Biol Fertil Soils (1995) 19:343–347

ORIGINAL PAPER

O. Dilly · J.-C. Munch

Microbial biomass and activities in partly hydromorphic agricultural and forest soils in the Bornhöved Lake region of Northern Germany

Received: 7 January 1994

Abstract The soil microbial biomass and activity were estimated for seven field (intensive and extensive management), grassland (dry and wet), and forest (beech, dry and wet alder) sites. Three of the sites (wet grassland, dry and wet alder) are located on a lakeshore and are influenced by lake water and groundwater. Four different methods were selected to measure and characterize the microbial biomass. Values of microbial biomass (weight basis) and total microbial biomass per upper horizon and hectare (volume basis) were compared for each site.

Fumigation-extraction and substrate-induced respiration results were correlated but did not give the same absolute values for microbial biomass content. When using the original conversion factors, substrate-induced respiration gave higher values in field and dry grassland soils, and fumigation-extraction higher values in soils with low pH and high water levels (high organic content). Results from dimethylsulfoxide reduction and arginine ammonification, two methods for estimating microbial activity, were not correlated with microbial biomass values determined by fumigation-extraction or substrate-induced respiration in all soils examined. In alder forest soils dimethylsulfoxide reduction and arginine ammonification gave higher values on the wet site than on the dry site, contrary to the values estimated by fumigation-extraction and substrate-induced respiration. These microbial activities were correlated with microbial biomass values only in field and dry grassland soils. Based on soil dry weight, microbial biomass values increased in the order intensive field, beech forest, extensive field, dry grassland, alder forest, wet grassland. However, microbial biomass values per upper horizon and hectare (related to soil volume) increased in agricultural soils in the order intensive field, dry grassland, extensive field, wet grassland and in forest soils in the order beech, wet alder, dry alder. We conclude that use of the original conversion factors with the soils in the present study for fumigation-extraction and substrate-induced respiration measurements does not give the same values for the microbial biomass. Furthermore, dimethylsulfoxide reduction and arginine ammonification principally characterize specific microbial activities and can be correlated with microbial biomass values under specific soil conditions. Further improvements in microbial biomass estimates, particularly in waterlogged soils, may be obtained by direct counts of organisms, ATP estimate, and the use of ^{14}C-labelled organic substrates. From the ecological viewpoint, data should also be expressed per horizon and hectare (related to soil volume) to assist in the comparison of different sites.

Key words Ecosystem research · Agricultural soils · Forest soils · Microbial biomass · Microbial activities

O. Dilly (✉)
Projektzentrum Ökosystemforschung,
Universität Kiel, Schauenburger Straße 112,
D-24118 Kiel, Germany

J.C. Munch
Institut für Bodenbiologie,
Bundesforschungsanstalt für Landwirtschaft (FAL),
Bundesallee 50, D-38116 Braunschweig, Germany

Introduction

In order to examine and model structure and functions of ecosystem complexes with different interactive parts (fields, grasslands, forests, lakes), an interdisciplinary project was started in 1988 by the Ecosystem Research Center from the University of Kiel (Germany).

Microbial biomass is of crucial importance for transformation processes in soils. Soil microorganisms are the driving force for nutrient transformations and thus have a major role in soil fertility and the functioning of an ecosysystem (Smith and Paul 1992). Estimates of microbial biomass are essential for the explanation and understanding of processes in different soils. For an accurate determination of microbial, more than one method should be applied (Nannipieri et al. 1978; West et al. 1986), as recommended by Jenkinson and Ladd (1981). In addition, the limitations of each method should be taken

into account as described previously (Jenkinson and Ladd 1981; Alef 1993; Martens 1994).

The aim of the present study was to quantify the microbial biomass in the upper horizons of soils of the research area. In addition methods for assessing microbial activity, which is generally correlated with the microbial biomass, were tested for potential use as an index for microbial biomass, particularly in waterlogged soils.

Quite different soils had developed in the inner research area. Since upper horizons are places of intensive elemental transformation, the focus was on these horizons. Four measurement methods were selected, two "indirect" microbial biomass methods and two methods for microbial activity. According to Alef and Kleiner (1986, 1989), Alef et al. (1988), and Sparling and Searle (1993) these two methods for microbial activity give results that are well correlated with microbial biomass values.

