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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

**Table 1**
Basic chemical and biological characteristics of the studied soils (mean ± standard deviation). The data are shown as means (n = 3) ± SD.

| Horizon (Depth, cm) | $C_{org}$, % | $N_{tot}$, % | C/N | SIR-$C_{mic}$, μg $g^{-1}$ | pH | Carbonates, % |
|---|---|---|---|---|---|---|
| Haplic Chernozem, virgin | | | | | | |
| Ah1 (0–18) | 4.65 ± 0.25 | 0.35 ± 0.02 | 13.3 ± 1.5 | 472 ± 11 | 6.51 ± 0.12 | – |
| Ah2 (18–45) | 3.29 ± 0.27 | 0.26 ± 0.02 | 12.7 ± 2.0 | 456 ± 10 | 6.85 ± 0.10 | – |
| AhB (45–74) | 2.54 ± 0.25 | 0.21 ± 0.01 | 12.1 ± 1.8 | 232 ± 34 | 7.63 ± 0.01 | 0.1 |
| Haplic Chernozem, arable | | | | | | |
| Ap (0–23) | 3.61 ± 0.22 | 0.28 ± 0.01 | 12.9 ± 1.2 | 335 ± 59 | 7.10 ± 0.10 | – |
| Ah (23–39) | 3.42 ± 0.14 | 0.27 ± 0.01 | 12.7 ± 1.0 | 249 ± 36 | 7.64 ± 0.09 | – |
| AhB (39–69) | 2.72 ± 0.07 | 0.21 ± 0.01 | 13.0 ± 1.0 | 191 ± 35 | 7.72 ± 0.02 | 0.1 |
| Haplic Calcisol, virgin | | | | | | |
| Ah (0–17) | 0.56 ± 0.01 | 0.06 ± 0.00 | 9.3 ± 0.2 | 47 ± 7 | 8.55 ± 0.10 | – |
| Bt1 (17–30) | 0.57 ± 0.01 | 0.07 ± 0.00 | 8.1 ± 0.1 | 45 ± 17 | 8.42 ± 0.11 | – |
| Bk (30–40) | 0.53 ± 0.01 | 0.07 ± 0.00 | 7.6 ± 0.1 | 37 ± 1 | 8.55 ± 0.05 | 5.2 |
| BkcC (40–60) | 0.35 ± 0.02 | 0.04 ± 0.01 | 8.8 ± 0.5 | 24 ± 6 | 8.63 ± 0.03 | 10.9 |
| Ckv (85–100) | 0.14 ± 0.04 | 0.02 ± 0.00 | 7.0 ± 2.0 | – | 8.34 ± 0.01 | 11.3 |
| Ck (120–140) | 0.12 ± 0.05 | 0.02 ± 0.00 | 6.0 ± 2.5 | – | 9.08 ± 0.01 | 8.0 |
| Haplic Calcisol, arable | | | | | | |
| Ap1k (0–10) | 0.89 ± 0.01 | 0.08 ± 0.01 | 11.1 ± 0.2 | 124 ± 18 | 8.46 ± 0.06 | 7.7 |
| Ap2k (10–27) | 0.40 ± 0.01 | 0.05 ± 0.00 | 8.0 ± 0.1 | 91 ± 17 | 8.42 ± 0.03 | 7.9 |
| Bkc (27–45) | 0.23 ± 0.04 | 0.02 ± 0.01 | 11.5 ± 2.0 | 23 ± 3 | 8.63 ± 0.01 | 11.8 |
| BkcC (45–65) | 0.12 ± 0.02 | 0.02 ± 0.00 | 6.0 ± 1.0 | 9 ± 5 | 8.66 ± 0.05 | 9.6 |
| Ck1 (85–100) | 0.11 ± 0.01 | 0.02 ± 0.01 | 5.5 ± 0.5 | 22 ± 6 | 8.37 ± 0.01 | 8.2 |
| Ck2 (120–140) | 0.11 ± 0.01 | 0.02 ± 0.01 | 5.5 ± 0.5 | – | 8.94 ± 0.01 | 8.8 |

experiment. Soil samples with glucose were incubated (3–4 h, 22 °C), and thereafter the air probes were sampled with a syringe to analyze $CO_2$ (GC 6000 VEGA series 2, Carlo Erba instruments, UK).

Microbial biomass C was calculated according to the original equation (Anderson and Domsch, 1978) where 1 mL $CO_2$ corresponds to 40 mg $C_{mic}$:

$$C_{mic}\ (\mu g\ C\ g^{-1}\ soil) = SIR\ (\mu L\ C\text{-}CO_2\ (g\ soil \times h)^{-1}) \times 40.04 + 0.37 \quad (1)$$

The conversion factor of 40 was also used in studies considered the correlations between dsDNA and microbial biomass values (Anderson and Martens, 2013; Blagodatskaya et al., 2003, 2014).

The second approach for microbial biomass C was chloroform fumigation-extraction (CFE) (Brookes et al., 1985; Vance et al., 1987). Unfumigated moist soil samples (5 g) were extracted with 20 ml of 0.05 M $K_2SO_4$ and agitated for 1 h on an overhead shaker (40 rev $min^{-1}$). The same soil amount was fumigated with ethanol-free chloroform and then extracted in the same way. The fumigation was done in desiccators at room temperature for 24 h (Joergensen and Mueller, 1996). After 5 min centrifugation of soil suspension, the supernatant was filtered through Rotilabo-rondfilters (type 15A, Carl Roth GmbH & Co. KG). Centrifugation of soil suspension was applied to shorten a filtration time (Rousk and Jones, 2010). The organic C content of the $K_2SO_4$ extracts was measured using a multi N/C analyzer (multi N/C analyzer 2100S, Analytik Jena, Germany).

