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Second edition
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Soil quality — Direct extraction of soil DNA

Qualité du sol — Extraction directe de l'ADN du sol

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Foreword

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This document was prepared by Technical committee ISO/TC 190/SC 4, *Soil quality,* Subcommittee SC 4 *Biological characterization*

This second edition cancels and replaces the first edition (ISO 11063:2012), which has been technically revised. The main changes compared to the previous edition are as follows (see details in Annex A):

— the amount of soil used (1 g instead of 0,25 g dry mass equivalent);

— the nature and amount of beads (2,5 g of ceramic beads, 2 g of 0,1 mm silica beads and 4 glass beads instead of 0,5 g of 106 µm glass beads and 2 glass beads);

— the duration of the mechanical lysis (90 s instead of 30 s);

— the salt used to precipitate the proteins (potassium acetate instead of sodium acetate).

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Introduction

DNA (deoxyribonucleic acid) is an essential component of any living organism coding for enzymes responsible for any biological activities. The study of DNA sequences from DNA sources extracted from different matrices, by means of numerous molecular approaches, provides molecular markers that can be used to sharply distinguish and identify different organisms (bacteria, archaea and eucaryotes).

Up to now, most of the studies aiming to develop microbial soil quality indicators applicable to complex environments, such as soil, were biased by the unculturability of many microorganisms and the lack of sensitivity of traditional microbiological methods.[1] The recent development of numerous molecular biology methods based primarily on amplification of soil-extracted nucleic acids have provided a pertinent alternative to classical culture-based microbiological methods, providing unique insight into the composition, richness, and structure of microbial communities.[2],[3],[4],[5],[6] DNA-based approaches are now well-established in soil ecology and serve as genotypic (molecular genetic) markers for determining microbial diversity.

The results of molecular analyses of soil microbial communities and/or populations rely on two main parameters:

a) the extraction of DNA representative of the indigenous bacterial community composition;

b) PCR bias, such as the choice of primers, the concentration of amplified DNA, errors in the PCR, or even the method chosen for analysis.[4],[7],[8],[9] Recently, numerous studies have investigated new methods to improve extraction, purification, amplification, and quantification of DNA from soils[10].

The first edition (ISO 11063:2012) described the procedure used to extract DNA directly from soil samples suitable for the study of the composition of microbial community by both quantitative (q-PCR) and qualitative (A-RISA) approaches[11].

The aim of this document is to describe a new method recently reported[12] derived from the first edition procedure to analyse the diversity of soil microorganisms by next-generation sequencing of ribosomal amplicons generated by polymerase chain reactions (PCR) using soil DNA as template. This new method was shown to increase the DNA recovery and to better represents the abundance and the structure of archaeal and fungal communities as compared to the original method[12].