For ecological studies microbial biomass values related to soil dry weight are not as useful as those related to horizon or hectare (volume-related). In comparing different sites for microbial transformation potential or microbial-incorporated nutrients, possible differences in soil densities and horizon thickness must be taken into account (Table 1). Therefore microbial biomass values expressed on a dry soil basis were compared with those expressed per upper horizon and hectare.

Materials and methods

Description of site and soils

The research area is located 30 km south of Kiel in Schleswig-Holstein, Germany (59°97'N, 35°81'E). In this region the soils have developed predominantly from sediments deposited in the Last Glaciation in a region known as Ostholsteinisches Hügelland.

The inner terrestrial research area is divided into two catenas, one under agricultural use and one under forest. The predominantly sandy soils have varying contents of organic matter and pH values. Seven sites were selected (Table 1). The agricultural soils included an extensively managed field under a crop rotation [rye (*Secale cereale* L.) in the year of the present study], fertilized predominantly with organic manure, an intensively managed field in maize (*Zea mays* L.) monoculture, fertilized with pig-cattle slurry and additionally mineral fertilizer, a dry grassland and a wet grassland. Forest soils were selected under beech (*Fagus sylvatica* L.) and black alder [*Alnus glutinosa* (L.) Gaertn.]. In the alder forest, two sites were selected, one directly beside a lake (called wet alder), the other only about 50 m inside the forest at the foot of a hill (dry alder).

The wet grassland and both alder forest sites are located on the lakeshore of Belauer See, are poorly drained, seasonally waterlogged, and have partly hydromorphic properties (FAO soil classification: gelic and stagnic).

Sampling procedure

Samples of the upper horizons were taken monthly from January to June 1992, in order to confirm the results at different times. Multiple cores (50 cores in the field and grassland sites with a Puerckhauer drill, 20 in the beech and 8 in the alder sites with a 4-cm diameter drill) were mixed together, gently sieved, and stored at 4 °C for not longer than 4 weeks until analysis. The fraction < 2 mm was used for all subsequent analyses.

Analytical methods

The fumigation-extraction method (CFE) was applied according to Vance et al. (1987) except that the samples were incubated for 3 days at room temperature and field-moist soils were extracted with 0.5 *M* K_2SO_4 in a ratio of 1:4 on a soil dry weight basis. Samples of field, grassland, and beech soils were analyzed with a water content of 11–20% (g g^{-1} dry soil), equivalent ot about 40–70% water-holding capacity (undisturbed soil), and wet grassland and alder soils were treated without any soil moisture modification (Table 1). C was determined in the extracts using a Shimadzu TOC 5000 and ASI 5000.

Substrate-induced respiration (SIR) was measured according to Anderson and Domsch (1978), using equipment developed by Heinemeyer et al. (1989). Samples were incubated for 3 days at room temperature before analysis. A substrate concentration of 5 mg glucose-monohydrate g^{-1} dry soil for field, dry grassland, and beech soil and of 12.5 mg g^{-1} dry soil for wet grassland and alder soil was determined as optimal. The glucose was mixed with talcum in the ratio 3:5. Samples of field, grassland, and beech soil were analyzed with a water content of 11–20% (g g^{-1} dry soil), equivalent to about 40–70% water-holding capacity (undisturbed soil), and wet grassland and alder soil samples were treated without any soil moisture modification (Table 1).

Dimethylsulfoxide reduction (DMS) was carried out using the general method described by Alef and Kleiner (1989) with the modifications that 0.125 ml 6.6% (v/v) dimethylsulfoxide solution was added to 0.5 g field-moist soil (in an 8.8-ml glass container sealed with a teflon-rubber septum) and then incubated at 30 °C in a waterbath (three replicates). One control was prepared using water instead of dimethylsulfoxide solution. Gas samples from the container (two samples of 0.25 ml each) were taken after 3 h with a gastight syringe and the dimethylsulfide concentration was determined by gas chromatography using a Shimadzu GC 14 with a 30-m

Table 1 Some properties of upper soil horizons in the Bornhöved Lakes region, northern Germany