Microbial biomass C was calculated by dividing the difference between extracted C from fumigated and unfumigated soil samples, by a $k_{EC}$ factor of 0.45 (Joergensen and Mueller, 1996; Wu et al., 1990).

### 2.3. DNA kit selection

Two widely used commercial kits were used to isolate DNA from soil: the MP Biomedicals FastDNA® SPIN kit for Soil and the MO BIO PowerSoil® DNA Isolation kit. Efficiency of dsDNA extraction was verified by adding a known amount of lambda dsDNA as an external standard to the sterile (3 times autoclaved) samples, as the sterile samples did not contain any DNA after extraction. MO BIO PowerSoil® DNA Isolation kit was characterized by low DNA yields and recovery (see in Results). The DNA recovery ranged from 94% to almost 100% for all samples tested by FastDNA® SPIN kit for Soil. Since FastDNA® SPIN kit for Soil provided sufficient DNA yields with high recovery, we chose it for the further work.

### 2.4. dsDNA extraction and quantification

DNA was extracted according to the manufacturer's protocol with 0.5 g of soil using the FastDNA® SPIN kit for Soil (MP Biomedicals, Germany). Bead beating and a silica matrix were used to isolate DNA from soil. Before extraction, soils were placed in a freezer overnight to ensure higher DNA yields. Soil samples were added to lysing matrix tubes containing silica and glass spheres of different diameters and treated with sodium phosphate buffer ($Na_2HPO_4$; pH 8.0, 0.12 M) and MT buffer (1% sodium dodecyl sulfate – SDS, 0.5% Teepol, and PVP40 with EDTA and Tris). The tubes were subjected to bead beating in the FastPrep® instrument and processed by protein precipitation solution (150 μL of 3 M $CH_3COOK$ and 4% glacial acetic acid). DNA was bound to a DNA binding matrix then (1 mL of glassmilk diluted 1:5 with 6 M guanidine isothiocyanate), washed by a salt ethanol wash solution (SEWS – ultra-pure 100% ethanol and 0.1 M sodium acetate), and, finally, eluted in DNase-free water (DES). Purified DNA extracts were stored at −20 °C until analysis.

The quantity of DNA in the extract was determined by a 150-fold dilution of the extract in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). Aliquots of 0.1 ml were then transferred to 96-well microplates (Brand pureGrade, black). For labeling the dsDNA, a 200-fold dilution of the dsDNA fluorescence stain PicoGreen® (Molecular Probes, Life Technologies, Germany) was prepared in plastic containers. The dye (0.1 mL) was added to the wells with diluted DNA extract (final 300-fold dilution of the extracts) and left to react at room temperature protected from light for 2 min. Fluorescence intensity was measured with an automated fluorometric plate-reader (Wallac 1420, Perkin Elmer, Turku, Finland) of excitation 485 nm, emission 525 nm and measurement time 1.0 s. The dsDNA of bacteriophage lambda was used as a standard; samples for the standard curve were prepared in TE-buffer in the same way as the experimental samples (Blagodatskaya et al., 2014).

The conversion factor from dsDNA into microbial biomass-C ($F_{DNA}$) of 5.10 was derived as described in the Results section and was used for all samples, as consistent with the range of $F_{DNA}$ (5–6) mentioned above. Microbial biomass-C was calculated as:

$$C_{mic}\ (\mu g\ g^{-1}\ soil) = F_{DNA} \times dsDNA\ (\mu g\ g^{-1}\ soil) \quad (2)$$

### 2.5. Soil carbon, nitrogen and pH determinations

Soil moisture content was determined gravimetrically by drying soil samples for 24 h at 105 °C. $C_{org}$ was analyzed in dried samples which had been pounded with a mortar to a fine powder. $C_{org}$ and $N_{tot}$ contents were determined using a multi N/C analyzer (multi N/C analyzer 2100S, Analytik Jena, Germany). Soil samples for organic carbon measurements were pretreated with 0.5 M HCl to remove carbonates (Harris et al., 2001). Soil pH was measured in 1 M KCl with a 1:2 soil-to-solution ratio.

### 2.6. Calculations and statistics

Experimental $CO_2$ retention correction factors were calculated as the ratios of actually produced SIR-$CO_2$ effluxes to the evolved (measured) SIR-$CO_2$ reduced by $CO_2$ retention in soil solution. Actually produced SIR-$CO_2$ quantities were derived by reverse calculation based on dsDNA-$C_{mic}$ considering the fact that dsDNA-$C_{mic}$ values demonstrated a clear linear relationship with $CO_2$ evolution during substrate-induced microbial respiration (equations (1) and (2)). Theoretical pH-based $CO_2$ retention correction factors for semi-arid soils were calculated as suggested by Oren and Steinberger (2008), by regression equation $F_{cor} = 0.002 \times \exp(0.923 \times pH)$ with $R^2 = 0.91$. Calculations are based on the dry soil weight. The data are expressed as mean of three replications ± standard deviation. Statistical analyses were done using software Statistica 7.0 (StatSoft Inc., Tulsa, OK). To consider different number of horizons in Chernozem and Calcisol the Spearman's correlation coefficient was applied when the data were not equally distributed. All nonlinear regression analyses were performed using SigmaPlot 4.01(SPSS Inc, Chicago, ILL).

## 3. Results

### 3.1. Basic soil properties

The $C_{org}$ content in Chernozem (2.54–4.65%) was 2–5 times higher than in Calcisol (0.11–0.89%) (Table 1). Chernozem characterized by a neutral or slightly alkaline pH (6.51–7.72), whereas Calcisol was alkaline or strongly alkaline (8.34–9.08). Horizons of Chernozem were non-carbonaceous (0–0.1%). In contrast to Chernozem, only two upper horizons of virgin Calcisol (Ah and Bt1) didn't contain carbonates. In other horizons of Calcisol, the carbonate content varied from 5.2 to 11.8% (Table 1).

