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DNA-based determination of soil microbial biomass in alkaline and carbonaceous soils of semi-arid climate

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ABSTRACT

The applicability of DNA-based analysis of soil microbial biomass was proven under conditions when the common approaches – *chloroform fumigation-extraction* (CFE) and *substrate-induced respiration* (SIR) – are restricted. These restrictions include certain soil properties typical for arid areas (alkaline or carbonaceous soils) or limitation by sample preparation (frozen samples). To prove the suitability and correspondence of the methods, microbial biomass was determined by CFE, SIR and by DNA quantification in slightly alkaline Chernozem and strongly alkaline Calcisol of semi-arid climate under contrasting land use. Quantification of double-stranded DNA (dsDNA) revealed an excellent agreement ($R^2 = 0.96$) with SIR-based microbial biomass (SIR-C_{mic}) for soils with pH lower than 8. DNA- and CFE-based microbial biomass (CFE-C_{mic}) correlated well ($R^2 = 0.97$) for all studied soils. The conversion factors from dsDNA to SIR-C_{mic} of 5.1 and to CFE-C_{mic} of 4.4 were obtained. In alkaline soils (pH > 8), microbial biomass measured by SIR was strongly underestimated because of CO_2 retention in soil solution due to high pH and CO_3^{2-} exchange with carbonates.

Thus, the dsDNA quantification provides a simple and durable approach for microbial biomass analysis as alternative for SIR and CFE and can be successfully used in alkaline or calcareous semi-arid soils.

1. Introduction

Soil microbial biomass is a sensitive indicator of land use and management effects (Powlson et al., 1987) and consequently of soil fertility changes. Therefore, it is frequently used in several eco-physiological indexes, such as qCO_2 (microbial community respiration per biomass unit) or C_{mic}:C_{org} ratio (microbial biomass C to soil organic C). The two traditional approaches, namely chloroform fumigation-extraction (CFE) and substrate-induced respiration (SIR), are commonly used to determine microbial biomass carbon in soil. Fresh pre-incubated soil samples are required both for SIR and CFE. This reflects the high sensitivity of both approaches and microorganisms to environmental changes such as drying, freezing, mixing, or sieving.

Number of studies on geographical gradients of spatially remote soils from tundra to arid and tropical soils, as well as studies with numerous samplings within short periods, require immediate soil freezing or drying after sampling for transporting to the lab and for storage (German et al., 2012; Kandeler et al., 2006; Schmidt and Bölter, 2002). Sample storage is required due to restricted number of the samples, which can be analyzed simultaneously. Both the SIR and CFE approaches, however, are not applicable for frozen or dry soil samples due to a partial destruction of microbial cells during freezing-thawing and drying-rewetting. This calls for approaches enabling correct estimation of microbial biomass in frozen or dried soil samples.

Application of SIR and CFE is not solely limited to fresh soil samples: both approaches are also restricted by other factors. Although SIR can be determined by O_2 uptake (Dilly, 2003), the standard and more sensitive SIR version is based on CO_2 release. Therefore, the applicability of the SIR is restricted for soils with pH < 7 (Lindsay, 1979) due to the high solubility of CO_2 in alkaline soils (Blagodatskaya and Kuzyakov, 2008; Oren and Steinberger, 2008). Alkaline soils cover more than 30% of Earth's land surface generally spreading in arid and semi-arid regions. The deeper horizons of soils with slightly acid and neutral pH at the surface (e.g., Chernozems) might also be alkaline due to calcareous parent materials. Various pH-depending correction factors have been suggested to consider the CO_2 retention in alkaline soil

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solutions (Oren and Steinberger, 2008). Such correction factors relate measured CO_2 emission to the theoretical absorption of CO_2 in the solution. The correction is based on the theoretical distribution of CO_2 between the gaseous and liquid phases depending on pH (Oren and Steinberger, 2008). This makes the determination of such correction factors quite complicated, time consuming and very uncertain. Even though the theoretically calculated and experimentally derived factors may be similar, their efficiency applied to natural soil conditions needs to be proven on a broad range of samples. The exchange of dissolved HCO_3^- with the carbonates (Kuzyakov et al., 2006), common in soils with pH above 6.5, leads to unpredictable underestimation of microbial biomass.

The CFE technique is commonly applied for a wide range of soils to assess C content in microbial biomass (Brookes et al., 1985; Vance et al., 1987). The CFE reliability is strongly affected by the presence of root residues (Mueller et al., 1992), which quantity may vary depending on soil management, depth, plant development and analysis procedure (e.g. sieving) (Powlson, 1980; Joergensen, 2000). Molar concentration of extractant and soil pH affect estimation of extractable C with CFE (Haney et al., 2001). This method may not be suitable to estimate soil microbial biomass if soils have different pH, which often occurs due to management effects on soil (Haney et al., 2001).

Quantification of microbial double-stranded DNA (dsDNA) may be applied as an alternative approach to determine soil microbial biomass, when the SIR and CFE are restricted. In contrast to approaches based on indirect characteristics (respiration, *etc.*), the DNA-based approach enables evaluating microbial biomass using the content of universal cell compound. Highly sensitive fluorescent dye PicoGreen (Life Technologies) is applied for dsDNA quantitative analysis (Fornasier et al., 2014; Terrat et al., 2012). Specifically binding to dsDNA, PicoGreen fluorescence increases more than 1000-fold proportionally to the dsDNA concentrations. Therefore, PicoGreen can be used for quantification of total dsDNA yields even in the presence of co-extracted and co-purified components such as humic acids, cellular debris and organic solvent residues (Bachoon et al., 2001). A conversion factor (F_{DNA}) of μg dsDNA (g soil)$^{-1}$ to μg SIR-C_{mic} (g soil)$^{-1}$ suggested by the range of independent studies, varied in a surprisingly narrow range of 5.0 (Anderson and Martens, 2013), 5.4 (Blagodatskaya et al., 2003) and 5.6 (Lloyd-Jones and Hunter, 2001). This was very close to the literature-based F_{DNA}-factor of 6, which indicates that approximately 13% of C_{mic} stems from DNA (Joergensen and Emmerling, 2006).