	Horizon	Depth (cm)	pH (H_2O)	H_2O content (% w/w)	Organic C (mg g^{-1} dry soil)	Organic N (mg g^{-1} dry soil)	C:N	Soil density (g cm^{-3})
Agricultural soils								
Extensive field	Ap	0–20	6.4	13.1 ± 5.9	12.8	1.32	10	1.3
Intensive field	Ap	0–20	5.4	11.3 ± 3.1	12.1	1.01	12	1.3
Dry grassland	Ah	0–10	6.4	10.5 ± 4.9	16.5	1.53	11	1.3
Wet grassland	M	0–20	5.8	84.3 ± 13.0	75.7	6.45	12	0.6
Forest soils								
Beech	Ah	0–5	4.1	21.3 ± 3.4	24.5	1.79	14	1.1
Dry alder	H	0–20	4.1	311.6 ± 40.3	275.4	18.34	15	0.2
Wet alder	H	0–10	6.0	455.8 ± 64.8	230.0	15.14	15	0.2

capillary column (J & W, DB 5) and flame ionization detector. The carrier gas was N_2 at pressure of 3 kg cm^{-2} and the injector, column, and detector temperatures were 220, 120, and 200 °C respectively. The retention time of dimethylsulfide under the conditions described was only 27 s. To prepare standards, 10 µl cooled dimethylsulfide (about 4 °C) was transferred to a 28.5-ml glass container, which was warmed for 3 min in the hand; 25 µl was then injected into a second 8.8-ml glass container, aliquots of 0, 10, 20, 30, 40, 50, 75, and 100 µl were removed, and the amount of dimethylsulfide determined.

Arginine ammonification (ARG) was determined according to Alef and Kleiner (1986) with modifications. Samples of 2.0 g field moist soil (placed in 12-ml centrifuge tubes) were pre-incubated for 15 min at 30 °C. Then 0.5 ml of a 0.47 m*M* arginine solution was added dropwise. Three samples were incubated for 3 h at 30 °C and three were stored at −20 °C. After incubation, 8 ml 2 *M* KCl was added for extraction, shaken for 15 min, and centrifuged for 10 min at 3000 rpm. To 1 ml of the supernant, 4 ml 2 *M* KCl, 0.2 ml of a 6.71 m*M* nitroprusside sodium dihydrate/1.062 *M* sodium salicylate solution, 0.2 ml of a mixture (4:1) of 0.68 *M* trisodium citrate/0.134 *M* sodium hydroxide, and 22.66 m*M* dichloroisocyanuric acid sodium salt dihydrate were added subsequently. After 90 min, the absorbance was determined at 690 nm. To prepare standards, 0, 0.25, 0.5, 1, 1.5, and 4 ml of a 100-fold diluted 71.4 m*M* ammoniumchloride solution were made up to 10 ml using 2 *M* KCl and then aliquots of 1 ml were treated as samples. Each analysis was carried out with three replicates. Regression analysis (Table 2) was performed with the mean of each sampling (the mean of the three replicates, $n = 42, 12, 18$) and the mean of the six samplings as well ($n = 7$). SD (Fig. 1) are given from the different sampling times ($n = 6$). A median test (significance level $P = 0.05$) was used to determine differences between the methods ($n = 18$ for each site).

Results and discussion

Reproducibility

The covariance of the three replicates was usually below 10%. In less than 20% of the analyses, higher variances were measured. Higher variation was observed for sampling times, particularly for arginine ammonification in alder forest and wet grassland soils (Fig. 1).

Comparison of fumigation-extraction, substrate-induced respiration, dimethylsulfoxide reduction, and arginine ammonification related to soil dry weight

Figure 1 shows the results given for the four methods, expressed per gram dry soil. For field and grassland soils all methods showed similar tendencies. The values increased in the order intensive field, extensive field, dry grassland, wet grassland. In beech soil the values were lower than in both alder soils. For the two alder forest soils there was no agreement between the four methods. Despite dry alder soil showing significantly higher substrate-induced respiration and extractable C than wet alder soil, opposite results were obtained with dimethylsulfoxide reduction and arginine ammonification.

