TECHNICAL SPECIFICATION

ISO/TS 29843-2

Second edition
2021-07

Soil quality — Determination of soil microbial diversity —

Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method

Qualité du sol — Détermination de la diversité microbienne du sol — Partie 2: Méthode par analyse des acides gras phospholipidiques (PLFA) en utilisant la méthode simple d'extraction des PLFA

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Contents

Page

Foreword

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO/TS 29843-2:2011), which has been technically revised.

The main changes compared to the previous edition are as follows:

— addition of specification for qualitative and quantitative analysis of PLFAs;

— use of BAME (qualitative) or FAME (quantitative) standards;

— use of GC-MS apparatus;

— precisions in 7.2 and 7.3;

— possibility to use commercial cartridges in addition to, or replacement of, home-made cartridges;

— update of bibliographic references.

A list of all the parts in the ISO/TS 29843 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Phospholipids are essential components of membranes of all living cells. Extracted from soil samples in fatty acid form (phospholipid fatty acids, PLFA) or ether-linked isoprenoid side chains (phospholipid ether lipid, PLEL), they provide quantitative and qualitative insights into the soil's viable/active microbial biomass. Cellular enzymes hydrolyse and release the phosphate group within minutes to hours following cell death[1]. The determination of total PLFA and PLEL provides a quantitative measure of the viable biomass of soil, i.e. microorganisms of all three primary domains of the biosphere (bacteria, archaea and microeukaryota). PLFA and PLEL can also allow for rough taxonomic differentiation within complex microbial communities[2],[3]. Each microbial species contains several fatty acids, with a total composition in PLFA subject to the environmental conditions[4]. The approach is performed to evaluate biomass and shifts in microbial community composition[5], in what regards dominance of main groups of organisms[6]. Furthermore, combined with the use of isotope (^{13}C or ^{14}C) labelled substrates, the lipid methods can also be used to identify the metabolically active part of the microbial community. This approach is well established in soil ecology and serves as a phenotypic, and thus complementary, tool to genotyping approaches for determining microbial diversity. Apart from taxonomic descriptions, the PLFA technique enables the determination of physiological changes within microbial consortia. For instance, the monoenic PLFA 16:1ω7c and 18:1ω7c are increasingly converted to the cyclopropyl fatty acids cy17:0 and cy19:0 in Gram-negative bacteria in response to environmental stress[7].

Different methodologies are available for the determination of soil fatty acids. These methodologies present different levels of complexity when applied and provide different levels of resolution in the description of soil microbial communities. ISO/TS 29843-1 deals with the generally called “extended PLFA extraction method” while this document deals with the generally called “simple PLFA extraction method”[8],[9].

This document is accessible to most research and analytical laboratories involved in soil sciences. This methodology can be used for a wide range of soils. It provides a broad diversity measurement of a soil microbial community at the phenotypic level. It can be applied to biomass estimation and can be used to differentiate microbial communities among different soil samples (with the aid of an adapted statistical method). This method is especially adapted for detecting rapid changes in the soil microbial community structure. It can also be used to give a rough description of microbial groups present in soil samples (e.g. Gram-positive bacteria, actinomycetes, fungi[6]). Table 1 (adapted from Table 1 in Reference [8]), presents a comparison of the sensitivity of the “extended PLFA” versus “simple PLFA” techniques.

Table 1 — Comparison of the sensitivity of the “simple” and “extended” PLFA techniques in characterizing shifts in the composition of microbial communities

Property	PLFA (simple)	PLFA (extended)
Ability to differentiate between two communities (with the aid of multivariate statistical methods)	Yes	Yes
Applicability for biomass estimation	Yes	Yes
Ability to register all single components of an entire community structure (“fingerprint”)	No	Yes
Ability to register FAs other than EL-FAs	No	Yes
Estimation of number of FAs in soil samples	<50	200 to 400
Capacity to determine the linkage of the FAs to lipids in the molecule	Yes, EL	Yes, EL, NEL
Capacity to detect defined FAs in lower concentrations in the soil extract	No	Yes
Capacity to detect unusual FAs in the soil extract	No	Yes
Number of available signatures of FAs for defined organisms	Few	High numbers
Relationships of FAs widespread in the profile	High	Natura

Table 1 *(continued)*

Property	PLFA (simple)	PLFA (extended)
Ability to identify the organisms causing the shift in microbial community	No	Basically yes
FA fatty acid EL ester-linked NEL non-ester–linked		

This method has been derived from the one first proposed in Reference [10]. This revised method has been widely used[11] and has also been discussed and compared to the extended PLFA extraction method in peer-reviewed articles[8],[9].