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A unified protocol for the high-throughput measurement of PLFA, NLFA, GLFA and sterols from soil

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Phospholipid fatty acids (PLFA) are widely used as biomarkers for soil microbial biomass. In more recent years, neutral lipid fatty acids (NLFA) have additionally been used as storage biomarkers. Both lipid classes are usually separated via silica solid phase extraction (SPE) after extraction with a mixture of chloroform, methanol and citric acid buffer. However, in recent years several studies reported incomplete or inconsistent separation of lipid classes, depending on minor differences in the polarity of the eluents used during the SPE. Moreover, while PLFA profiles have been tested on microbial pure cultures, the taxonomic specificity of NLFA is only assumed to equal that of PLFA.

Complementary to fatty acid based biomarkers, many studies quantify ergosterol as a reliable indicator for fungal biomass because the fungal-specific PLFA 18:1ω9 and 18:2ω6,9 also occur in plants, which compromises their use for detecting fungal biomass in plant tissue (for example mycorrhizal fungi in plant roots). Measuring ergosterol requires an additional extraction method, but existing protocols include silylation for further gas chromatography analysis and are thus not compatible with determining ^{13}C by IRMS.

Here, we aimed to quantify the recovery of polar and non-polar lipid classes as well as ergosterol following lipid extraction and silica SPE fractionation. We used pure standards of representative phospholipids, glycolipids and neutral lipids with unique fatty acid chain lengths for unambiguous identification of the lipid class after SPE. Lipid fractionation was tested on a 96-well SPE plate with different eluents. Subsequently, we applied the modified method to characterize lipid fractions in microbial pure cultures from bacteria (Proteobacteria, Firmicutes, Actinobacteria), and saprotrophic and ectomycorrhizal fungi (Ascomycota, Basidiomycota).

Separation of lipid classes was achieved by successively eluting NLFA and sterols with a mixture of chloroform and ethanol (v:v = 98:2), glycolipid fatty acids (GLFA) with acetone, and PLFA with a mixture of methanol, chloroform and water (v:v:v = 5:5:1). GLFA were partially recovered in the NLFA or PLFA fraction depending on the nature of the lipid, which should be considered when interpreting PLFA data. Ergosterol recovery was unaffected by subsequent mild alkaline methanolysis of the NLFA fraction in which it was collected, allowing further analysis of both lipid classes in the same mixture. The gas-chromatographic method may be extended to elute both NLFA and (non-silylated) sterols in one run, assuming that the concentration of ergosterol in soil samples is high enough. Therefore, the method can be optimized by using an internal standard

added to the NLFA fraction and simultaneously quantify ergosterol. Finally, we show how different lipid classes and attached fatty acid chains distribute in pure cultures of soil micro-organisms.