Correlation between the methods

The best correlation was given between fumigation-extraction and substrate-induced respiration (Table 2). Neither dimethylsulfoxide reduction nor arginine ammonification were correlated with fumigation-extraction for all soils or for the acid soils. Kaiser et al. (1992) also found no correlation between arginine ammonification and fumigation-extraction or substrate-induced respiration results in 24 field and 3 grassland soils. In the field and dry grassland soils of the present study, which were low in organic matter and had pH (H_2O) values of 5.4–6.4,

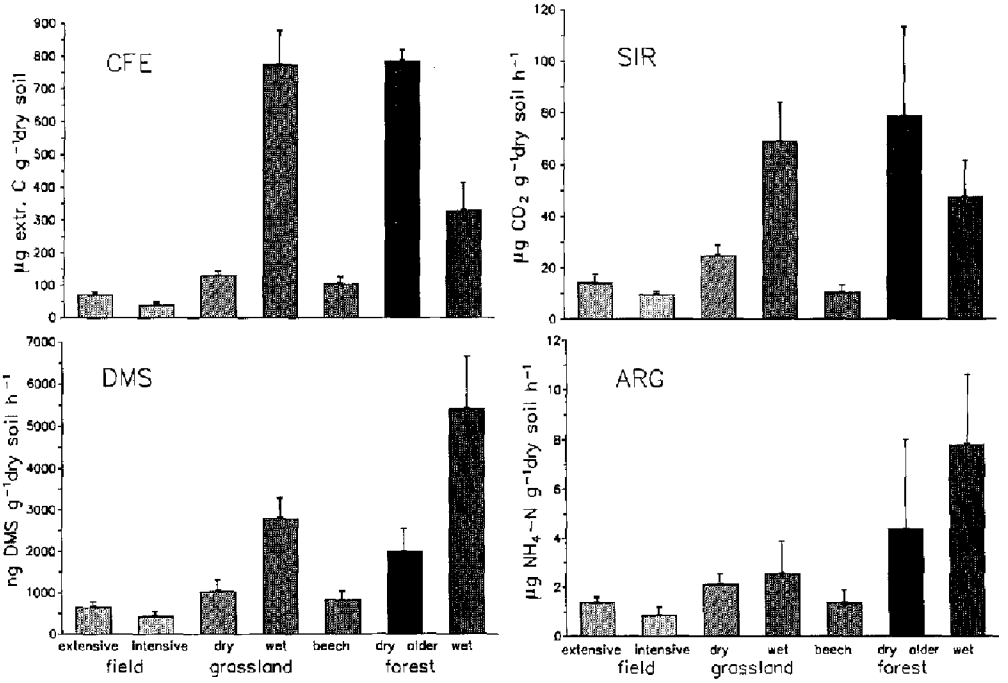

Fig. 1 Potassium sulfate-extractable microbial C (*CFE*) substrate-induced respiration (*SIR*), dimethylsulfoxide reduction (*DMS*), and arginine ammonification (*ARG*) in upper horizons of soils of the Bornhöved Lakes region

Table 2 Correlations between analysis data (see Fig. 1) for fumigation-extraction (*CFE*), substrate-induced respiration (*SIR*), dimethylsulfoxide reduction (*DMS*), and arginine ammonification (*ARG*)

		CFE-SIR	CFE-DMS	CFE-ARG
All soils	($n = 42$)	$r^2 = 0.71$	$r^2 = 0.15$	$r^2 = 0.06$
Mean	($n = 7$)	$r^2 = 0.94$	$r^2 = 0.15$	$r^2 = 0.10$
Beech/dry alder (acid soils)	($n = 12$)	$r^2 = 0.65$	$r^2 = 0.57$	$r^2 = 0.12$
Field/dry grassland	($n = 18$)	$r^2 = 0.94$	$r^2 = 0.81$	$r^2 = 0.73$

dimethylsulfoxide reduction and arginine ammonification were correlated with fumigation-extraction. A correlation between microbial biomass values obtained by dimethylsulfoxide reduction and those obtained by arginine ammonification was also reported by Alef et al. (1988) and Sparling and Searle (1993).

Under extreme conditions, as in alder or wet grassland soils (high organic matter, low O_2 availibility), the processes of dimethylsulfoxide reduction and arginine ammonification are independent of the amount of microbial biomass. These activities seem to be more closely related to population structure, as reported for bacteria in extensive field and wet alder forest soils by Bach and Munch (1993).

Microbial biomass calculated by fumigation-extraction and substrate-induced respiration related to soil dry weight

The conversion factors 2.64 for fumigation-extraction (Vance et al. 1987) and 40.04 for substrate-induced respiration (Anderson and Domsch 1978) gave differing microbial biomass values (Table 3). In field and dry grassland soils the values were overestimated by substrate-induced respiration (in agreement with Harden et al. 1993) while in acid beech and dry alder soils as well as in the wet grassland soil, the fumigation-extraction method to higher values.

