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A simplified rapid, low-cost and versatile DNA-based assessment of soil microbial biomass

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ABSTRACT

We set up a simple, culture independent, low-cost and high-throughput method for DNA-based quantitative assessment of soil microbial biomass using eight soils covering a wide range of physico-chemical properties. DNA was extracted with a 0.12 M, pH 8 Na_2HPO_4 buffer using bead beating; double stranded DNA (*ds*DNA) was quantified in a *crude* (not purified) extract using PicoGreen reagent. In contrast to yields obtained by using a commercial standard method (FastDNA Kit for soil, MP-Biomedicals), our yields of *ds*DNA were generally higher, most probably because any purification method for obtaining highly pure DNA for downstream analyses leads to DNA loss. These results suggest the *new method* provides more reliable quantitative data; thus it is a good environmental indicator, as an underestimation of the soil microbial biomass due to DNA loss during purification can be excluded. The ratio between microbial C (Cmic) obtained by the traditional, widely used fumigation-extraction method and *ds*DNA ranged from 12.0 to 63.5 μg Cmic per μg *ds*DNA. *Crude* DNA obtained by the new method as well as purified DNA obtained by using the commercial kit were compared in terms of quantity (fluorometry; spectrophotometry) and quality (purity indices: A_{260}/A_{280}, A_{260}/A_{230}; PCR compatibility; gel electrophoresis: molecular weight and molecular integrity). Our results suggest that the *new method* provides a high-throughput estimator of microbial biomass (expressed as μg *ds*DNA g^{-1} soil) in soils having widely different properties without the need for high-cost commercial extraction kits and/or cumbersome individual methods. Due to its simplicity, speed and low-cost, our method is capable for routine quantitative assessments of soil microbial biomass, assessable also for soil scientists with laboratories that are otherwise not equipped for molecular analyses.

1. Introduction

Soil microbial biomass acts as an important transformation agent of soil organic matter (SOM), representing both a source and sink of the most labile fraction of principal nutrients (C, N, P and S) with a key role in nutrient cycling (e.g. transforming newly deposited OM into mineral forms of carbon dioxide and ammonium or nitrate ions) (Jenkinson and Ladd, 1981; Miltner et al., 2011). The amount of living microbes in soil is large and corresponds to 2–5% of the overall SOM in temperate grassland soils (Killham, 1994). Thus, knowledge of the microbial biomass in terms of size, turnover, functional diversity and community structure is of basic concern to understand biological activity in soil.

For detailed assessment of the soil microbiome (microbial structure and composition, evenness, richness), specific microorganisms and specific functions, a holistic approach and molecular techniques based on biochemical markers are necessary (Nannipieri et al., 2003; Ramsey et al., 2006; Zelles et al., 1992). However, the impact of soil management or pollution on soil can be sensitively and effectively assessed by a simple quantification of soil microbial biomass (Moscatelli et al., 2005; Insam, 2001).

Due to a lack of suitable and standardised methods in soil microbiology, the microbial biomass was long neglected or underestimated because it was based on culture dependent microbial counts (Insam, 2001). Jenkinson (1976) revolutionised soil ecology

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studies by introducing the fumigation-incubation (FI) method, an indirect approach based on killing and lysing microbial cells in a soil sample by chloroform fumigation and measuring respiration in comparison to an unfumigated control soil; the enhanced CO_2 production in the fumigated sample was attributed to the killed and subsequently decomposed microbial biomass C. Anderson and Domsch (1978) introduced the substrate-induced respiration (SIR) method. Brookes et al. (1982, 1985) proposed the fumigation-extraction (FE) method based on the direct measurement of microbial biomass P and N after their extraction from chloroform fumigated soils. Vance et al. (1987) adopted the FE method for biomass C measurements, which has become the most frequently used standard reference technique to measure microbial biomass in soil. The FE method is still used in its original form (Insam, 2001; Nannipieri et al., 2003) or with modifications (Blagodatskaya et al., 2011). Next to the above-mentioned *physiological approaches*, soil microbial biomass can be assessed by measuring the concentration of cellular compounds. Reliable biomass indicators should be permanently present in representative concentrations in all the living parts of the SOM. In addition, biomass indicators should be accurately extractable and quantifiable by adequate methods, both directly from soil and in soil extracts (Jenkinson and Ladd, 1981). Among the biochemical markers fulfilling these criteria there are adenosintriphosphate (ATP), phospholipid fatty acids (PLFA), and double stranded deoxyribonucleic acids (*ds*DNA) (Frostegård et al., 2010).

Initially (80s), the PLFA method was categorised as technically very demanding (Frostegård and Bååth, 1996) but has now been accepted and scheduled as rapid and inexpensive way of assaying microbial biomass and composition of microbial communities in soils. The most recent molecular method for estimating the soil microbial biomass is based on the direct extraction and quantification of *ds*DNA from soil (Ascher et al., 2009a,b, 2012; Gangneux et al., 2011; Marstorp and Witter, 1999; Noe et al., 2012). Quantitative assessment of soil microbial biomass is much faster and easier when using *ds*DNA than PLFA but it usually requires multi-step time-consuming methods or expensive commercial kits.