### 3.2. Comparison of microbial biomass C derived by CFE, SIR and DNA-based approaches

The dsDNA content isolated with FastDNA® SPIN kit for Soil varied from 3 to 35 μg dsDNA (g soil)$^{-1}$ in Calcisol and from 31 to 91 μg dsDNA (g soil)$^{-1}$ in Chernozem, whereas PowerSoil® DNA Isolation kit has never extracted more than 20 μg dsDNA (g soil)$^{-1}$ and was not suitable for successful quantitative DNA extraction from soils.

The SIR-$C_{mic}$ correlated very well ($R^2 = 0.96$; $p < 0.01$) with total soil dsDNA extracted with FastDNA® SPIN kit for Soil for the Chernozem (Fig. 1). From the regression equation, the conversion factor $F_{DNA}$ of 5.10 was calculated for the Chernozem. In Calcisol, the $CO_2$ retention at alkaline pH (> 8) and by low microbial biomass C resulted in an inability to obtain any $CO_2$ at deeper horizons, i.e. the $CO_2$ retention potential of the soil was higher than the $CO_2$ release by SIR. Consequently, the SIR-$C_{mic}$ was equal to 0 at the deeper horizons with pH > 8.0 (Table 1). Nevertheless, reliable dsDNA values (4.12 and 3.14 μg dsDNA (g soil)$^{-1}$ in Ckv and Ck horizons of virgin Calcisol, and 2.35 μg dsDNA (g soil)$^{-1}$ in Ck2 of arable Calcisol) were obtained also in these horizons. Due to the above-mentioned restrictions of the SIR approach under alkaline conditions and the absence of SIR in some samples, the conversion factor of 2.70 was obtained for Calcisol which

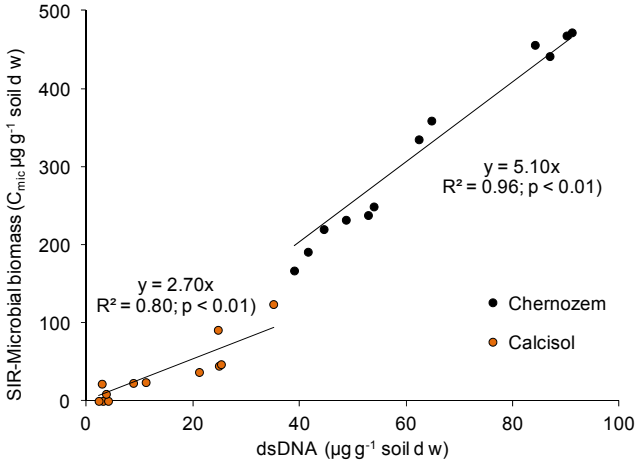


**Fig. 1.** Regressions between extractable dsDNA and microbial biomass-C (SIR-$C_{mic}$) for the studied horizons of Chernozem and Calcisol (Table 1).

reflected the underestimation of $C_{mic}$ by SIR. Thus, we were not able to combine the results for Chernozem and Calcisol in coupled dsDNA - SIR regressions (Fig. 1). Since the conversion factor $F_{DNA}$ of 5.10 calculated for Chernozem was very close to the conversion factors obtained for wide range of soils in previous studies, we applied this factor to calculate DNA-based microbial biomass C in Calcisol.

Thus, the $C_{mic}$ values determined by SIR and dsDNA were similar in neutral and slightly alkaline Chernozem (Fig. 2a). In Calcisol, however, the SIR-$C_{mic}$ was strongly underestimated (by a factor of 2–3), whereby the $CO_2$ emission declined to zero in subsoil deeper than 1 m with low total C content and a pH of about 9 (Fig. 2b).

CFE and DNA quantification approaches showed a good correspondence for both soil types (Fig. 3) with the conversion factor $F_{DNA}$ of 4.41 ($R^2 = 0.97$; $p < 0.01$) and a coefficient of variation of 19.4%. The CFE-$C_{mic}$ to DNA-$C_{mic}$ ratio was 0.87, indicating slight (about 13%) difference of microbial biomass-C obtained by CFE comparing with the DNA approach.

### 3.3. $C_{mic}$:$C_{org}$ as dependent on soil type and depth

The DNA-based $C_{mic}$ values were positively correlated with soil $C_{org}$ content for both soil types. However, an increasing $C_{org}$ content led to a higher rise of $C_{mic}$ in Calcisol than in Chernozem: 212 μg $C_{mic}$ g$^{-1}$ soil per each 1% of $C_{org}$ content for Calcisol versus 96 μg $C_{mic}$ g$^{-1}$ soil for Chernozem (data not shown).

SIR-$C_{mic}$:$C_{org}$ ratios for various depths varied in the range of 0.98–1.38% for virgin and 0.74–0.91% for arable Chernozem (Fig. 4). It was not possible to calculate the $C_{mic}$:$C_{org}$ by SIR-$C_{mic}$ for the deeper horizons of Calcisol because of the absence of SIR-$CO_2$ evolution. However, dsDNA-$C_{mic}$ solved this problem of soil alkalinity. Despite the much higher $C_{org}$ in Chernozem, the dsDNA-$C_{mic}$:$C_{org}$ ratios in Calcisol were 2–3 times higher (Fig. 4). Accordingly, Chernozem turned out to be characterized by a low proportion of microbial C in total $C_{org}$.

Agricultural land use of Chernozem reduced $C_{mic}$:$C_{org}$ at all depths due to the sharp fall of microbial biomass content (Fig. 4). In Calcisol, the land-use decreased the $C_{org}$ in the deeper horizons, but, interestingly, at the same time the $C_{org}$ and $C_{mic}$ values of the top horizon Ap1k rose. Moreover, we observed a 70% and 40% increase of the dsDNA-$C_{mic}$:$C_{org}$ ratio in the Ap2k and Bkc horizons, respectively.