To overcome the discrepancy between fumigation-extraction and substrate-induced respiration results other conversion factors were proposed (Sparling et al. 1990; Kaiser et al. 1992). An additional influence on microbial biomass values estimated by fumigation-extraction may be the use of different soil-extractant ratios. Water contents were especially high in the wet grassland and both alder soils. In the present study a soil-extractant ratio of 1 : 4 on a soil dry weight basis was applied. Sparling and West (1988) used 1 : 5 for mineral soils and 1 : 25 for low-density organic samples with results related to the soil dry weight, and Martikainen and Palojärvi (1990) used 1 : 22.5 for fresh soils with a high organic content. In some cases different K_2SO_4 concentrations (Zagal 1993; Mazzarino et al. 1993) and extractants (Joergensen and Brookes 1991) have been applied. Other conversion factors were pronounced (Wu et al. 1990; Joergensen and Brookes 1991) using a methodology for determination of organic C that differed from that reported by Vance et al. (1987). Moreover, the substrate-induced respiration method estimates the living, non-resting biomass in contrast to the fumigation-extraction method which relies on the total C compounds in both dead and living, resting and non-resting (micro)organisms in soils. Further improvements in microbial biomass estimates, particularly in waterlogged soils, may be obtained by direct counts of organisms, ATP estimate (first by Inubushi et al. 1989), and by the use of ^{14}C-labelled organic substrates.

Compared on a soil dry weight basis (Table 3), microbial biomass values increased in the order intensive field, beech forest, extensive field, dry grassland, alder forest, wet grassland. The results are comparable to data reported by Jenkinson and Ladd (1981) and Smith and Paul (1992). Microbial biomass values in wet grassland soil were very high. Even higher soil microbial biomass values per gram dry soil were reported for old grassland by Jenkinson and Ladd (1981) and in the litter horizon (using substrate-induced respiration) by Anderson and Domsch (1978).

Microbial biomass per upper horizon and hectare (per unit volume)

The second pair of columns in Table 3 show microbial biomass values obtained by fumigation-extraction and substrate-induced respiration for each site per upper horizon and hectare (per unit volume), calculated by multiplying microbial C per gram dry soil by soil density and horizon thickness (Table 1).

Table 3 Microbial biomass in soil from the Bornhöved Lakes region calculated from fumigation-extraction (*CFE*) and substrate-induced respiration (*SIR*) expressed per gram dry weight and per upper soil horizon and hectare

	CFE ($\mu g\ C\ g^{-1}$ dry soil)	SIR ($\mu g\ C\ g^{-1}$ dry soil)	CFE ($kg\ C\ ha^{-1}$)	SIR ($kg\ C\ ha^{-1}$)
Agricultural soils				
Extensive field	194	288	505	750
Intensive field	111	215	289	558
Dry grassland	349	526	454	685
Wet grassland	2050	1402	2460	1682
Forest soils				
Beech	284	227	156	125
Dry alder	2082	1305	833	522
Wet alder	877	907	351	363

The following sequences of microbial biomass values per upper horizon and hectare were obtained. In agricultural soils the microbial biomass increased in the order intensive field, dry grassland, extensive field, wet grassland. However, the microbial biomass in the dry grassland soil was to be calculated to a depth of 10 cm only, in contrast to a 20-cm depth in the other sites. In forest soils the microbial biomass increased in the order beech, wet alder, dry alder, but values for the beech forest soil were calculated only to a depth of 0–5 cm in contrast to a depth of 0–20 cm in the alder forest. For a complete comparison of agricultural and forest soils, it is necessary to calculate the biomass in all important horizons, particularly in the litter horizon of forests.

These values are comparable to results reported by Smith and Paul (1992). In the present investigation, the highest microbial biomass values were found in wet grassland soil but even higher data were reported by Smith and Paul (1992).

The order of increasing microbial biomass values per upper horizon and hectare was different from that for values expressed on a dry soil basis. The effect was quite clear, particularly in the organic soils of the alder forest (Table 3). From an ecological viewpoint the values per horizon and hectare, or per unit soil volume, should allow a comparison of different sites.

Acknowledgements We thank Mrs. A. Buckenauer, Mrs. E. Erlebach, Mrs. F. Schütze, and Mr. J. Sprenger for technical assistance, and Dr. O. Heinemeyer, Dr. R. Martens and coworkers, of the Institute, for the use of the laboratory facilities. These studies were supported by the country of Schleswig-Holstein and the German Ministry of Research and Technology (BMFT), Bonn, Germany.

