns based on SMILES generation, retention time prediction, feature-based molecular networking, as well as the relative quantification of these compounds using ionization efficiency prediction models. The developed method was capable of analyzing and identifying over 2000 unique intact polar lipid molecules from more than 12 classes in a variety of samples, covering Archaea, Gram-positive and Gram-negative bacteria, fungi, arthropods, algae and higher plants. Thus, our method can provide valuable insights into the complex and diverse soil food web by accurately identifying and quantifying a wide range of intact polar lipid molecules and further can be used for biomarker analysis and isotope tracing in soil microbial communities.