### 3.4. Comparison of theoretical and experimental $CO_2$ retention correction factors

For virgin Calcisol, experimental $CO_2$ retention correction factors varied from 2.38 to 2.93 and were 1.7–6.6 times lower than the

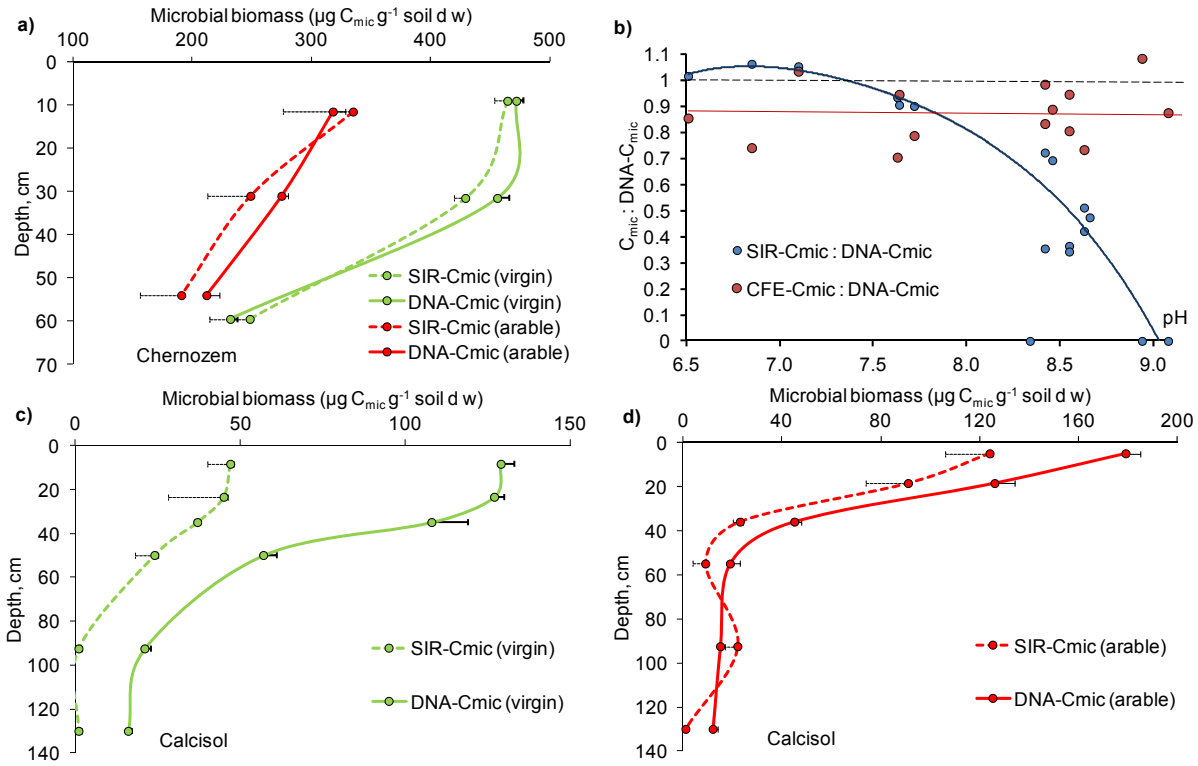


**Fig. 2.** DNA- and SIR-based microbial biomass distribution with depth: a) Comparison of SIR-$C_{mic}$ and DNA-$C_{mic}$ in virgin and arable Chernozem; b) Effect of pH on underestimation of SIR-$C_{mic}$; c) Comparison of SIR-$C_{mic}$ and DNA-$C_{mic}$ in virgin Calcisol and d) in arable Calcisol.

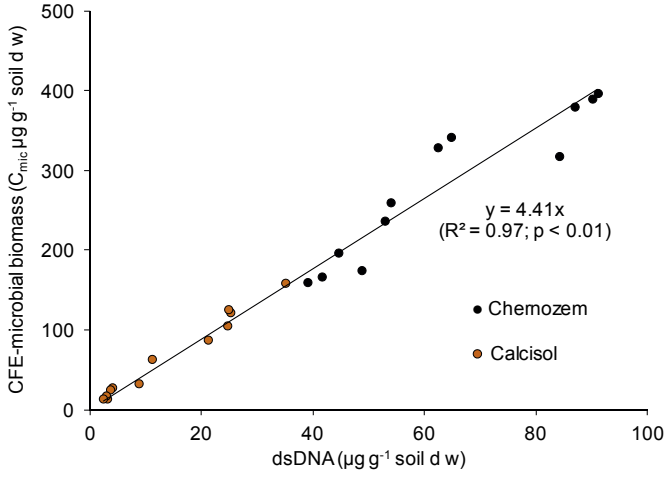


**Fig. 3.** Regression between extractable dsDNA and microbial biomass (CFE-$C_{mic}$) for the horizons of two soil types (Table 1). The regression line is based on means of 24 samples of Chernozem and Calcisol.