Since soil microbial pool size has proven to be a robust and reliable indicator of soil quality, there is the need of a simple, rapid, possibly cheap and high-throughput method for its quantitative assessment.

We aimed to set up a DNA-based method which meets the above requirements so it could be used even by non-molecular microbiology oriented soil scientists. To prevent for possible artefacts, extracts of *crude* DNA (i.e., obtained by the *new method*) vs. purified DNA (using a commercial kit; *reference method*) were characterised both quantitatively (spectrophotometrically and fluorometrically), and qualitatively by agarose gel electrophoresis, purity indexes and PCR-compatibility.

2. Materials and methods

2.1. Study sites, soil sampling, physical and chemical analyses

Eight Italian soils located in the Friuli region (North-Eastern Italy), chosen to cover up a wide spectrum of physico-chemical properties, were sampled in May 2012 (Table 1). Six sub-samples were randomly collected and pooled. Samples were sieved at <2 mm and aliquots were (i) immediately analysed for microbial biomass (FE method), (ii) stored at −20 °C for DNA-based analyses, and (iii) air dried for physical and chemical analyses.

Soils were characterised for texture using pipette method; pH was determined potentiometrically in water (soil/water ratio 2.5); total nitrogen (N_{tot}) was analysed using a NA1500 CN analyzer (Carlo Erba); and organic carbon content (C_{org}) by dry combustion (Table 1).

2.2. Soil microbial biomass by fumigation-extraction procedure

Soil microbial biomass was determined using the standard reference technique of fumigation-extraction (Vance et al., 1987). Briefly, unfumigated soil samples (5 g dw) were extracted with 20 mL of 0.5 M potassium sulphate for 30 min. Fumigated samples were treated as follows: 200 μL liquid chloroform were added to the soil surface before placing them in a desiccator containing liquid chloroform in beakers, so to guarantee the efficiency of the fumigation process (Ocio and Brookes, 1990). Vacuum was applied to desiccator for 20 min to boil chloroform. After 24 h the desiccator was evacuated and soil samples were extracted as described for the unfumigated ones. Organic C was quantified in the extracts using a Shimadzu TOC-V-CSN analyser. Microbial biomass was calculated using a K_C of 0.45 and expressed as $\mu g\, C_{mic}\, g^{-1}$ soil.

2.3. DNA extraction

2.3.1. Reference-method

Total community DNA was extracted from soil by using a commercial kit (FastDNA Kit for Soil, MP-Biomedicals) as described in Ascher et al. (2009b). Here we provide a detailed description of the single steps to highlight differences and complexity compared to our *new method*, that will be described in Section 2.3.2; in addition, we aimed to provide interesting details of used reagents which are not provided by the manufacturer.

Soil samples (0.5 g dw) were transferred to 2 mL Multimix FastDNA tubes containing a lysing matrix consisting of silica and glass spheres of different diameters. To this, 122 μL of MT buffer (1% sodium dodecyl sulfate – SDS, 0.5% Teepol, and PVP40 with EDTA and Tris) were added together with 978 μL of sodium phosphate buffer (Na_2HPO_4; pH8, 0.12 M). Cells were disrupted using a FastPrep Instrument (MP-Biomedicals) at 5.5 m/s for 45 s. Following centrifugation at 15,000 × *g* for 5 min, the DNA-containing supernatant (1 mL) was transferred to a sterile tube (2 mL microcentrifuge tube). A protein precipitation solution (150 μL of 3 M potassium acetate and 4% glacial acetic acid) was added. Then, the sample was mixed end-over-end and incubated on the bench at room temperature for 5 min. After centrifugation (as above), the supernatant containing DNA was transferred to a sterile 15 mL tube and amended with DNA binding matrix (1 mL of glassmilk diluted 1:5 with 6 M guanidine isothiocyanate, MP-Biomedicals). The tubes were placed on a rotor-agitator for 15 min, to allow specific binding of DNA to the silica binding matrix in presence of high salt concentration source (chaotropic salt, guanidine isothiocyanate, MP-Biomedicals). Afterwards, the DNA-glassmilk was placed on the bench for 10 min, to settle down the glassmilk with the entrapped DNA; 700 μL of the supernatant were discarded and the DNA-glassmilk pellet was gently re-suspended in the remaining supernatant and sequentially (max. 600 μL) transferred to Catch-Tubes spiked with SpinFilters (MP-Biomedicals); after spinning (at 15,000 g for 5 min) the DNA-glassmilk pellet was recovered in the spin filter while the flow-through was discarded. Pellet was washed two times with guanidine isothiocyanate (5.5 M; 500 μL) to denature proteins (Chomczynski and Sacchi, 1987), and three times with a salt ethanol wash solution (SEWS, MP-Biomedicals; 70% ethanol and 0.1 M sodium acetate); each wash step was terminated by spinning and discarding the relative flow-through. The DNA-glassmilk pellet was then air-dried, to allow evaporation of ethanol residuals. Finally, DNA was eluted in 100 μL distilled water (DNA elution solution, DES, MP-Biomedicals); DNA elutes were collected (flow-through) by a two times centrifugation (spinning at 15,000 × *g* for 5 min).