Further advantage is that DNA content increases with microbial growth and can therefore be used to assess microbial growth dynamics after substrate addition to soil (Nannipieri et al., 2003; Anderson and Martens, 2013), as well as in eco-physiological indexes, metabolic quotients, and activity parameters (Blagodatskaya et al., 2003, 2014), which are important for accessing nutrient cycling and organic carbon decomposition in arid and semi-arid environments (Vishnevetsky and Steinberger, 1997). Another advantage of DNA-based approach is related to the relatively small contribution of plant dsDNA to total dsDNA in comparison with the contribution of root residues to CFE quantifications. Because of much lower DNA content in plants compared to microbial cells, the plant dsDNA never exceeded 2.6% of total dsDNA content for a wide range of soils (Gangneux et al., 2011). The DNA-based determination of microbial biomass is possible in frozen samples because freezing is a necessary step for successful soil DNA extraction enhancing DNA release from already lysed cells (Smalla et al., 1993). Finally, the use of DNA in the microbial biomass determination is very convenient when combined with soil metagenomic studies. Thus, dsDNA quantification approach has a strong potential as an alternative to CFE and SIR for measuring microbial biomass in soil.

Intensification of land use during the last decades calls for careful monitoring of soil health and quality. However, there is a lack of suitable parameters to assess “soil quality” from a microbiological point of view. This calls for new microbiological tools to evaluate the consequences of soil resource management (Anderson, 2003). These tools may be based on microbial DNA content in soils. Vegetation and agricultural practices significantly affect soil microbial biomass and microbial community structure, and consequently the conversion values to be used for converting the data obtained by SIR and CFE into microbial biomass C (Hintze et al., 1994). Agricultural soil management practices also result in a decrease of the fungal biomass, and the fungal: bacterial ratio is usually substantially lower in agricultural soils than in more natural soils (Bailey et al., 2002). The DNA content of fungi per unit biomass is lower than that of bacteria. This requires comparing DNA-based microbial biomass determination with other approaches in soils varying in pH at different depths, as well as varying in total organic C content, and being under different land-use type during a long time.

This study compares the DNA-based microbial biomass estimation with the commonly used SIR and CFE approaches and wants to demonstrate their applicability for carbonate-containing, slightly and strongly alkaline soils of semi-arid areas. Moreover, we consider the theoretical CO_2 retention correction factors and want to prove their applicability by SIR-C_{mic}:dsDNA-C_{mic} conversion ratios in a range of soils with varying pH and carbonate contents. Finally, we compare the sensitivity of DNA-based eco-physiological indice (C_{mic}:C_{org}) to land-use effects on the range of Chernozem and Calcisol soils. For this purpose, we compared two commonly used DNA extraction kits and applied that with the highest total DNA yields.

2. Material and methods

2.1. Soils and sampling sites

The samples of Haplic Chernozem and Haplic Calcisol (IUSS Working Group WRB, 2014) of natural and agricultural ecosystems were taken throughout the soil profile from long-term static field experiments located in the European part of Russia. Environmental conditions at these sites correspond to a semi-arid climate. The arable soils were annually plowed to a depth of 23 cm. Field crop rotation included cereals (wheat, barley, maize) and tuber crops (sugar beet, sunflower). Mineral fertilizers were applied at a rate of 45–60 kg NPK ha^{-1}.

Chernozem was sampled in the Russian Federal Nature Preserve “Kamennaya Step” established by V.V. Dokuchaev in 1892. “Kamennaya Step” locates in Talovsky District in the Voronezh region, in the watershed of the rivers Bitug and Khoper (51°02′ N, 40°72′ O). This region has a annual mean precipitation of 520 mm and air temperature of 6.9 °C. Soils were sampled from 0 to 70 cm, corresponding to the Ah1 or Ap, Ah2 or Ah, and AhB horizons in virgin and closely adjacent arable ecosystems.

Calcisol was sampled in the Astrakhan region (47°93′ N, 46°11′ O). This region has a annual mean precipitation of 233 mm and an air temperature of 10.5 °C. Virgin soil and arable soils were considered. Soils were sampled from 0 to 140 cm at depths corresponding to the Ah or Ap, AhBkc, Bkc, BkC, Ck1 and Ck2 horizons (Table 1).

Soil samples (about 100 g of each sample) were collected from each horizon by digging vertically from the surfaces at six locations within one horizon to take into account the soil heterogeneity. The collected samples within one horizon were then mixed and stored at −20 °C. Prior to microbial biomass analyzes, soil samples were thawed in the refrigerator, sieved (< 2 mm), and fine roots and other plant debris were carefully removed with tweezers. The sieved field-moist soil samples were pre-incubated for 72 h at 22 °C. Three analytical replicates per sample per assay were used.

2.2. Estimation of microbial biomass-C in soils

To determine microbial biomass-C by substrate-induced respiration (SIR), an original method was used (Anderson and Domsch, 1978). The concentration of added glucose solution (4 mg C g^{-1} soil) was saturating for all studied soils and was determined in preliminary