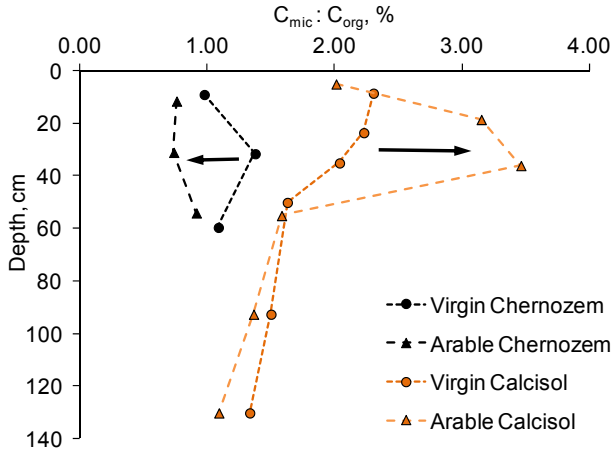


**Fig. 4.** The $C_{mic}$:$C_{org}$ ratios for horizons of Chernozem and Calcisol. Microbial biomass-C values obtained by DNA-based method were used. The two arrows indicate opposing shifts in the $C_{mic}$:$C_{org}$ values during prolonged land-use of two soils.

theoretical correction factors. Experimental factors for arable Calcisol were lower than for virgin soil (1.38–2.11), although pH values were at the same level (Fig. 5). Furthermore, despite alkaline conditions, in the Ck1 horizon the SIR-$CO_2$ value was even higher than that obtained by DNA-$C_{mic}$. For deeper horizons, no experimental $CO_2$ retention correction factors could be calculated due to the absence of $CO_2$ evolution. Thus, experimentally derived correction factors were much lower for all soils and horizons studied than theoretically calculated pH-based correction factors (Fig. 5).

The experimental correction factors ($F_{corr}$) were approximated by a non-linear multiple regression to reveal how the correction factor is dependent on pH and carbonates ($CO_3$):

$$F_{corr} = \exp(pH - a) + (b \times CO_3^{2-}, \%) + c \times (CO_3^{2-}, \%)^2$$

$$R^2 = 0.86\ p < 0.0004,$$

$$a = 7.43 \pm 0.06,\ b = -2.35 \pm 0.48 \text{ and } c = 0.97 \pm 0.26.$$

According to this equation, experimental correction factors were exponentially dependent on soil pH while a quadratic function explained an effect of carbonate content.

## 4. Discussion

### 4.1. The DNA-to-microbial biomass conversion factors

The obtained $F_{DNA}$-factor of 5.10 agreed well with the SIR-$C_{mic}$ of Chernozem (Fig. 1). It was also close to the values reported for

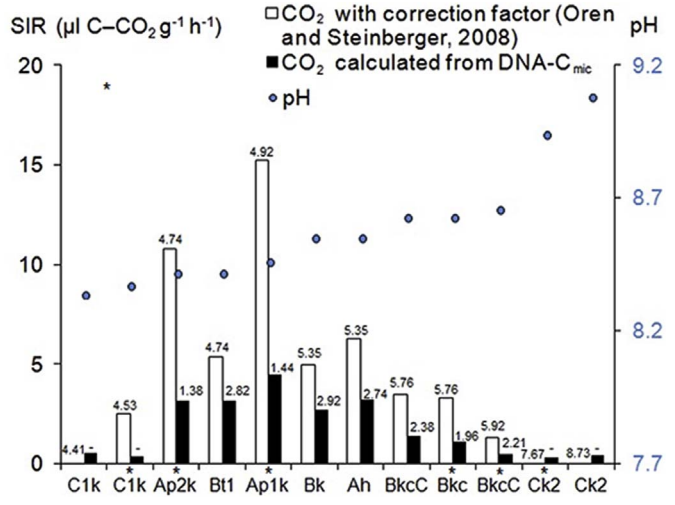


**Fig. 5.** Comparison of theoretically calculated SIR-$CO_2$ using pH correction factors for arid soils (Oren and Steinberger, 2008) and obtained $CO_2$ by recalculation of DNA-$C_{mic}$ for Calcisol samples depending on pH. The data are arranged in order of increasing pH. The axis X indicates the studied horizons of two soil profiles. Horizons of arable Calcisol are marked with an asterisk. The numbers above the black and white columns correspondently indicate the theoretical pH-based and experimentally derived correction factors.

Cambisols (5.0 – Anderson and Martens, 2013) and for the range of various soils (6.0 – Joergensen and Emmerling, 2006).

The conversion factor of 4.41 from dsDNA to CFE-$C_{mic}$ (Fig. 3) was in the range of already suggested values from 3.9 (Marstorp and Witter, 1999) to 4.6 (Gong et al., 2001). Since the range of the conversion factors is very narrow, variability of possible results does not exceed 16%. Thus, the DNA-based approach indicates a good reproducibility of both SIR and CFE for studied soils where both approaches are applicable.

The obtained CFE-$C_{mic}$ to DNA-$C_{mic}$ ratio of 0.87 indicates either slight underestimation of microbial biomass by CFE or, overestimation by SIR. This over-/underestimation may be related to high variability of conversion factors in CFE and SIR approaches depending on soil properties (Kaiser et al., 1992).

The $F_{DNA}$-factor demonstrated a surprisingly narrow range because of very high sensitivity of PicoGreen dye to detect DNA content from strongly diluted samples. Thus, DNA quantification can serve as effective and suitable alternative to basic approaches of microbial biomass determination in highly alkaline and carbonaceous soils.

It is important to note that DNA-based approach also has certain shortcomings for soil microbial biomass determination. Primarily, $F_{DNA}$ can be affected by various factors such as extracellular DNA or insufficient extraction (Bakken and Frostegård, 2006; Pietramellara et al., 2009). Extracellular DNA content, however, does not usually exceed 3% of total DNA (Gangneux et al., 2011). The efficiency of total soil DNA extraction may vary among soils differing in particle-size distribution and chemical composition. The complete extraction may also be limited by incomplete cell lysis, losses due to shearing and enzymatic degradation, sorption to colloids, and losses during purification from mono- and oligophenols and humic materials (Marstorp and Witter, 1999). In our study, however, the efficiency of DNA extraction was close to 98%. Selection of the suitable kit and/or DNA recovery test strongly depends on the goal of the analyses. For many molecular biology methods (for instance, community analysis based on *Taq* DNA PCR), high DNA extraction efficiency is of a lesser importance than the necessity of high grade DNA purification. For such methods, a soft extraction is necessary to avoid the risks of forming chimeric products with smaller template DNA. For successful total soil DNA quantification in turn, it is required to select most efficient extraction method using severe chemical and physical treatments such as bead beating and sonication to lyse microbial cells, and own reducing number of washing/purification steps as well. Our preliminary experiments have demonstrated that DNA yield varies depending on the kit used. Therefore, comparison of different studies is possible only when the same extraction procedure was used.