References

Alef K (1993) Bestimmung mikrobieller Biomasse im Boden: Eine kritische Betrachtung. Z Pflanzenernaehr Bodenkd 156: 109–114

Alef K, Kleiner D (1986) Arginine ammonification, a simple method to estimate microbial activity potentials in soils. Soil Biol Biochem 18:233–235

Alef K, Kleiner D (1989) Rapid and sensitive determination of microbial activity in soils and in soil aggregates by dimethylsulfoxide reduction. Biol Fertil Soils 8:349–355

Alef K, Beck T, Zelles L, Kleiner D (1988) A comparison of methods to estimate microbial biomass and N-mineralization in agricultural and grassland soils. Soil Biol Biochem 20:561–565

Anderson JPE, Domsch K-H (1978) A physiological method for measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221

Bach H-J, Munch J-C (1993) Zusammensetzung mikrobieller Populationen als prägendes Merkmal von Böden des Gebietes „Bornhöveder Seenkette". Mitt Dtsch Bodenkd Ges 72:487–490

Harden T, Joergensen RG, Meyer B, Wolters V (1993) Soil microbial biomass estimated by fumigation-extraction and substrate-induced respiration in two pesticide-treated soils. Soil Biol Biochem 25:679–683

Heinemeyer O, Insam H, Kaiser E-A, Walenzik G (1989) Soil microbial biomass and respiration measurements: An automated technique based on infra-red gas analysis. Plant and Soil 116: 191–195

Inubushi K, Brookes PC, Jenkinson DS (1989) Adenosine 5'-triphosphate and adenylate energy charge in waterlogged soil. Soil Biol Biochem 21:733–739

Jenkinson D, Ladd JN (1981) Microbial biomass in soil: Measurements and turnover. Soil Biochem 5:415–471

Joergensen RG, Brookes PC (1991) Soil microbial biomass estimation by fumigation extraction. Mitt Dtsch Bodenkd Ges 66:511–514

Kaiser E-A, Mueller T, Joergensen R, Insam H, Heinemeyer O (1992) Evaluation of methods to estimate the soil biomass and the relationship with soil texture and organic matter. Soil Biol Biochem 24:675–683

Martens R (1995) Current methods for measuring microbial biomass-C in soil. Potentials and limitations. Biol Fertil Soils 19:87–99

Martikainen PJ, Palojärvi A (1990) Evaluation of the fumigation-extraction method for the determination of microbial C and N in a range of forest soils. Soil Biol Biochem 22:797–802

Mazzarino MJ, Szott L, Jimenez M (1993) Dynamics of soil total C and N, microbial biomass and water-soluble C in tropical agroecosystems. Soil Biol Biochem 25:205–214

Nannipieri P, Johnson RL, Paul EA (1978) Criteria for measurement of microbial growth and activity in soil. Soil Biol Biochem 10:223–229

Smith JL, Paul EA (1992) The significance of soil microbial biomass estimations. Soil Biochem 7:357–396

Sparling GP, Searle PL (1993) Dimethylsulfoxide reduction as a sensitive indicator of microbial activity in soil: The relationship with microbial biomass and mineralization of nitrogen and sulphur. Soil Biol Biochem 25:251–256

Sparling GP, West AW (1988) A direct extraction method to estimate soil microbial C: Calibration in situ using microbial respiration and ^{14}C labelled cells. Soil Biol Biochem 20:337–343

Sparling GP, Feltham CW, Reynold J, West AW, Singleton P (1990) Estimation of soil microbial C by a fumigation-extraction method: Use on soils of high organic matter content, and a reassessment of the k_{ec}-factor. Soil Biol Biochem 22:301–307

Vance ED, Brookes PC, Jenkinson DJ (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

West AP, Sparling GP, Grant WD (1986) Correlation between four methods for estimate total microbial biomass in stored, air-dried and glucose-amended soils. Soil Biol Biochem 18: 569–576

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation-extraction – an automated procedure. Soil Biol Biochem 22:1167–1169

Zagal E (1993) Measurement of microbial biomass in rewetted air-dried soil by fumigation–incubation and fumigation-extraction techniques. Soil Biol Biochem 25:553–559