### 4.2. Determination of microbial biomass-C in alkaline soils

Extraction and subsequent quantification of dsDNA revealed an excellent relationship with substrate-induced respiration for determining the microbial biomass-C in soils with pH below 8. In the case of alkaline soil pH, the SIR showed significantly lower values of microbial biomass-C than DNA-$C_{mic}$ and CFE-$C_{mic}$ (Fig. 2). Experimental analysis of the SIR in alkaline soils strongly underestimated (25–100%) $C_{mic}$, while using theoretical pH-based correction factors of $CO_2$ retention for SIR (Oren and Steinberger, 2008), overestimated the results. Furthermore, the temperature and time of $CO_2$ release and trapping can significantly affect the values of correction factors. $CO_2$ retention in soil solutions with pH above 8 can be estimated by continuous flushing of soil air, followed by adsorption by an alkali in a closed system (Martens, 1987). Another approach considering the $CO_2$ retention in alkaline solution is based on a correction factor, which relates measured $CO_2$ emission to the theoretically calculated allocation of $CO_2$ to solution (Oren and Steinberger, 2008; Sparling and West, 1990). Although such correction factors are effective in certain cases, the procedure for their determination is complicated and time consuming. The use of correction factors was also hampered in our study by the absence of any $CO_2$ emission, especially at low absolute values of microbial biomass-C. This situation is particularly typical for the deep horizons of alkaline semi-arid and arid soils.

Another reason for the underestimation of SIR-$C_{mic}$ in alkaline soils could be the $CO_2$ retention and exchange by soil carbonates (Kuzyakov et al., 2006), resulting in corresponding interference of microbial biomass estimation. However, we observed an ambivalent effect of carbonates on $CO_2$ underestimation in the set of soils tested. The multiple regression analysis revealed a parabolic relationship between carbonate content and $CO_2$ retention. We assume that one-week preincubation of soil leads to equilibration between $CaCO_3$ and $HCO_3^-$ in soil solution. Consequently, additional $CO_2$ produced by SIR was released from soil and was measured using gas chromatography for some of the samples. Note that besides the measurable effect of $CaCO_3$ presence on released $CO_2$, the exchange between $HCO_3^-$ in solution and produced $CO_2$ may lead to exchange of C isotopes. The SIR approach therefore cannot be used in studies applying $^{13}C$ natural abundance or $^{13}C$ and $^{14}C$ labeling in the soils containing carbonates.

Thus, our study demonstrated the inapplicability of the theoretical conversion factors of $CO_2$ retention for the SIR recalculation to determine soil microbial biomass under alkaline conditions. The dsDNA quantification provides a simple and suitable approach for microbial biomass analysis as alternative for SIR and can be successfully used in alkaline or calcareous semi-arid soils. In contrast to the CFE, the DNA-based method may be suitably combined with the molecular analysis of soil microbial communities, e.g., quantitative PCR or high-throughput sequencing.

### 4.3. Sensitivity of DNA-based estimations to land-use effect

For monitoring long-term land-use effects in arable soils with extreme properties (e.g., high alkalinity), it was suggested to use at least two methods for measuring soil microbial biomass as independent controls, considering the fact that the changes in some soil properties due to land use lead to systematic deviations between two methods (Joergensen and Emmerling, 2006). However, the good reproducibility of SIR, CFE and DNA-based methods in Chernozems, and CFE and DNA methods in Calcisols was revealed both for soils under virgin and agricultural ecosystems. These results demonstrate sustainability of

DNA as an indicator of soil microbial biomass in arable soils. Thus, the DNA-based method can be successfully used to assess the land-use effects on soils.

Agricultural land use of Chernozem led to a decrease of $C_{org}$, $C_{mic}$ as well as of the proportion of $C_{mic}$ in $C_{org}$ at all depths in arable versus virgin soil. This primarily indicates a reduction of the total reserves of $C_{org}$ and the predominance of mineralization processes (Blagodatskii et al., 2008), which in turn reduces microbial biomass-C. Thus, DNA-$C_{mic}$:$C_{org}$ demonstrated the negative impact of agricultural land use on C sequestration in Chernozem.

Quantification of DNA enabled determining the $C_{mic}$:$C_{org}$ ratio in alkaline Calcisol, where the SIR-$CO_2$ efflux was totally or partially absorbed. Remarkably, the $C_{mic}$:$C_{org}$ ratio was higher in Calcisol than in Chernozem, despite the greater total organic carbon and microbial biomass content in the latter. Since the $C_{mic}$:$C_{org}$ ratio is an indicator of the availability of soil C to microorganisms (Anderson, 2003; Anderson and Domsch, 1989), this refers to the highly recalcitrant $C_{org}$ in Chernozems usually characterized by a very low pool of potentially mineralizable (active) C (Semenov et al., 2008). That explains why the $C_{mic}$:$C_{org}$ ratio of Chernozems is commonly the lowest when compared for example with polar Cryosols, temperate Albeluvisols and Luvisols, or semi-arid Kastanozems (Ananyeva et al., 2008).

In Calcisol, the changes in the biological cycle by agricultural land use led to two opposite trends: 1) the parallel increase of $C_{org}$ and $C_{mic}$ in the top horizon Ap1k and of the $C_{mic}$:$C_{org}$ ratio in the underlying horizons Ap2k and Bkc, and, concurrently, 2) a general decrease of $C_{org}$ in the deeper horizons. This strong decline of $C_{org}$ content resulted in a 40–70% increase of the $C_{mic}$:$C_{org}$ at 10–45 cm depth. At the same time, in top horizon $A_p1$ of arable Calcisol, the land-use effect resulted in a nearly 60% increase of $C_{mic}$ and a 40% increase of $C_{org}$ compared with virgin soil. This effect can be due to a very low C content in native Calcisols. Regular plowing leads to grinding up, redistribution and accelerated decomposition of plant residues, resulting in soil enrichment by available organic carbon (Tian et al., 2012). In the long-term, a higher efficiency of organic matter utilization for microbial growth increases the humification intensity under dry climate conditions. We thus conclude that agrogenic impact is not always a negative phenomenon. For arid soils with high $C_{mic}$:$C_{org}$ index but low organic C-content, land use can even increase such eco-physiological indexes as $C_{mic}$, $C_{org}$ and the $C_{mic}$:$C_{org}$ ratio.

## 5. Conclusions

We successfully applied the DNA-based approach to estimate microbial biomass in alkaline and carbonaceous soils. Extraction and subsequent quantification of dsDNA revealed a strong agreement with substrate-induced respiration and chloroform fumigation extraction measured microbial biomass content in soils with pH below 8. The conversion factors ($F_{DNA}$) from dsDNA to SIR-$C_{mic}$ (5.10) and CFE-$C_{mic}$ (4.41) were obtained based on a range of soil samples with $C_{org}$ content from 0.1 to 4.6%, and the DNA-based microbial biomass indicated a good reproducibility.

High alkalinity and the presence of carbonates in arid and semi-arid soils lead to strong underestimation of microbial respiration and microbial biomass C by the SIR approach. Thus, DNA quantification is a very useful alternative approach at least for alkaline and carbonaceous soils.

Possible shifts in microbial biomass measurements due to land-use effects were not detected both for CFE and DNA quantification methods. These results demonstrate sustainability of DNA as an indicator of soil microbial biomass in arable soils. DNA-based estimations of the $C_{mic}$:$C_{org}$ ratios in virgin and arable Calcisols revealed that agrogenic impact does not always lead to negative consequences for soil status and cannot be considered a solely negative phenomenon. For arid soils with high $C_{mic}$:$C_{org}$ index but low organic C content, land use can even increase eco-physiological indexes such as $C_{mic}$, $C_{org}$ and the $C_{mic}$:$C_{org}$ ratio.

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## References

Ananyeva, N.D., Susyan, E.A., Chernova, O.V., Wirth, S., 2008. Microbial respiration activities of soils from different regions of European Russia. Eur. J. Soil Biol. 44, 147–157.
Anderson, J.P.E., Domsch, K.H., 1978. Physiological method for quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. 10, 215–221.
Anderson, T.-H., 2003. Microbial eco-physiological indicators to assess soil quality. Agric. Ecosyst. Environ. 98, 285–293.
Anderson, T.-H., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total organic-C in arable soils. Soil Biol. Biochem. 21, 471–479.
Anderson, T.-H., Martens, R., 2013. DNA determinations during growth of soil microbial biomasses. Soil Biol. Biochem. 57, 487–495.
Bachoon, D.S., Otero, E., Hodson, R.E., 2001. Effects of humic substances on fluorometric DNA quantification and DNA hybridization. J. Microbiol. Methods 47, 73–82.
Bailey, V.L., Smith, J.L., Bolton, H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biol. biochem. 34 (7), 997–1007.
Bakken, L.R., Frostegård, A., 2006. Nucleic acid extraction from soil. In: Nannipieri, P., Smalla, K. (Eds.), Soil Biology. me 8. Springer-Verlag, Berlin Heidelberg, pp. 49–73.
Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol. Fertil. Soils 45, 115–131.
Blagodatskaya, E., Blagodatsky, S., Anderson, T.-H., Kuzyakov, Y., 2014. Microbial growth and carbon use efficiency in the rhizosphere and root-free soil. PLoS One 9 (4).
Blagodatskaya, E.V., Blagodatskii, S.A., Anderson, T.-H., 2003. Quantitative isolation of microbial DNA from different types of soils of natural and agricultural ecosystems. Microbiology 72 (6), 744–749.
Blagodatskii, S., Bogomolova, I., Blagodatskaya, E., 2008. Microbial biomass and growth kinetics of microorganisms in chernozem soils under different land use modes. Microbiology 77 (1), 99–106.
Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biol. Biochem. 17 (6), 837–842.
Dilly, O., 2003. Regulation of the respiratory quotient of soil microbiota by availability of nutrients. FEMS Microbiol. Ecol 43 (3), 375–381.
Fornasier, F., Ascher, J., Ceccherini, M.T., Tomat, E., Pietramellara, G., 2014. A simplified rapid, low-cost and versatile DNA-based assessment of soil microbial biomass. Ecol. Indic. 45, 75–82.
Gangneux, C., Akpa-Vincesla, M., Sauvage, H., Desaire, S., Houot, S., Laval, K., 2011. Fungal, bacterial and plant dsDNA contributions to soil total DNA extracted from silty soils under different farming practices: relationships with chloroform-labile carbon. Soil Biol. Biochem. 43, 431–437.
German, D.P., Marcelo, K.R.B., Stone, M.M., Allison, S.D., 2012. The Michaelis–Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. Glob. Change Biol. 18, 1468–1479.
Gong, P., Guan, X., Witter, E., 2001. A rapid method to extract ergosterol from soil by physical disruption. Appl. Soil. Ecol. 17, 285–289.
Haney, R.L., Franzluebbers, A.J., Hons, F.M., Hossner, L.R., Zuberer, D.A., 2001. Molar concentration of $K_2SO_4$ and soil pH affect estimation of extractable C with chloroform fumigation–extraction. Soil Biol. Biochem. 33 (11), 1501–1507.
Harris, D., Horwath, W.R., van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci. Soc. Am. J 65, 1853–1856.
Hintze, T., Gehlen, P., Schriider, D., 1994. Are microbial biomass estimations equally valid with arable soils and forest soils? Soil Biol. Biochem. 26, 1207–1211.
IUSS Working Group WRB, 2014. World Reference Base for Soil Resources 2014. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports No 106. FAO, Rome.
Joergensen, R.G., 2000. Ergosterol and microbial biomass in the rhizosphere of grassland soils. Soil Biol. Biochem. 32, 647–652.
Joergensen, R.G., Emmerling, C., 2006. Methods for evaluating human impact on soil microorganisms based on their activity, biomass, and diversity in agricultural soils. J. Plant Nutr. Soil Sci. 169, 295–309.
Joergensen, R.G., Mueller, T., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the $k_{EN}$ value. Soil Biol. Biochem. 28, 33–37.
Kaiser, E.-A., Mueller, T., Joergensen, R.G., Isam, H., Heinemeyer, O., 1992. Evaluation of methods to estimate the soil microbial biomass and the relationship with soil texture and organic matter. Soil Biol. Biochem. 24, 675–683.
Kandeler, E., Mosier, A.R., Morgan, J.A., Milchunas, D.G., King, J.Y., Rudolph, S., Tscherko, D., 2006. Response of soil microbial biomass and enzyme activities to the transient elevation of carbon dioxide in a semi-arid grassland. Soil Biol. Biochem. 38 (8), 2448–2460.
Kuzyakov, Y., Shevtzova, E., Pustovoytov, K., 2006. Carbonate re-crystallization in soil revealed by $^{14}C$ labeling: experiment, model and significance for paleo-environmental reconstructions. Geoderma 131, 45–58.

Lindsay, W.L., 1979. Carbonate equilibria. In: Lindsay, W.L. (Ed.), Chemical Equilibria in Soils. Wiley, New York, pp. 78–84.
Lloyd-Jones, G., Hunter, D.W.F., 2001. Comparison of rapid DNA extraction methods applied to contrasting New Zealand soils. Soil Biol. Biochem. 33 (15), 2053–2059.
Marstorp, H., Witter, E., 1999. Extractable dsDNA and product formation as measures of microbial growth in soil upon substrate addition. Soil Biol. Biochem. 31 (10), 1443–1453.
Martens, R., 1987. Estimation of microbial biomass in soil by the respiration method: importance of soil pH and flushing methods for the measurement of respired $CO_2$. Soil Biol. Biochem. 19, 77–81.
Mueller, T., Joergensen, R.G., Meyer, B., 1992. Estimation of soil microbial biomass C in the presence of living roots by fumigation-extraction. Soil Biol. Biochem. 24, 179–181.
Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. Eur. J. Soil Sci. 54 (4), 655–670.
Oren, A., Steinberger, Y., 2008. Coping with artifacts induced by $CaCO_3$–$CO_2$–$H_2O$ equilibria in substrate utilization profiling of calcareous soils. Soil Biol. Biochem. 40, 2569–2577.
Pietramellara, G., Ascher, J., Borgogni, F., Ceccherini, M.T., Guerri, G., Nannipieri, P., 2009. Extracellular DNA in soil and sediment: fate and ecological relevance. Biol. Fertil. Soils 45, 219–235.
Powlson, D.S., 1980. The effects of grinding on microbial and non-microbial organic matter in soil. J. Soil Sci. 31, 1–9.
Powlson, D.S., Brookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil Biol. Biochem. 19, 159–164.
Rousk, J., Jones, D.L., 2010. Loss of low molecular weight dissolved organic carbon (DOC) and nitrogen (DON) in $H_2O$ and 0.5 M $K_2SO_4$ soil extracts. Soil Biol. Biochem. 42, 2331–2335.
Schmidt, N., Bölter, M., 2002. Fungal and bacterial biomass in tundra soils along an arctic transect from Taimyr Peninsula, central Siberia. Polar Biol. 25, 871–877.
Semenov, V.M., Ivannikova, L.A., Kuznetsova, T.V., Semenova, N.A., Tulina, A.S., 2008. Mineralization of organic matter and the carbon sequestration capacity of zonal soils. Eurasian Soil Sci. 41 (7), 717–730.
Smalla, K., Cresswell, N., Mendonca-Hagler, L.C., Wolters, A., van Elsas, J.D., 1993. Rapid DNA extraction protocol from soil for polymerase chain reaction-mediated amplification. J. Appl. Bacteriol. 74 (1), 78–85.
Sparling, G.P., West, A.W., 1990. A comparison of gas chromatography and differential respirometer methods to measure soil respiration and to estimate the soil microbial biomass. Pedobiologia 34 (2), 103–112.
Terrat, S., Christen, R., Dequiedt, S., Lelievre, M., Nowak, V., Regnier, T., Bachar, D., Plassart, P., Wincker, P., Jolivet, C., Bispo, A., Lemanceau, P., Maron, P.-A., Mougel, C., Ranjard, L., 2012. Molecular biomass and MetaTaxogenomic assessment of soil microbial communities as influenced by soil DNA extraction procedure. Microb. Biotechnol. 5, 135–141.
Tian, J., Fan, M.F., Guo, J.H., Marschner, P., Li, X.L., Kuzyakov, Y., 2012. Effects of land use intensity on dissolved organic carbon properties and microbial community structure. Eur. J. Soil Biol. 52, 67–72.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19 (6), 703–707.
Vishnevetsky, S., Steinberger, Y., 1997. Bacterial and fungal dynamics and their contribution to microbial biomass in desert soil. J. Arid Environ. 37, 83–90.
Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction—an automated procedure. Soil Biol. Biochem. 22 (8), 1167–1169.